

## RESEARCH ARTICLE

# A salamander that chews using complex, three-dimensional mandible movements

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## ABSTRACT

Most non-mammal tetrapods have a hinge-like jaw operation restricted to vertical opening and closing movements. Many mammal jaw joints, by contrast, operate in more complex, three-dimensional (3D) ways, involving not only vertical but also propalinal (rostral-caudal) and transverse (lateral) movements. Data on intraoral food processing in lissamphibians and sauropsids has prompted a generally accepted view that these groups mostly swallow food unreduced, and that in those cases where lissamphibians and sauropsids chew, they mostly use simple vertical jaw movements for food processing. The exception to this generally accepted view is the occurrence of some propalinal chewing in sauropsids. We combined 3D kinematics and morphological analyses from biplanar high-speed video fluoroscopy and micro-computed tomography to determine how the paedomorphic salamander *Siren intermedia* treats captured food. We discovered not only that *S. intermedia* uses intraoral food processing but also that the elaborated morphology of its jaw joint facilitates mandibular motions in all three planes, resulting in complex 3D chewing. Thus, our data challenge the commonly held view that complex 3D chewing movements are exclusive to mammals, by suggesting that such mechanisms might have evolved early in the tetrapod evolution.

**KEY WORDS:** Intraoral food processing, Feeding, Amphibia, Kinematics, Functional morphology, Form and function

## INTRODUCTION

Food processing refers to any mechanical reduction or preparation of food before it is swallowed (Bels and Goosse, 1989; Bramble and Wake, 1985; Schwenk and Rubega, 2005) and involves rhythmic, cyclical and usually tightly coordinated movements of the cranium, mandible and tongue (or hyobranchial apparatus in gill bearing vertebrates) (Hiimäe and Ardran, 1968; Lauder, 1981; Schwenk and Rubega, 2005). Such behaviours are generally considered essential for immobilization and reduction of food prior to swallowing (Reilly et al., 2001; Schwenk and Schwenk, 2000; Throckmorton, 1976). The most familiar and commonly utilized processing mechanism in gnathostomes is chewing, which involves puncturing, shearing or crushing of food items by dentition set in the mandibular jaws, while cyclic motions of the hyobranchial apparatus act to move food onto the occlusal table (Davis, 1961;

Hiimäe and Ardran, 1968; Schwenk and Schwenk, 2000). Mammal chewing results in real comminution of the food (i.e. bolus formation) while non-mammals typically pierce, crush or rasp food items, resulting in little, if any, fragmentation (Schwenk and Rubega, 2005). Chewing jaw movements are diverse and involve three major elements that are directionally distinguishable: (i) vertical (arcuate or orthal, open–close) movements, (ii) propalinal (longitudinal, retraction–protraction) movements and (iii) transverse (lateral) movements of the mandible. While non-mammal tetrapods tend to use simple vertical jaw movements, most mammals combine movements in all three planes (i.e. 3D mandible movements) to varying extents (Bhullar et al., 2019; Crompton et al., 2010; Hiimäe and Crompton, 1985; Grossnickle, 2017).

Data on chewing exist for fishes (Gintof et al., 2010; Kolmann et al., 2016; Lauder, 1980; Laurence-Chasen et al., 2019; Rutledge et al., 2019) and sauropsids (Reilly et al., 2001; Ross et al., 2010; Throckmorton, 1976) but the behaviour has mostly been studied in mammals (Bhullar et al., 2019; Crompton et al., 2010; Hiimäe and Ardran, 1968), whereas relatively few data exist for lissamphibians (Schwenk and Wake, 1993). In fact, it has been suggested that lissamphibians generally only transport food (Dockx and De Vree, 1986; Lauder and Reilly, 1990; Schwenk and Schwenk, 2000) without processing it (De Vree and Gans, 1994; Schwenk and Rubega, 2005). The few known exceptions include the ‘head tucking’ behaviour of plethodontid salamanders following prey capture (Deban and Richardson, 2017; Schwenk and Wake, 1993) where rhythmic vertical jaw movements are used to inflict a series of strong bites to the prey. A recent study also described food processing in a salamandrid newt that rasps prey against its palatal dentition using cyclic loop movements of the tongue (Heiss et al., 2019). Together, these findings suggest that food processing could be more widespread and diverse amongst salamanders than previously thought.

Food processing depends on head, jaw and hyobranchial morphology (Herrel et al., 2012; Schwenk and Rubega, 2005), which vary substantially with ontogeny and phylogeny in lissamphibians (Heatwole and Rose, 2003; Wiedersheim, 1877; Ziermann, 2019). While at least two intraoral food processing mechanisms are known for metamorphosed salamanders, no data are currently available on the processing behavior of salamanders with larval characteristics (i.e. larval or paedomorphic morphotypes). Therefore, we analysed food processing in *Siren intermedia*, whose differentiation of somatic features is arrested in early ontogeny (Noble and Marshall, 1932; Reiss, 2002; Rose and Reiss, 1993), including retention of larval head muscles and skeletal characteristics (Clemen and Greven, 1988; Davit-Béal et al., 2007; Diogo and Abdala, 2010) that are clearly distinct from those of metamorphosed salamanders (Carroll and Holmes, 1980; Estes, 1965). For example, the prominent tooth pads on the surfaces of the mouth roof (specifically the palatine and vomerine bones) comprise the functional upper jaw, as the actual upper jaw (maxillary and

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premaxillary) bones that typically carry teeth in metamorphic tetrapods are small and toothless. Similarly, the presence of teeth on the coronoid elements of the mandible, as well as the arrested state of development of the hyobranchial elements signal an early developmental stage in larval head growth (Davitt-Béal et al., 2007; Heatwole and Rose, 2003). Therefore, we propose *S. intermedia* to be a suitable model for studying food processing in salamanders with early larval traits.

The preferred prey of *S. intermedia* includes potentially damage-inflicting organisms (Hampton, 2009; Hanlin, 1978; Scroggen and Davis, 1956) so we predict that *S. intermedia* uses food processing to incapacitate and reduce food prior to swallowing. Indeed, our initial observations revealed that *S. intermedia* engages in rhythmic movements of the head, jaw and hyobranchial apparatus following prey capture. We hypothesized that these movements represent a hitherto undescribed mechanism for food processing, an idea we tested using a combination of morphological and experimental approaches.

## MATERIALS AND METHODS

### Specimens and animal care

Two female and two male *Siren intermedia* Barnes 1826 were chosen from the animal stock of the Institute of Zoology and Evolutionary Research at Friedrich-Schiller-University of Jena, Germany. Snout-vent length (SVL) of the animals was  $29.5 \pm 2.5$  cm (mean  $\pm$  s.d.) and their mass was  $135.9 \pm 45$  g (mean  $\pm$  s.d.). The specimens were kept paired (SiF2/SiM2 and SiF3/SiM1). The two pairs were housed in separate glass aquariums ( $120 \times 40 \times 50$  cm) with a temperature of  $23 \pm 2^\circ\text{C}$ , a 12 h/12 h photoperiod and fed a varied diet of maggots, small fish and mussel flesh. Husbandry and experiments were approved by the Committee for Animal Welfare of the State of Thuringia (Germany) (code for animal experiments: 02-008/15, code for animal husbandry: J-SHK-2684-05-04-05-07/14).

### Marker implantation, data collection and data processing

Surgical implantation of radio-opaque tantalum markers (Bal-Tec, Los Angeles, CA, USA) on the skeletal structures of interest followed a protocol modified from prior studies (Herrel et al., 2000). The specimens were anaesthetized with buffered (pH 7.2) aqueous 0.05% MS222 (tricaine methanesulfonate) solution and the depth of the anaesthetic plane was verified by toe pinch and gently squeezing varying parts of the body with blunt tweezers. The fully anaesthetized specimen was placed on a sterile surgery tray and covered with a towel soaked in the anaesthesia solution. A hypodermic needle was used to implant spherical tantalum markers of 0.45 mm diameter to mark the tip of the upper jaw (pt), tip of the lower jaw (dt), tip of the basibranchial (indicating the hyobranchial apparatus) (bb) and back end of one hemimandible (pa) (Fig. 1A). Our initial analysis revealed that the hemimandibles of both sides move symmetrically during the transverse mandible movement. Hence, we only implanted one marker in the back end of one hemimandible and duplicated the transverse hemimandible movement of this side (Fig. 1E) to reduce the potential burden from an additional marker implant. Once recovered from anaesthesia, the animals were housed separately in recovery tanks for 1 day, after which they were returned to paired housing. The specimens were given a recovery time of at least 2 weeks to ensure complete surgical wound healing and that regular feeding had resumed.

*Siren intermedia* were fed maggots (*Lucilia* sp.) in water and to ensure that these food items were visible in the X-ray recordings, we glued tantalum markers of 0.45 mm diameter to their cuticle. We chose maggots as they are part of the natural prey spectrum of

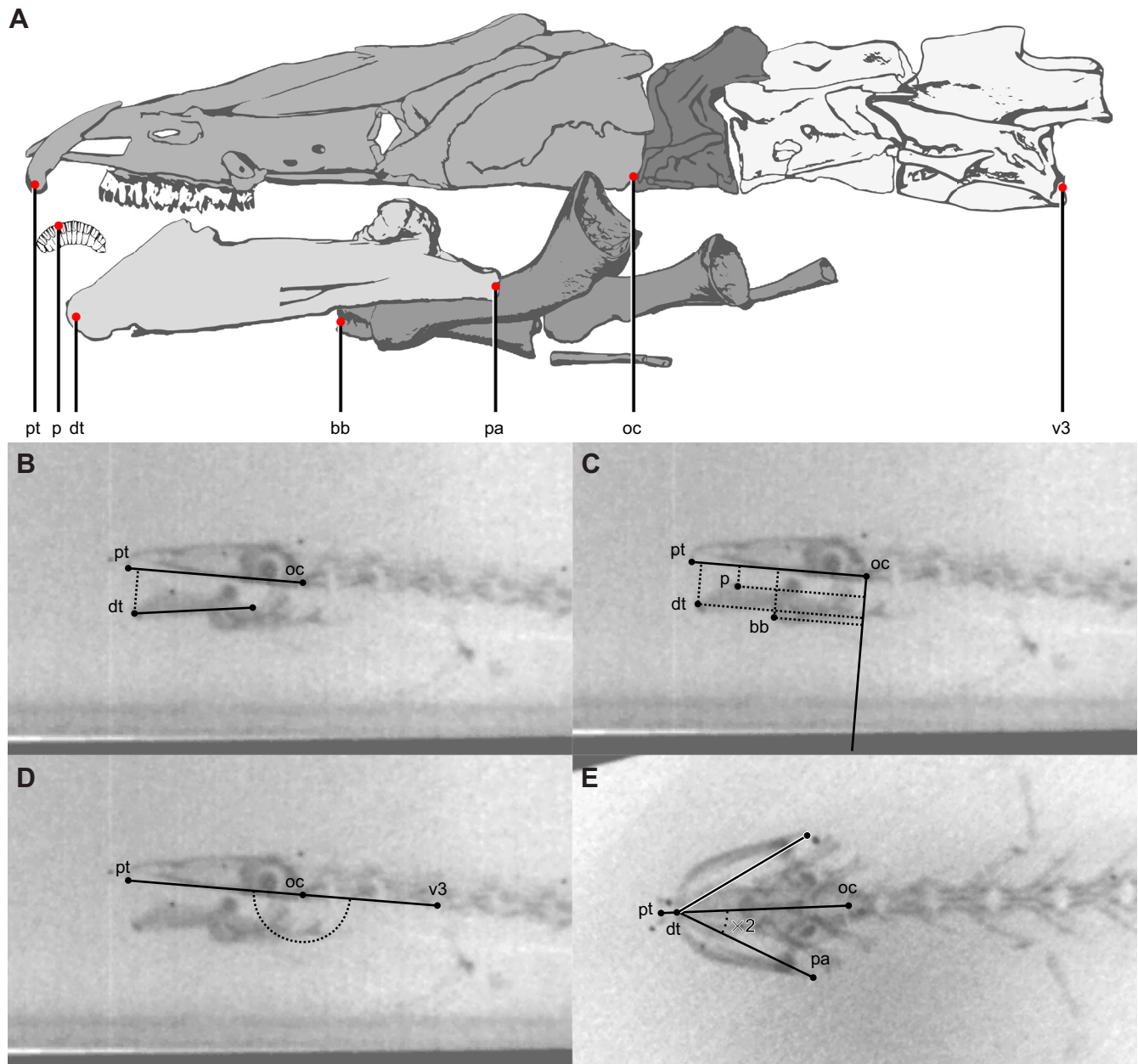
*S. intermedia*, though also potentially damage inflicting. It has been reported that maggots of the genus *Lucilia* can survive in the digestive tract and tissue of amphibians under certain circumstances, where they can cause severe damage (Boie, 1865; Brumpt, 1934; Zumpt, 1965).

The biplane high-speed X-ray setup consisted of two customized Neurostar TOP devices (Siemens Medical Solutions Inc.), which were mounted on two independently movable C-arms, and an acrylic aquarium. X-ray recordings were taken from the (A) dorso-ventral and (B) latero-lateral projections with a sampling frequency of 500 Hz. The following technique was used: aperture 10 mm (A), 12 mm (B); image intensifier zoom  $2\times$  (A, B); electric current 175 mA (A, B); voltage 50 kV (A, B); frame resolution  $1536 \times 1024$  pixels. A total of 39 videos were recorded of which 16 recordings were selected based on the orientation of the specimen for further manual and automatic analysis.

This selection resulted in 97 cycles of post-capture movements (SiF2, 29; SiF3, 24; SiM1, 24; SiM2, 20). The videos were digitized in XMALab 1.5.0 (Knörlein et al., 2016) using a mixture of manual landmark tracking and automatic marker tracking. We calculated eight componential motions from the 3D landmark coordinates in Excel (Office 2016, Microsoft Corporation, WA, USA).

The component motions were: (1) propalinal mandible movement, measured as displacement of the tip of the lower jaw relative to the normal slope of the line connecting the points pt and oc through point oc (Fig. 1C); (2) vertical mandible movement as the distance between the tip of the lower jaw (dt) perpendicular to the line connecting the points pt and oc (Fig. 1B); (3) transverse mandible movement as the angle between the line connecting the posterior end (pa) and anterior tip (dt) of one hemimandible and the line connecting points pt and oc, times two (Fig. 1E); (4) longitudinal basibranchial movement as displacement of the anterior tip of the basibranchial (bb) relative to the normal slope of the line connecting the points pt and oc through point oc (Fig. 1C); (5) vertical basibranchial movement as normal displacement of the anterior basibranchial tip (bb) relative to the line connecting the points pt and oc (Fig. 1C); (6) longitudinal prey transport as prey (p) displacement relative to the normal slope of the line connecting the points pt and oc through point oc (Fig. 1C); (7) vertical prey transport as normal displacement of the prey (p) relative to the line connecting the points pt and oc (Fig. 1C); and (8) vertical cranial movement as the angle enclosed by the slope connecting the tip of the upper jaw (pt) and the occipital (oc) and the slope connecting the occipital (oc) and the third vertebra (v3) (Fig. 1D). The kinematic variables describing translations were normalized to the individual cranial length (% CL).

The kinematic profiles (changes in angles and distances over time) show repetitive cycles of component motions. Subdivision of the kinematic profiles into component motion cycles was achieved using a custom graph analyser tool for MATLAB 2017b (The Mathworks, Inc., Natick, MA, USA). A cycle was defined as a movement event containing three extrema: two of either low or high point and one of the other. Visual inspection of sequences suggested that propalinal movement of the mandible was the main motion component. Therefore, to generate mean kinematic profiles, the graph of the propalinal mandible movement was subdivided into componential phases (i.e. component motion cycles). All related component motion graphs were generated automatically using propalinal mandible movement as reference (Fig. 3). The kinematic variables (translations and their duration from extrema to extrema) were generated by fragmentation of the component motion cycles according to their extremes (Table S1).



**Fig. 1. Landmarks used for kinematics analyses.** (A) Anatomical sketch of the *Siren intermedia* skull (lateral view) with landmarks used for kinematic analyses. (B–E) X-ray screenshots from (latero-lateral and ventro-dorsal views): (B) landmarks used for calculation of vertical mandible movements; (C) landmarks used for calculation of prey, basibranchial (indicating the hyobranchial apparatus), and mandible translations; (D) landmarks used for calculation of neck flexion–extension; and (E) landmarks used for calculation of transverse hemimandible movements. bb, basibranchial; dt, dentary tip; oc, occipital condyle; p, prey; pa, prearticular; pt, premaxilla tip; and v3, third vertebra.

### Anatomical analysis

The musculoskeletal components of the feeding apparatus of two specimens (SiF2/SiM1) were reconstructed from micro-computed tomography ( $\mu$ CT) scans. Specimens were euthanized in a buffered 0.5% MS222 solution, fixed in 4% formaldehyde for 1 month, dehydrated in a graded series of ethanol, immersed for 2 weeks in an alcoholic iodine solution, rinsed in absolute ethanol and mounted in Falcon tubes. Two scans of the entire head region of each of the two specimens were acquired using a  $\mu$ CT scanner (XRadia MicroXCT-400, Carl Zeiss X-ray Microscopy, Pleasanton, CA, USA) at VetCore Facility for Research (University of Veterinary Medicine, Vienna, Austria). To apply the dual-energy  $\mu$ CT workflow (Handschuh et al., 2017), the first scan was performed with 40 kVp, 200  $\mu$ A and the second

scan with 80 kVp, 100  $\mu$ A, with an isotropic voxel size of 20.3  $\mu$ m for both approaches (resulting in two scans per specimen). Next, separate material fractions of mineralized and soft tissue were reconstructed according to the dual-energy  $\mu$ CT workflow. Volume rendering of the resulting  $\mu$ CT scans was performed using the Amira 6.4 software package (<https://www.fei.com/software/amira>). Based on tomographic image data, we threshold segmented relevant structures. The resulting 3D reconstructions were visualized in reference orientations using volume rendering from which snapshots were taken. For a more detailed anatomical analysis of the jaw joint, the skull of SiF2 and a specimen that died prior to the study were prepared for histological sections. Both specimens were decalcified using Osteomol (Merck KGaA, Darmstadt, Germany), and after complete decalcification, dehydrated in a graded

ethanol series and embedded in paraffin (Histoplast-S, SERVA Electrophoresis GmbH, Heidelberg, Germany). Serial sections (8 µm) were taken on a rotary microtome (Microm HM 355 S, Thermo Fisher Scientific Inc., Waltham, MA, USA), mounted on glass slides and stained with AZAN using standard protocols (Kiernan, 1999), and documented using a light microscope (Olympus BX-51 with XC10 camera, Olympus, Tokyo, Japan).

### Analyses of mandible and squamosal motion potential

To examine the range of motion of the mandible at the jaw joint and to test for cranial kinesis between the squamosal and skull, we dissected two specimens (SIF3/SIM2) and the skeletal elements of interest were manually manipulated following *in vivo* data collection. The specimens were euthanized in a buffered 0.5% MS222 solution, fixed in 4% formaldehyde, rinsed in tap water and dissected. After careful removal of the head muscles, propalinal and transverse movability of the mandible and the squamosal was tested by pulling and pushing on the lower jaw and squamosal using forceps.

## RESULTS

### Chewing motion kinematics

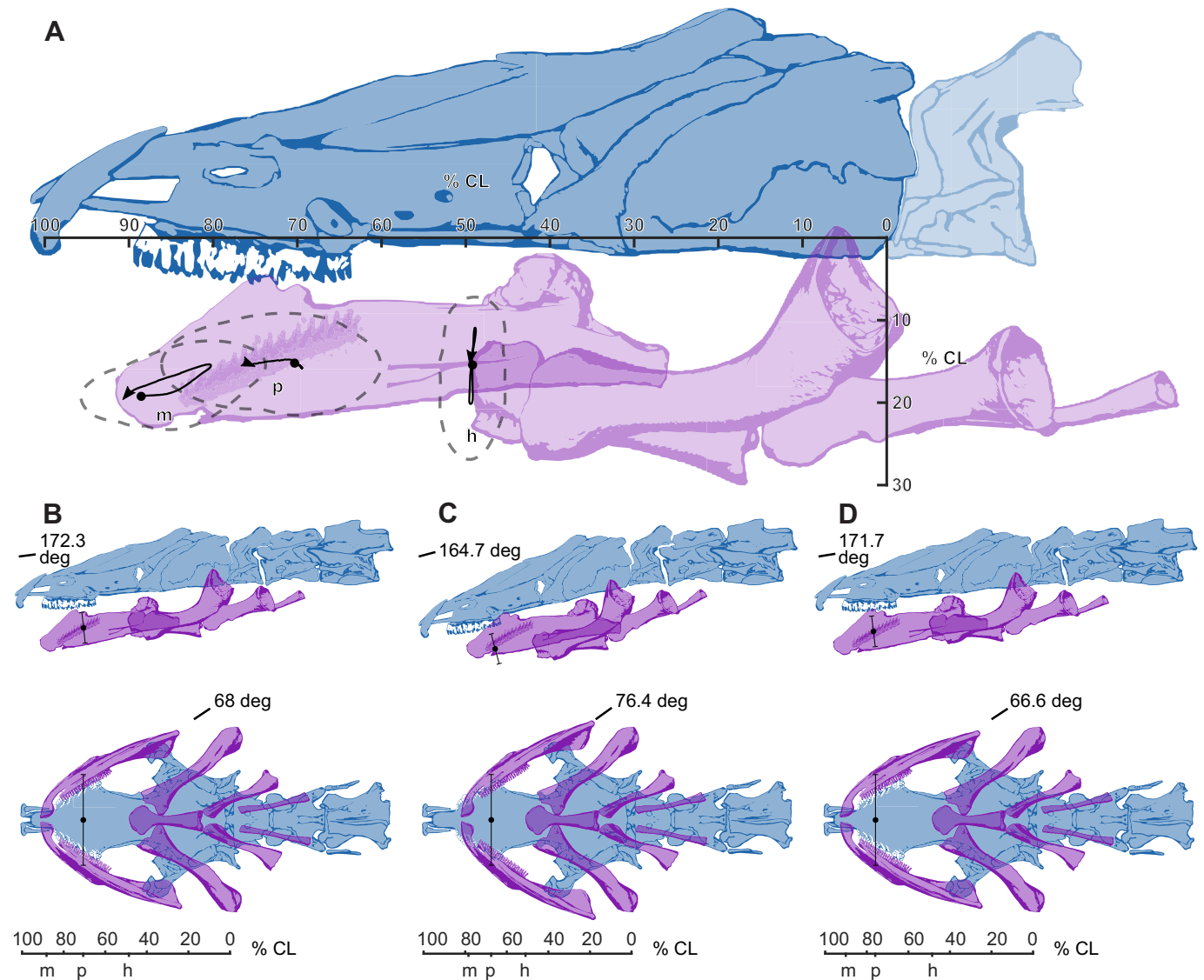
Following prey capture, all specimens used rhythmic jaw and hyobranchial movements to process food. Processing involved cyclic jaw opening and closing (i.e. vertical mandible movement) (Fig. 2 and Fig. 3B), mandible retraction and protrusion (propalinal mandible movement) (Fig. 2B–D and Fig. 3A), and lateral abduction and adduction of the lower jaw ‘arms’ or hemimandibles (transverse hemimandible movement, i.e. mandible wishboning) (Fig. 2B–D and Fig. 3H). At the same time, there was rhythmic and cyclic flexion and extension of the neck (vertical cranial movement) (Fig. 2B–D and Fig. 3G), hyobranchial retraction and protrusion (longitudinal hyobranchial movement) (Fig. 2B–D and Fig. 3E) as well as hyobranchial elevation and depression (vertical hyobranchial movement) (Fig. 2B–D and Fig. 3F). During these movements, prey debris was expelled from the oral cavity, indicating that the behaviour caused significant prey disintegration (see Movies 1 and 2 in figshare: <https://doi.org/10.6084/m9.figshare.11881110.v1>).

A processing cycle is defined from the start of lower jaw retraction to the end of its protrusion. Using these propalinal jaw movements as a reference, all cycles were divided into preparatory (I) and power stroke (II) phases (see vertical dotted lines in Fig. 3A–H). In the first phase (preparatory phase), as the skull was depressed and the lower jaw retracted, the hemimandibles were spread apart along the transverse axis (i.e. abducted laterally, wishboning over the symphysis of the mandible) and the mouth closed slightly (Fig. 2C) (see Movie 3 in figshare: <https://doi.org/10.6084/m9.figshare.11881110.v1>). In the second phase (power stroke phase), as the skull was elevated and the lower jaw protruded, the mandibular rami were brought together along the transverse axis (adducted medially) and the mouth opened slightly (Fig. 2D). As the lower jaw was protruded, the prey was moved forward (anteriorly) and rasped against the functional upper jaw (anterior palatal dentition), whereby it was processed (Fig. 2D). During chewing, the prey was rasped forward bite by bite. When the prey was displaced too far anteriorly for further chewing (i.e. beyond the margins of the palatal dentition), water flows induced by hyobranchial depression transported the prey toward the oesophagus (posterior oropharynx) (see Movie 4 in figshare: <https://doi.org/10.6084/m9.figshare.11881110.v1>). The prey remained there for variable periods of time, after which it was either protruded and repeatedly processed or swallowed. Each chewing event consisted of one to several chewing bouts (or trains) and each bout consisted of between one and seven chewing cycles (or ‘rasping’ bites).

### Head anatomy of *S. intermedia*

Detailed descriptions of the cranial anatomy of *S. intermedia* are available elsewhere (Carroll and Holmes, 1980; Clemen and Greven, 1988; Diogo and Abdala, 2010; Iordansky, 2010; Kleinteich and Haas, 2011; Reilly and Altig, 2006; Wiedersheim, 1877); this study focuses on structures relevant to food processing. The upper jaw and mouth roof consists of the very small toothless premaxilla and maxilla, along with the prominent paired vomer and palatine elements. The vomer and palatine carry single-cusped teeth that are recurved backwards and inwards (i.e. postero-medially), arranged in rows and, accordingly, form the ‘functional upper jaw’. On the lower jaw, only the coronoid processes carry teeth, which show a similar morphology and orientation to the opposing dentition on the functional upper jaw. The hyobranchial apparatus (tongue) consists of massive bones, prominent muscles and cartilages filling most of the space of the floor of mouth between the hemimandibles (Fig. 4F).

The main muscles of the feeding system are shown in Fig. 4A–C and Fig. 5. The jaw closers (i.e. adductores mandibulae, am) contain an external (ame) and an internal (ami) part. The adductor mandibulae internus complex (ami) consists of a pseudotemporalis and a pterygoideus portion. The pseudotemporalis is further portioned into an anterior positioned pseudotemporalis profundus (also referred to as adductor mandibulae A3”) and a posterior pseudotemporalis superficialis (also referred to as adductor mandibulae A3’; Diogo and Abdala, 2010). The pseudotemporalis originates at the medial fission zone of both frontal and parietal bones, runs posteriorly and ventrally and inserts on the central and medial part of the hemimandible. Below the pseudotemporalis lies the pterygoideus muscle (also referred to as pterygomandibularis), which originates on the lateral wall of the skull (parasphenoid and orbitosphenoid). The pterygoideus reaches ventrally, laterally and posteriorly to the back end of the lower jaw where it wraps around the hemimandible behind the jaw joint to form a fleshy belly (Fig. 4B,C and Fig. 5) and inserts on the outer side of the mandible. Given this line of action, the pseudotemporalis is functionally a jaw opener that also protracts and adducts the mandible medially. The adductor mandibulae externus complex (ame) contains tightly interconnected muscles, originating on a tendon of the first vertebra (atlas), extending antero-ventrally and connecting to a tendon sheet (coronar aponeurosis) central on each hemimandible. The epaxial (e) neck musculature attaches on the back (occipital) of the skull and extends posteriorly along the whole body length (Fig. 4A,B and Fig. 5). The jaw opener complex (depressor mandibulae complex) lies adjacent to the adductor mandibulae complex and consist of two parts, the (anterior) depressor mandibulae (dm) and a depressor mandibulae posterior (dm’). The depressor mandibulae posterior descends from the levator hyoideus (Diogo and Abdala, 2010) and, consequently, it is often referred to as such. The smaller anterior depressor mandibulae originates at squamosal and exoccipital and inserts on the upper part of the retroarticular process (Fig. 4A,B and Fig. 5). The depressor mandibulae posterior originates on the dorsal fasciae of the neck muscles, runs along the ceratohyal cartilage and attaches on the back of the retroarticular process (Fig. 4A–C and Fig. 5). The hyobranchial skeleton, positioned within the floor of the mouth, forms the attachment site for four major muscles. The largest is the branchiohyoideus externus, extending from the upper part of the hyobranchial skeleton (cartilaginous ceratobranchial I) to the ceratohyal. The delicate geniohyoid muscle connects the foremost hyobranchial bone (basibranchial) anteriorly to the lower jaw (dentary) while the subarcualis rectus I connects the basibranchial posteriorly to the cartilaginous ceratobranchial I. The rectus cervicis



**Fig. 2. Movements of cranial elements during chewing in *S. intermedia*.** (A) Lateral view of the range of motion of the main chewing movements normalized to cranial length (CL). Solid lines represent means while dashed lines indicate 68% confidence interval of the 97 analysed cycles. The counter-clockwise loops indicate motion of the mandible (m) that moves prey (p) along the mouth roof while the hyobranchial apparatus (h) (tongue) moves mainly vertically. (B–D) The mean orientation of the head elements from lateral and ventral views at the different chewing stages: (B) resting, (C) fully retracted and (D) fully protracted state. Shifts in bone orientation from B to C indicate movements during stage I (preparatory phase); shifts in C to D indicate movements during stage II (power stroke phase). Mean displacements in Table S1 and raw data were used to generate the displacement panels B–D; the position of the prey (black dot) was added using the mean kinematic profiles.

(sternohyoid) muscle connects the central hyobranchial bone (hypobranchial I) posteriorly to the abdominal muscle (rectus abdominis) (Fig. 4B,C and Fig. 5).

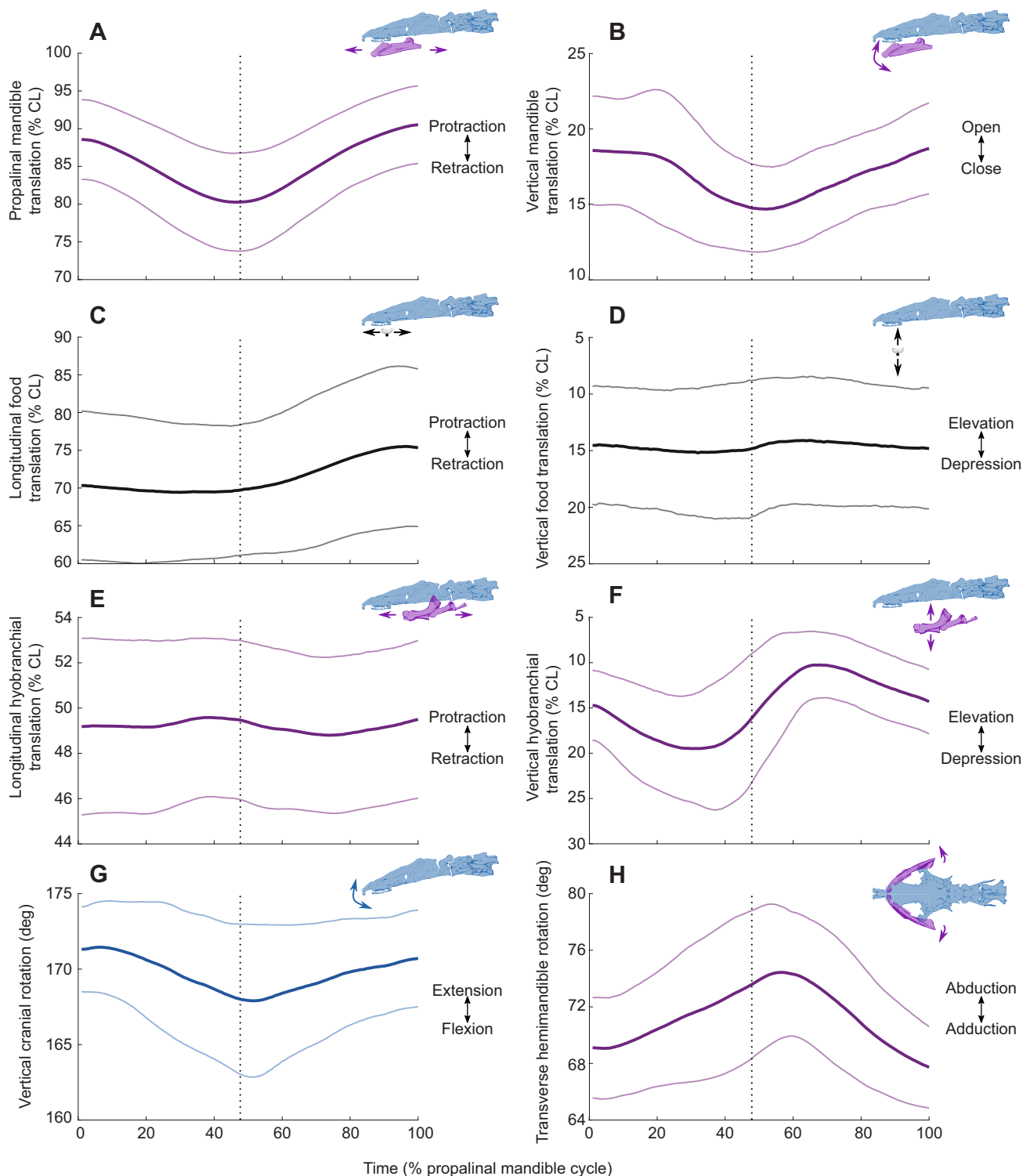
### Anatomy of the jaw joint complex

The jaw joint complex of *S. intermedia* is an elaborate structure built up by the cartilaginous mandibular suspension (quadrate), a set of cartilaginous processes and ligaments, the jaw joint with surrounding connective tissue, and parts of the mandible (Fig. 6; Fig. S1, Fig. S2). The contact area of the jaw joint consists of the cartilaginous quadrate that articulates with the cartilaginous articular of the lower jaw. The jaw joint resembles a half-saddle joint, with a saddle like or ball-and-socket like structure in its anterior region (Fig. 6B,E) that flattens up posteriorly (Fig. 6C,F). The quadrate carries two cartilaginous processes. The rear and descending (postero-ventral) branch (i.e. hyoquadrate process)

links the quadrate and ceratohyal (Fig. 6D,G; Fig. S1). The upper and rising (dorso-medial) branch (i.e. ascending process) connects the quadrate to a cartilaginous lateral part of the cranium, posterior to the orbitosphenoid (Fig. 6B,E).

### Analyses of the motion potential of the mandible and its squamosal suspension

To determine the potential magnitude and direction of jaw movement with respect to the skull, we dissected two *S. intermedia* specimens and used careful manipulations (Konow et al., 2008). Gentle pulling and pushing of the mandible in the longitudinal direction with respect to the long-axis of the skull resulted in clear propalinal and transverse stretching deformations at the jaw joint. The squamosal remained virtually fixed with respect to the skull. Greater forces directly applied to the squamosal only resulted in minor bending but no rotation of the

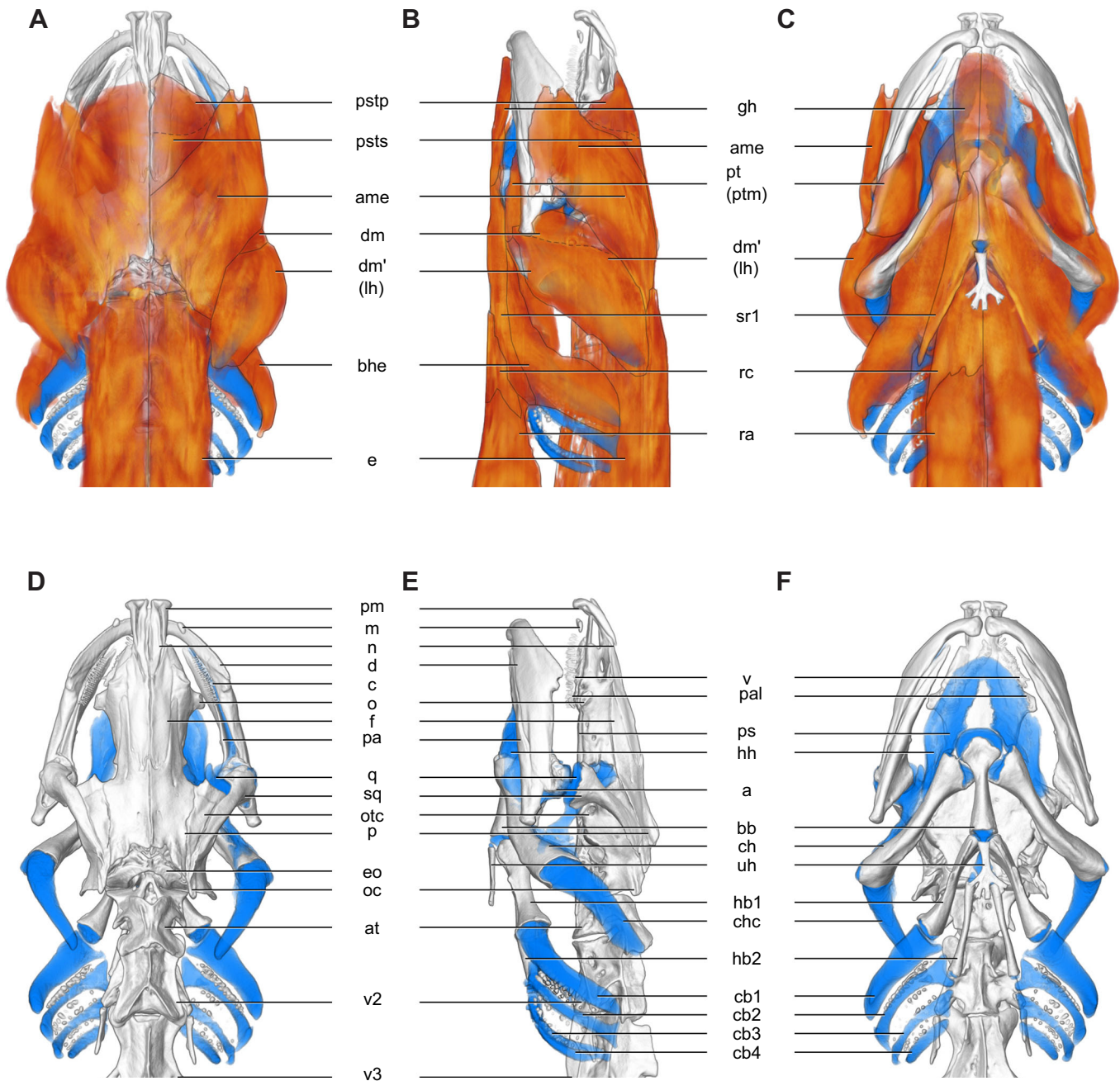


**Fig. 3. Mean kinematic profiles of aquatic chewing in *S. intermedia*.** Based on 97 chewing cycles (bold curves)  $\pm$ s.d. (narrow curves). The profiles show motions of the feeding apparatus and prey in the cranial coordinate system (see Fig. 2A for the coordinate system reference), normalized to the timing of propalinal mandible movement. Translations are normalized to cranial length (CL), and the dotted lines separate phase I (preparatory) and phase II (power stroke). Purple, mandible and hyobranchial movements; blue, cranial movement; and black, displacement of the prey item. The comparison of each kinematic profile with the corresponding movement (Fig. 2) demonstrates the position and translation or rotation of the underlying structures. Please see Table S1 for absolute kinematics values.

squamosal relative to the cranium was observed. Our dissections revealed that dense connective tissue encapsulates and reinforces the jaw joint.

## DISCUSSION

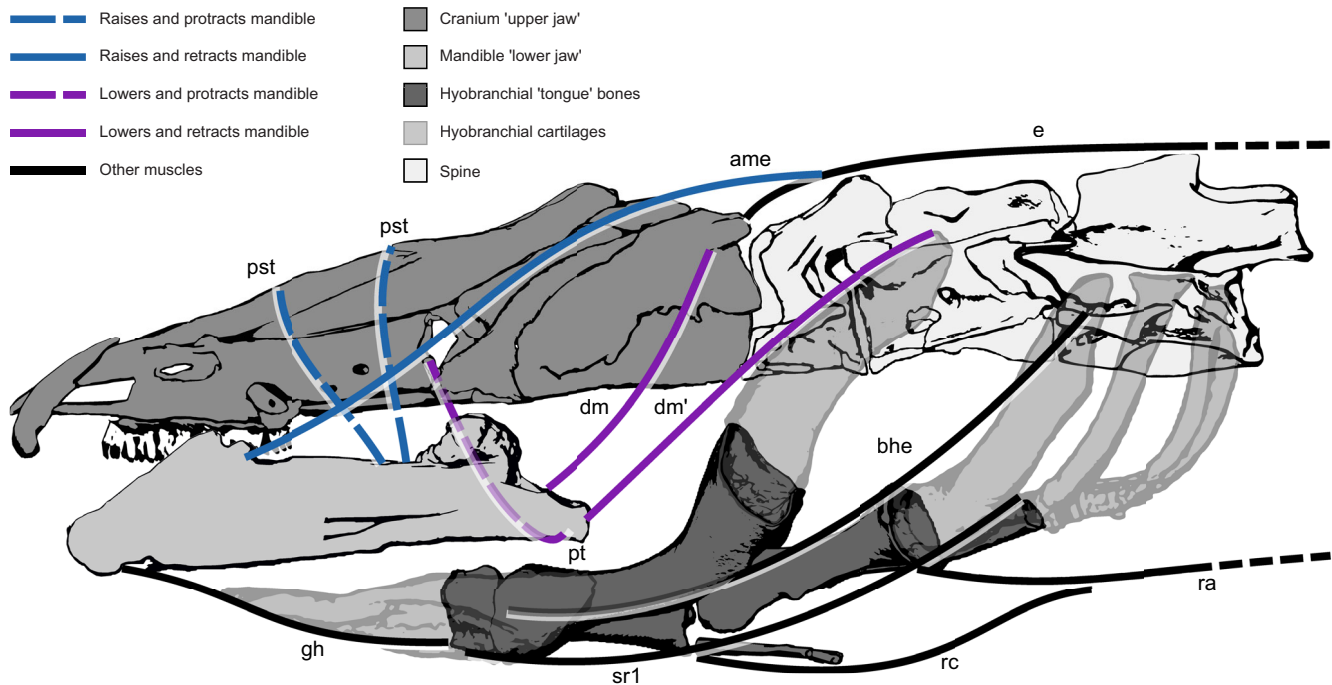
We used anatomical and biplanar high-speed video-fluoroscopy techniques to show that *S. intermedia*, after capturing its prey, uses



**Fig. 4. Head anatomy of *S. intermedia* based on dual-energy micro-computed tomography ( $\mu$ CT) scans.** Bone reconstruction is based on a hydroxyapatite fraction; cartilage reconstruction is based on a water fraction; and muscles were reconstructed using an iodine fraction. (A–C) Muscles (outlined to clarify their location): ame, adductor mandibulae externus (complex); bhe, branchiohyoideus externus; dm, depressor mandibulae (anterior); dm', depressor mandibulae posterior (or levator hyoideus, lh); e, epaxialis; gh, geniohyoideus; pstp, pseudotemporalis profundus; psts, pseudotemporalis superficialis; pt, pterygoideus (also referred to as pterygomandibularis, ptm); ra, rectus abdominis; rc, rectus cervici; and sr1, subarcualis rectus 1. (D–F) Bones and cartilages: a, articular; at, atlas; bb, basibranchial; c, coronoid; cb 1–4, ceratobranchial 1–4; ch, ceratohyal; chc, ceratohyal cartilage; d, dental; eo, exoccipital; f, frontal; hb1–2, hypobranchial 1–2; hh, hypohyal; m, maxilla; n, nasal; o, orbitosphenoid; oc, occipital condyle; otc, otic capsules; p, parietal; pa, prearticular; pm, premaxilla; ps, parasphenoid; pal, palatine; q, quadrate; sq, squamosal; uh, urohyal; v, vomer; v2, second vertebra; v3, third vertebra.

cyclic and rhythmic movements of the mandible, skull and hyobranchial apparatus (tongue) to process its food. The hyobranchial apparatus drives the food dorsally (compare Fig. 3D and F) after which the mandible translates the food longitudinally (compare Fig. 3A and C) and processing occurs as prey is rasped between the teeth of the lower jaw (i.e. mandible) and the functional upper jaw (i.e. palatal dentition) (compare Fig. 2C and D) (see Movies 3 and 4 in figshare: [https://doi.org/10.6084/m9.figshare.](https://doi.org/10.6084/m9.figshare.11881110.v1)

11881110.v1). Thus, food processing in *S. intermedia* qualifies as a form of chewing, which per definition, involves food processing by movements of the mandibular jaws (Reilly et al., 2001). The few processing mechanisms in other salamanders known so far differ considerably from chewing in *S. intermedia*. For example, plethodontids deploy cyclic flexion/extension at the neck, resulting in head pitching movements, and the power of this movement is amplified and transmitted to the mandible via the



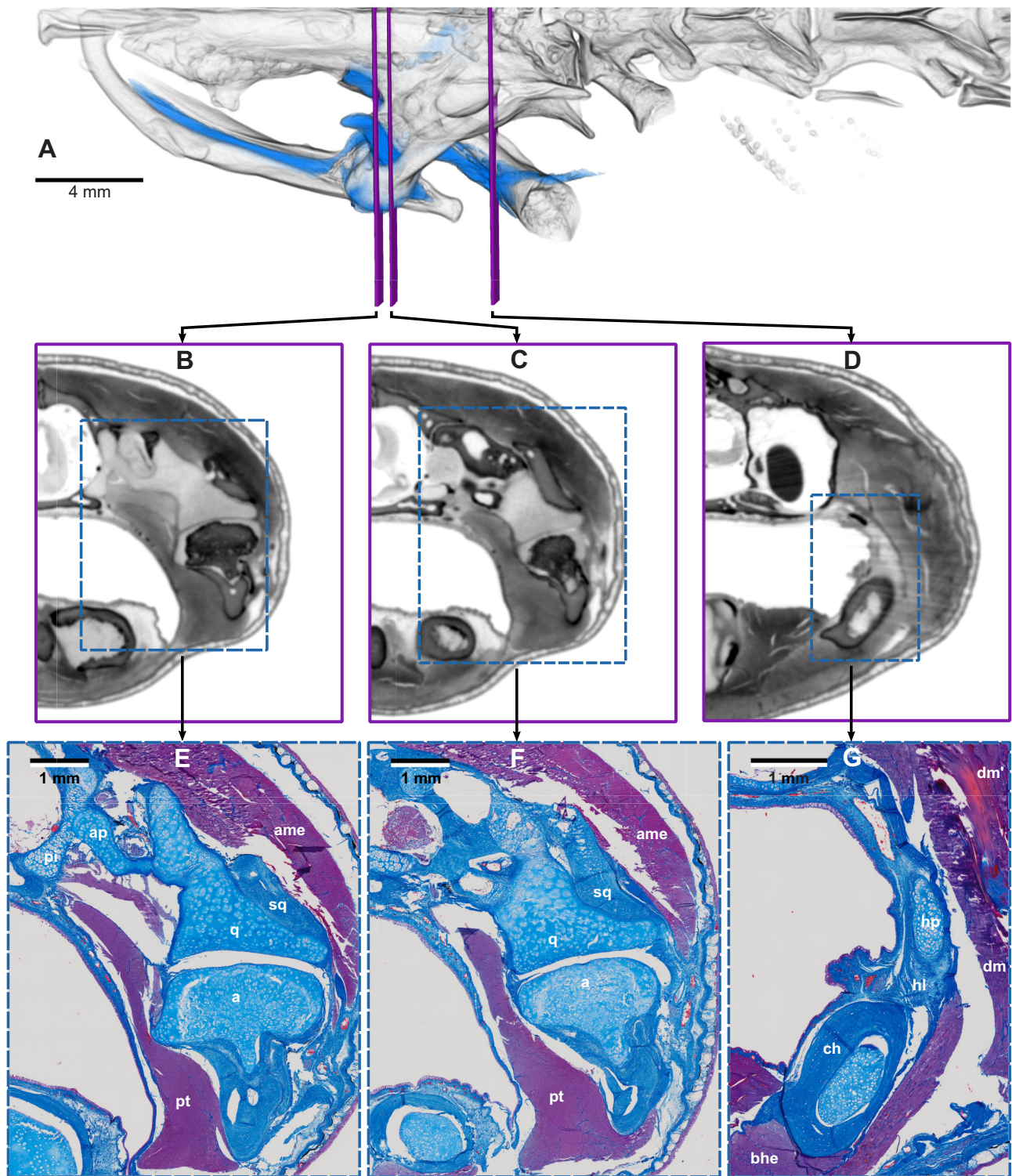
**Fig. 5. Schematic diagram of the functional key components of the cranial musculoskeletal system in *S. intermedia*.** Muscles: ame, adductor mandibulae externus; bhe branchiohyoideus externus; dm, depressor mandibulae; dm', depressor mandibulae posterior (or levator hyoideus, lh); e, epaxialis; gh, geniohyoideus; pstp, pseudotemporalis profundus; pst, pseudotemporalis superficialis; pt, pterygoideus (also referred to as pterygomandibularis, ptm); rc, rectus cervicis; ra, rectus abdominis; and sr1, subarcualis rectus 1. Dashed and opaque lines indicate muscle extending further than drawn, transparent lines (or parts) indicate muscle parts hidden from skeletal elements.

atlando-mandibular ligament in order to produce a series of strong bites onto the food (Deban and Richardson, 2017). Salamandrids use cyclic head pitching movements in combination with repetitive loop motions of the tongue to rasp the food across its palatal dentition (Heiss et al., 2019). In contrast, the chewing mechanism in *S. intermedia* is a remarkably complex 3D procedure that in addition to the rhythmic skull and hyobranchial movements involves three cyclical components of mandibular motion in the (i) median, (ii) horizontal and (iii) transverse planes. Hence, our results are at odds with the commonly accepted view that 3D mandible movements during chewing are exclusive to mammals (Reilly et al., 2001; Ungar and Sues, 2019).

Chewing is widespread across tetrapods in general (Gintof et al., 2010; Konow et al., 2011; Ross et al., 2007), but it has long been assumed that non-amniote tetrapods and many sauropsids – if they chew at all – mostly use ‘simple’ vertical mandible movements because their hinge-like jaw joints only permit dorso-ventral rotation of the lower jaw (Olson, 1961; Reilly et al., 2001; Ungar and Sues, 2019). Such vertical jaw movements are thought to be the ancestral tetrapod condition (Hotton et al., 1997; Olson, 1961) and have been assumed to be retained among lissamphibians (Olson, 1961). However, chewing movements can exhibit many degrees of freedom. For example, propalinal chewing movements have evolved independently several times in tetrapods (Reilly et al., 2001), and have been reported in mammals (Hiimäe and Crompton, 1985; Offermans and de Vree, 1990; Weijs, 1975), turtles (Bramble, 1974; Schumacher, 1973) and some lepidosaurs (Gorniak et al., 1982; Throckmorton, 1976). Chewing in turtles is initiated with mandibular protraction and simultaneous jaw opening before jaw closure and mandible retraction during the power stroke (Bramble, 1974). Chewing in lepidosaurs differs between herbivorous and carnivorous species: the herbivorous *Uromastix* uses a mechanism

similar to that of turtles (Throckmorton, 1976), whereas the carnivorous *Sphenodon* retracts its mandible during jaw opening and protracts its mandible during jaw closure during the power stroke (Gorniak et al., 1982). Among mammals, propalinal chewing movements are especially pronounced among rodents (Byrd, 1981; Cox et al., 2012; Offermans and de Vree, 1989), whose jaw is opened as the mandible is retracted, and the power stroke results from mandibular protraction, accompanied by jaw closing (De Vree and Gans, 1994; Offermans and de Vree, 1990; Weijs, 1975).

In contrast to vertical and propalinal chewing movements, jaw displacement along the transverse axis has exclusively been reported for mammals (Bhullar et al., 2019; Grossnickle, 2017; Hiimäe and Crompton, 1985) – but the present study suggests that at least one lissamphibian also performs transverse jaw movements during food processing. Transverse chewing movements require a flexible jaw joint and can be achieved either by the hemimandibles moving relative to one another, resulting in a change in the angle they subtend (i.e. wishboning: Hylander, 1985; Bhullar et al., 2019; Weijs, 1975), or by moving the mandible or hemimandible transversely, relative to a stationary upper jaw (transverse mandible displacement: Crompton et al., 2010). In mammals, transverse mandible displacement is characterized by a wide jaw gape followed by a lateral translation of the mandible towards and beyond the maxillary tooth row of the working side, as the jaw is closed (Crompton et al., 2010; Hiimäe and Crompton, 1985). The main vector of the power stroke is, therefore, lateral and ends as the mandibles begin to re-separate (De Vree and Gans, 1976; Weijs and Dantuma, 1981). It has also been suggested that wishboning accompanies the propalinal mandible movements in rodents (Hiimäe and Ardran, 1968). As shown in Figs 2 and 3, *S. intermedia* retracts and abducts its hemimandibles during jaw closure in the preparatory phase, and protracts and adducts its



**Fig. 6. Cross-sections from different areas of the jaw joint complex.** (A)  $\mu$ CT of the skull of *S. intermedia* in grey, with the jaw joint complex in blue. (B–D)  $\mu$ CT cross-section planes illustrating the position of histological cross-sections (E–G). a, articular; ame, adductor mandibulae externus; ap, ascending process; bhe, branchiohyoideus externus; ch, ceratohyal; dm, depressor mandibulae (anterior); dm', depressor mandibulae posterior; hl, hyoquadrate ligament; hp, hyoquadrate process; pt, pterygoideus; pi, pila antotica; q, quadrate; and sq, squamosal. Muscles are purple while bones and cartilages are blue in histological sections (E–G).

hemimandibles (i.e. wishboning) during jaw opening in the power stroke phase. Consequently, if we compare chewing kinematics of *S. intermedia* with that of turtles, lepidosaurs, rodents and other mammals, most similarities are seen between chewing in

*S. intermedia* and rodents. Both taxa use a power stroke that results from mandible protraction and exhibits transverse mandible movements. However, transverse mandible movements in *S. intermedia* are of the wishboning type and probably result from

the propalinal mandible movement in combination with the mandible morphology (i.e. loose symphysis and jaw joint) rather than being functionally necessary for food processing. By contrast, rodent chewing is likely to consist of wishboning as well as transverse mandible displacement as functionally important parts. Additionally, after maximal gape closure, *S. intermedia* opens its mouth during most of the anteriorly directed power stroke, probably attributed to the orientation of the mandibular tooth row that would collide with the teeth of the upper jaw during simple mandible protraction (Fig. 2C,D). The pterygoideus muscle that is likely to power the anteriorly directed mandible movement in *S. intermedia* also acts as a jaw opener (Fig. 5). In sum, the characteristics of the chewing mechanism in *S. intermedia* deviate strongly from those in other known tetrapod chewing systems, presumably reflecting the specialized morphology of the paedomorphic sirenid salamanders.

As noted above, mammals are generally considered to be the only tetrapod group that incorporates mandibular movements in the median, horizontal and transverse planes during chewing (Hiimäe and Crompton, 1985). These complex chewing movements have been argued to require a specialized jaw joint anatomy, which was suggested to be exclusive to mammals (Bhullar et al., 2019; Crompton and Hylander, 1986; Grossnickle, 2017; Herring, 1993; Turnbull, 1970). However, here we show that the salamander *S. intermedia* uses 3D chewing movements during food processing, resulting in a complex chewing pattern that challenges the commonly accepted view that amphibians only use their jaws for 'scissor-like' vertical movements (Olson, 1961; Reilly et al., 2001; Ungar and Sues, 2019). Complex 3D mandible movements demand either (i) a loose jaw joint that allows vertical, propalinal and lateral mandible movements with or without jaw joint disarticulation or (ii) a movable mandibular suspension (i.e. some form of cranial kinesis). Horizontal excursions at the jaw joint have so far not been described in lissamphibians, whereas cranial kinesis (Frazzetta, 1962; Fürbringer, 1900; Iordanski, 1966) has been suggested to be present in some salamanders (Iordansky, 1989; Natchev et al., 2016). It has also been suggested that some salamanders have a cranial architecture that might permit transverse movement of the jaw suspension during suction feeding and respiration (Carroll, 2007; Lauder and Shaffer, 1985). However, as the thin bony suspension of the mandible (i.e. squamosal) in *S. intermedia* was not always visible in our X-ray recordings, we used cadaver manipulations to test for cranial kinesis. Because the mandibular suspension and skull seem tightly connected and hardly movable against one another, we conclude that cranial kinesis is unlikely to explain the complex jaw movements in *S. intermedia* on its own. Rather, it appears that the complex jaw joint in *S. intermedia* permits extensive antero-posterior and lateral sliding of the articular along the quadrate, with the joint capsules stabilizing those flexible movements.

The complex jaw movements in *S. intermedia* appear to be feasible mainly because of its peculiar jaw joint morphology that integrates an anterior ball-and-socket joint and a posterior plane joint (Fig. 6E,F). This 'ball-socket-plane joint' allows vertical (pitch), propalinal (surge) and transverse (sway and yaw) movements, for a total of four degrees of freedom. This stands in remarkable contrast to the proposed hinge-like joint movements in lissamphibians, which only permit vertical jaw movements (one degree of freedom) (Olson, 1961). The high degree of mandibular mobility also appears to result from a derived ligament arrangement. The hyomandibular ligament, which connects the hyobranchial system and mandible in most actinopterygians and metamorphic salamanders, does not connect to the mandible in *S. intermedia* and is instead rerouted to the cartilaginous quadrate

(part of the mandible suspension) during early ontogeny (Reilly and Altig, 2006) and acts as a hyosuspensory ligament (Drüner, 1902; Huxley, 1874). Thus, the mandible can move relatively independently of the hyobranchial system. Propalinal jaw movements also require a specialized muscle-tendon morphology with muscles extending anteriorly and posteriorly from the mandible to the skull. Indeed, mandibular retraction in *S. intermedia* appears to result from contraction of the external mandible adductors (ame complex) while the mandible depressors (dm and dm') may support retraction to a certain extent. Similarly, mandibular protraction appears to result from contraction of the internal mandible adductors (ami complex) consisting of pseudotemporalis profundus (pstp), pseudotemporalis superficialis (psts) and the pterygoideus muscle (pt).

There is little doubt that cranial systems allowing propalinal jaw movements evolved from cranial systems with vertical jaw movements (Olson, 1961; Reilly et al., 2001). It had been suggested that the main factor allowing systems with propalinal jaw movements to form from systems with vertical jaw movements is the appearance of a jaw joint that allows the lower jaw to slide anteriorly from the hinge socket (Reilly et al., 2001). This theory might in fact be supported by the chewing apparatus in *S. intermedia*, where a loose and highly movable jaw joint enables propalinal chewing (Figs 2, 3 and 5). Aside from propalinal jaw movements, the loose jaw joint in *S. intermedia* also allows the lower jaw to slide laterally relative to the quadrate (wishboning of the lower jaw, see Fig. 2), resulting in extraordinary complex 3D chewing movements for lissamphibians (i.e. vertical, longitudinal and transverse). Still, given that the development of *S. intermedia* gets arrested early in its ontogeny (Noble and Marshall, 1932; Reiss, 2002; Rose and Reiss, 1993), and *S. intermedia* thereby shows a cranial morphology typical for most early salamander larvae, it might well be that such complex chewing motions are a common feature for early salamander larvae and that the ability to perform complex jaw movements is lost during ontogeny. Kinematic (and especially fluoroscopic) studies on early staged salamander larvae are technically challenging because of the small size of these creatures but macro-high-speed recordings could enable testing of this hypothesis.

In conclusion, we have revealed a previously unknown and peculiar chewing mechanism in *S. intermedia* and have shown that complex mandible movements during chewing are not exclusive to amniotes. We argue that as the development of *S. intermedia* is arrested in its early ontogeny, many anatomical characteristics connected with feeding resemble those of early salamander larvae (Heatwole and Rose, 2003). Given the generalized morphology of salamander larvae (Heatwole and Rose, 2003; Reilly, 1986; Rose and Reiss, 1993), it is possible that chewing movements similar to those seen in *S. intermedia* could be a generalized feature in salamanders with an early larval morphology.

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#### Competing interests

The authors declare no competing or financial interests.

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## Data availability

Data are available from the figshare digital repository:  
<https://doi.org/10.6084/m9.figshare.11881110.v1>

## Supplementary information

Supplementary information available online at  
<http://jeb.biologists.org/lookup/doi/10.1242/jeb.220749.supplemental>

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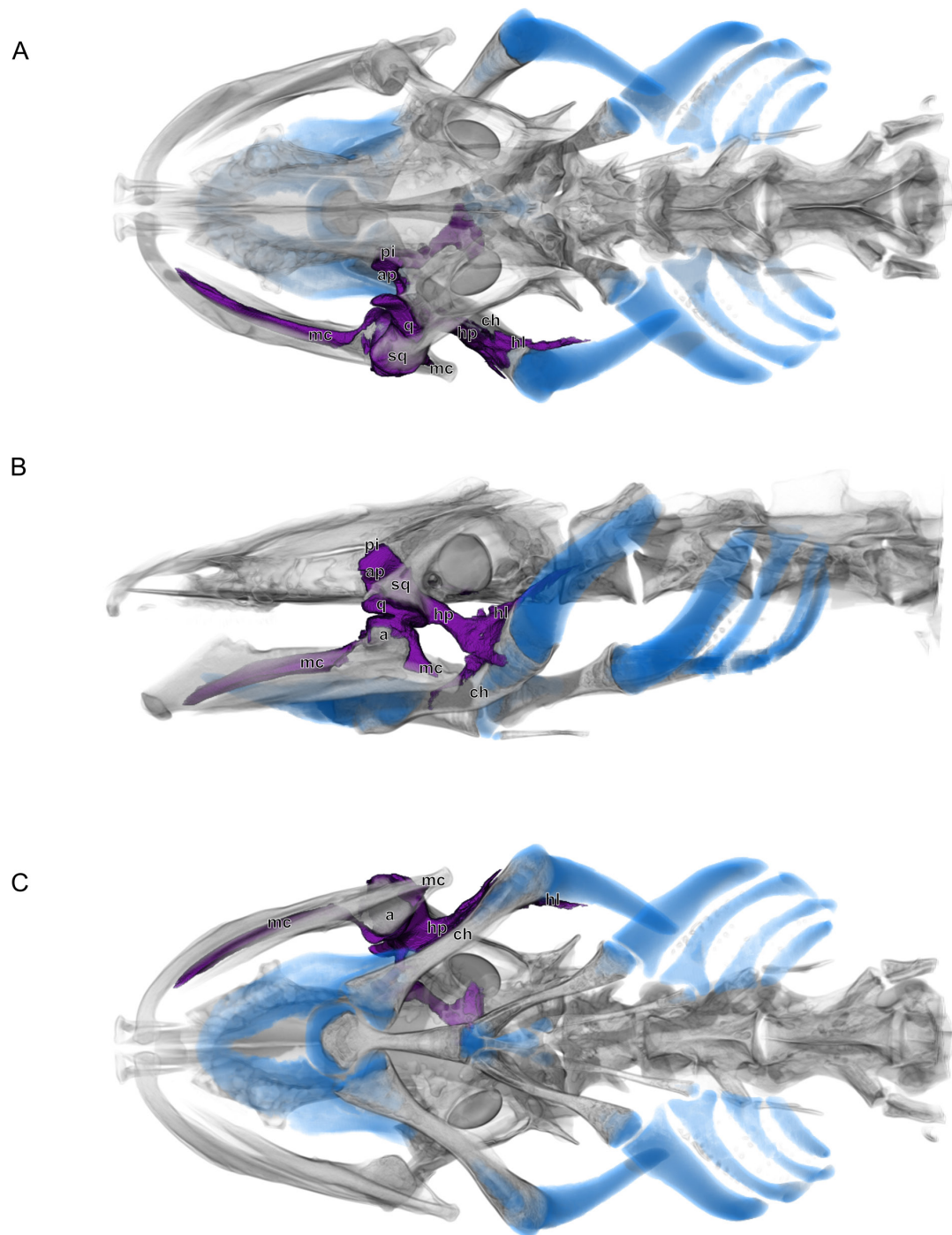


Fig. S1. Morphology of the jaw-joint-complex. A, dorsal; B, lateral and C, ventral views. Bony tissue is highlighted in grey, cartilages in blue, and the jaw-joint-complex in purple. Components of the jaw joint complex: a, articular; ap, ascending process; ch, ceratohyal; hl, hyoquadrate ligament; hp, hyoquadrate process; mc, Meckel's cartilage; pi, pila antotica; q, quadrate and sq, squamosal.

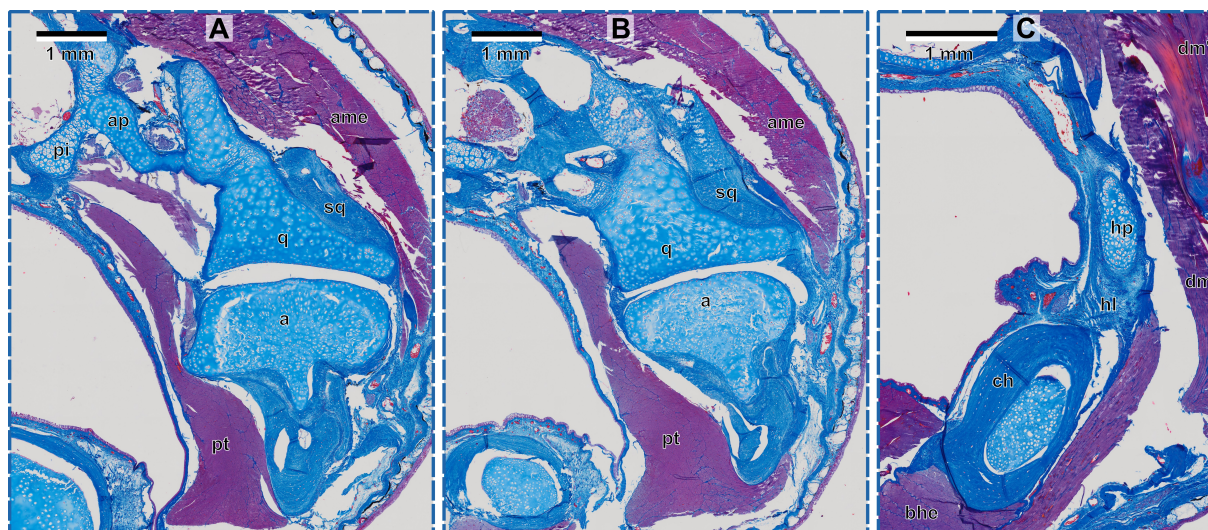


Fig. S2. Cross sections from different areas of the jaw joint-complex. Abbreviations: a, articular; ame, adductor mandibulae externus; ap, ascending process; bhe, branchiohyoideus externus; ch, ceratohyal; dm, depressor mandibulae (anterior); dm', depressor mandibulae posterior; hl, hyoquadrate ligament; hp, hyoquadrate process; pt, pterygoideus; pi, pila antotica; q, quadrate and sq, squamosal. Muscles are purple while bones and cartilages are blue in histological sections.

Table S1. Kinematic variables of chewing in *S. intermedia* with descriptive statistics

Component motion	Variable	Description	Value
Propalinal mandible movement	Mandible retraction	Distance from start to maximal retracted position of the mandible	9.77 ± 3.53 % CL
	Mandible protraction	Distance from maximal retracted to maximal protracted position of the mandible	11.72 ± 3.49 % CL
	Duration mandible retraction	Time from start retraction until maximal retraction of the mandible	146 ± 59 ms
	Duration mandible protraction	Time from start protraction until maximal protraction of the mandible	178 ± 41 ms
Vertical mandible movement	Gape closing	Distance from start of closure to maximal gape closure	7.74 ± 3.59 % CL
	Gape opening	Distance from closed gape to maximal gape opening	6.82 ± 2.88 % CL
	Duration gape closing	Time from start gape closure until maximal closure	99 ± 49 ms
	Duration gape opening	Time from gape closure until maximal gape opening	141 ± 57 ms
Longitudinal hyobranchial movement	Hyobranchial protraction	Distance from start to maximal protracted position of the hyobranchium	2.89 ± 1.57 % CL
	Hyobranchial retraction	Distance from maximal protracted position to maximal retracted position of the hyobranchium	3.12 ± 2.05 % CL
	Duration hyobranchial protraction	Time from start protraction until maximal protraction of the hyobranchium	95 ± 55 ms
	Duration hyobranchial retraction	Time from maximal protraction until maximal retraction of the hyobranchium	112 ± 57 ms
Vertical hyobranchial movement	Hyobranchial depression	Distance from start to maximal depressed position of the hyobranchium	11.79 ± 5.71 % CL
	Hyobranchial elevation	Distance from maximal depressed position to maximal elevation position of the hyobranchium	15.38 ± 5.36 % CL
	Duration hyobranchial depression	Time from start depression until maximal depression of the hyobranchium	129 ± 62 ms
	Duration hyobranchial elevation	Time from maximal depression until maximal elevation of the hyobranchium	114 ± 103 ms
Vertical cranial movement	Neck flexion (cranial 'ventroflexion')	Angular displacement from start to maximum neck flexion relative to the trunk	7.59 ± 3.16 °
	Neck extension (cranial 'dorsoflexion')	Angular displacement from maximum neck flexion to maximum neck extension relative to the trunk	7.10 ± 3.37 °
	Duration neck flexion	Time from start flexion until maximal flexion of the neck	99 ± 42 ms
	Duration neck extension	Time from maximal flexion until maximal extension of the neck	128 ± 67 ms
Transverse mandible movement	Medio-lateral mandible abduction	Angular displacement from start to maximum medio-lateral abduction of the mandible	4.22 ± 1.70 °
	Medio-lateral mandible adduction	Angular displacement from maximum medio-lat. abduction to maximum medio-lat. adduction of the mandible	4.95 ± 2.05 °
	Duration medio-lateral mandible abduction	Time from start medio-lateral abduction until maximal medio-lateral abduction of the neck	156 ± 60 ms
	Duration medio-lateral mandible adduction	Time from maximal medio-lateral abduction until maximal medio-lateral adduction of the neck	146 ± 53 ms

Data are means ± SD. Distances normalized by cranial length (% CL).