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RESEARCH ARTICLE

The Craniolingual Morphology of Waterfowl (Aves, Anseriformes) and Its Relationship with Feeding Mode Revealed Through Contrast-Enhanced X-Ray Computed Tomography and 2D Morphometrics

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Abstract Within Anseriformes, waterfowl (ducks, geese, and swans) exhibit three specialized feeding modes that are distinctive among Aves: filter-feeding with fine and dense keratinous lamellae on a flat, mediolaterally expanded bill; cropping or grazing vegetation with large and robust lamellae with a dorsoventrally expanded bill; and sharp lamellae associated with a narrow bill used in acquiring mixed invertebrates and fish underwater mainly by grasping. Here we assess morphometric variation in cranial and hyolingual structures as well as hyolingual myology in a diverse sample of Anatidae to explore the relationship of tongue variation and feeding mode. Phylogenetically informed principal component analysis (phyl.PCA) of cranial-lingual measurements for 67 extant and two extinct anatids recovers grazers and filter-feeding taxa in largely distinct areas of morphospace, while underwater graspers and other mixed feeders show less distinct clustering. The relationship between morphometric differences in skeletal features and muscular variation was further explored through a reassessment of hyolingual musculature enabled by contrast-enhanced X-ray computed tomography (CT) imagery acquired from three exemplar species (*Branta canadensis*, *Chen caerulescens*, and *Aythya americana*) with distinctive ecologies and morphologies of the bony hyoid. Data for these duck and geese exemplars reveal further significant, and previously unstudied, morphological differences between filter-feeding and grazing species. Grazers have a larger hyolingual apparatus with highly-developed extrinsic hyoid muscles; while filter-feeding species are characterized by relatively more diminutive extrinsic hyoid muscles and larger intrinsic hyoid muscles. The feeding modes of two extinct taxa (i.e., *Presbyornis* and *Thambetochen*) were also estimated from morphometric data. The results indicate a derived terrestrial browsing or grazing ecology for *Thambetochen* but do not unequivocally support a specialized filter-feeding ecology for *Presbyornis*, which is recovered with mixed feeders including swans. The combination of detailed, CT-mediated acquisition of fine muscular anatomy with morphometric approaches shows promise for illuminating form–function relationships in extant taxa more generally.

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Background

Anseriformes comprise 150 species with a global distribution (Carboneras 1992). Nearly all of these species are in Anatidae, a clade of waterfowl including ducks, geese, and swans (Baldassarre et al. 2006). Numerous anatomical cranial and postcranial features have been described as linked to both feeding ecology and habitat utilization in waterfowl (Tremblay and Couture 1986; Lagerquist and Ankney 1989; Nummi 1993; Nudds et al. 2000; Gurd 2007). Three broad categories of anatid feeding behavior and ecology have been recognized (Goodman and Fisher 1962; Batt et al. 1992; Kear 2005; Fig. 1). These include filter-feeding taxa or dabblers with a large number of fine ramphothecal lamellae on the bill, anatids that graze or

Fig. 1 Phylogeny and feeding mode of the 67 extant anatid species included in the analyses. Taxonomy follows Carboneras (1992) and Gonzalez et al. (2009). Parsimony-based ancestral state reconstruction of feeding mode is indicated by *different color*: grazing, *red*; filter-feeding, *blue*; diving grasping, *green*; mixed feeding, *black*
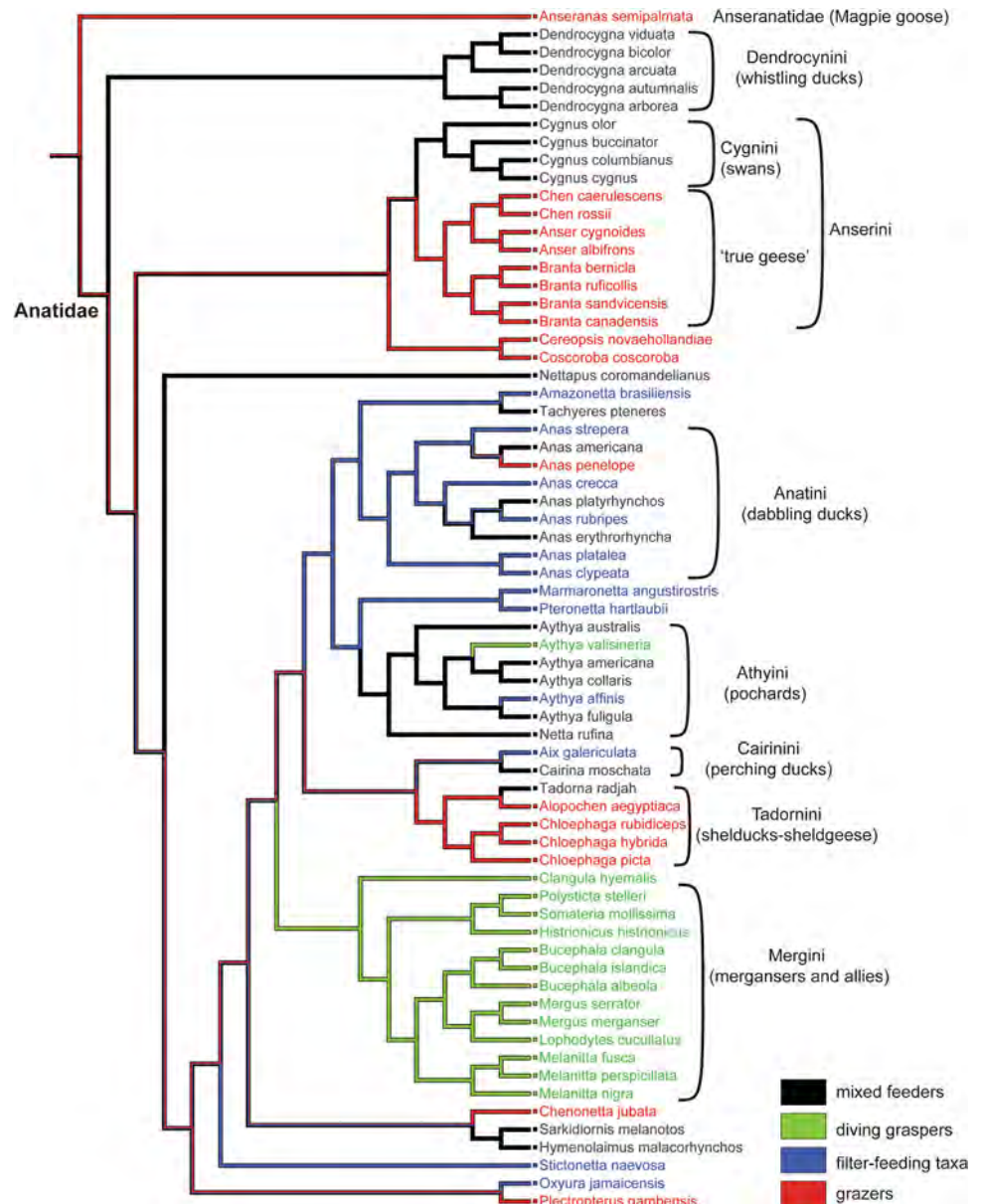
grub on land vegetation using stout ramphothecal lamellae, and taxa that feed primarily on invertebrates and fish while diving (Goodman and Fisher 1962; Carboneras 1992; Batt et al. 1992; Kear 2005). Filter-feeding has been hypothesized to be a key innovation in anseriform radiation with the earliest known filter-feeding taxon within Anseriformes, *Presbyornis*, known from the Paleocene and Eocene (Olson and Feduccia 1980; Feduccia 1999).

Anatidae that mainly obtain food through grazing or grubbing include geese and several duck species (e.g., wigeons), whose niches are mostly restricted to terrestrial open environments (e.g., grassland and farmland). The so-called "true geese" (*Anser*, *Branta*, and *Chen*; Carboneras 1992) and other geese (e.g., *Cereopsis*, *Plectropterus*, and *Anseranas*) are superficially similar in morphological

modifications inferred to be related to grazing, even though they are not phylogenetically related closely (Donne-Goussé et al. 2002; Fig. 1). For a few ducks (e.g., wigeons and the Maned Duck), grazing behavior has been considered to be a secondary adaptation based on their derived phylogenetic position and use of the feeding apparatus in both grazing and filtering (Van Der Leeuw et al. 2003). Food resources consumed by these grazers include mostly plant materials (e.g., grass, herbs, seeds, and leaves; Carboneras 1992; Durant 2013), while animal resources account for only a small portion of their diet and are much less important (Kear 2005). Specialized filter-feeding taxa include dabbling ducks (Anatini) and several diving ducks (Aythyini) (Goodman and Fisher 1962). The majority of these taxa are surface feeders or dabblers; the few exceptions sieve debris at the

water bottom (e.g., Freckled Duck; Carboneras 1992). Anatini is the most speciose group of Anatidae and cover a broader spectrum in both feeding ecology and diet variety than grazers (Green 1992; Kear 2005). Mergini (mergansers and allies) or the so-called sea-ducks, and a few pochards find food mainly by diving underwater (Tome and Wrubleski 1988) and use a strong grasping motion to acquire prey (Goodman and Fisher 1962). Both of these Mergini and pochards consume a large variety of animal material and some aquatic vegetation and show seasonal changes in diet (Kear 2005). For these sea ducks and diving ducks, invertebrates are important components of their diet. In addition to being superb divers, mergansers are specialized piscivores (Kear 2005). Other anatids do not exclusively specialize on a particular feeding mode but rather show a combination of two or more (Goodman and Fisher 1962). Here, we place them in a fourth category as "mixed feeders." This category includes whistling ducks, swans, and a few dabbling and diving ducks (Fig. 1).The variation in feeding ecology between these distinct groups makes Anatidae an ideal clade for investigating aspects of evolutionary adaptation (Rylander and Bolens 1974; Owen and Black 1990; Harvey and Pagel 1991). The general correlation of the cranial shape of waterfowl [e.g., skull length (SKL) and skull height (SKH)] with filtering-feeding and grazing in Anatidae has been noted (Goodman and Fisher 1962; Zweers 1974; Pöysä 1983; Kehoe and Thomas 1987; Owen and Black 1990) but not systematically investigated in a phylogenetic context. For instance, short-faced geese are specialized grazers while the more elongate, shallower cranial shape in ducks has been considered to be related to specialization for filter-feeding (Kear 2005). The fleshy tongue and bony hyoid are strikingly well developed within almost all anatids and are heavily involved in both filtering and grazing (Zweers 1974; Zweers et al. 1977; Kooloos et al. 1989; Lagerquist and Ankney 1989) but have not been the focus of comparative study. Extrinsic and intrinsic hyoid muscles are responsible for all lingual movement (Suzuki and Nomura 1975; Homberger and Meyers 1989). We investigate whether feeding mode will be reflected in systematic variation in cranial and hyolingual morphologies accessed via morphometric and contrast-enhanced CT-mediated assessment of myology. We also test previous hypotheses regarding the feeding modes of two extinct anatids, *Presbyornis*, and *Thambetochen* using the morphometric dataset.

Materials and Methods

Multivariate Analyses

Twelve measurements of the hyoid apparatus and skull (Fig. 2) were taken from 67 Anatidae species (34 of 41
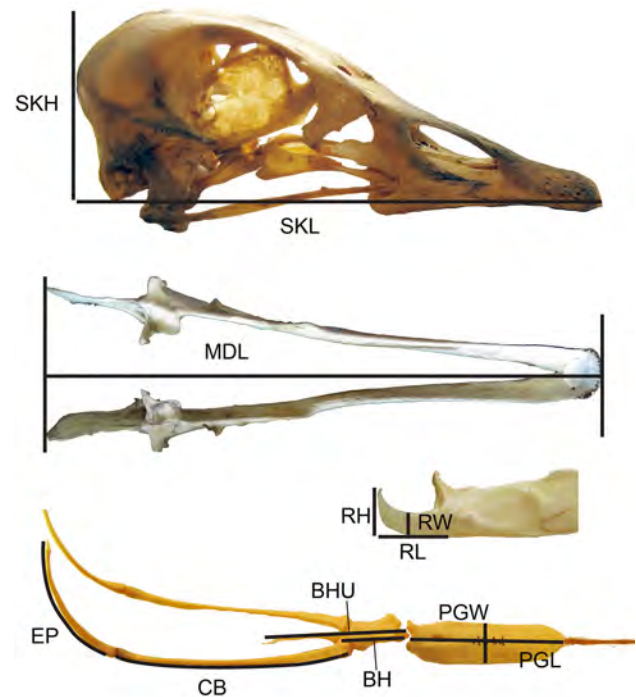

Fig. 2 Cranial and hyoid measurements used in the morphometric analyses. These variables include the SKH, SKL, *midline* length of mandible (MDL), the three dimension of retroarticular process: length (RL), and width (RW) and height (RH), paraglossal length (PGL) and width (PGW), ceratobranchial (CB) and epibranchial length (EP), basihyal length (BH), basihyal and urohyal length (BHU)

genera; Fig. 1) represented by 102 skeletal specimens (specimen number was indicated in Appendix 1 of ESM) from the National Museum of Natural History, Smithsonian Institution (NMNH) and American Museum of Natural History (AMNH). For a few specimens (Appendix 1 of ESM), the two skeletons with large size variation were chosen and the mean value was used. Most of these measurements are linear, except for ceratobranchial and epibranchial length, which were marked on a flexible plastic tape. The phylogeny and branch-length information used in the phylogenetic principle component analyses is from Jetz et al. (2012), Fig. 1, Appendix 2 of ESM. Sixty-seven species were identified to one of four feeding modes: (1) grazing, (2) filtering-feeding, (3) diving grasping, and (4) mixed feeding based on previous studies of anatid feeding ecology (e.g., Goodman and Fisher 1962); the assignment of each species by those authors was re-checked against multiple resources on anseriform ecology (e.g., Owen and Black 1990; Carboneras 1992; Kear 2005).

We evaluated the relationship between feeding modes and cranial and hyolingual measurements and estimate the feeding modes of extinct taxa using phylogenetic principal component analyses in phyl.PCA (Revell 2009). The skeletal measurements are 'size-corrected' utilizing log

body mass (phyl.resid. in 'R', Revell 2009) following standard procedures (e.g., Garland et al. 1992; Revell 2012; Blackburn et al. 2013) and residuals were analyzed. The body mass data for each species (mean values) were taken from the literature (Carboneras 1992; Iwaniuk et al. 2004; Dunning 2008; Zelenitsky et al. 2011; Appendix 1 of ESM). Phyl.PCA space reflects corrected shape differences by taking into account phylogenetic relationships (Monteiro 2013; Polly et al. 2013). These analyses cannot evaluate measurement sets for which there are missing values; thus fewer variables could be used to assess the extinct taxa. Nine out of the 12 variables could be used to assess these extinct taxa: *Thambetochen chauliodous* (USNM uncatalogued) and *Presbyornis* sp. (USNM PAL 299846, 299847; Appendix 1 of ESM). Their phylogenetic position within Anseriformes was based on previous hypotheses (Livezey 1997a, b; Sorenson et al. 1999). Branch lengths for these taxa were scaled using their reported geologic age (~25 Ka and ~50 Ma, respectively for *Thambetochen* and *Presbyornis*; Olson and James 1991; Leggitt et al. 1998). Phylomorphospace was visualized in 'R' and in 'Mesquite' using the Plot Tree 2D algorithm in the Rhetenor module (Dyreson and Maddison 2003; Maddison and Maddison 2011).

Computed Tomographic (CT) Data

Detailed hyolingual muscular features from contrast-enhanced high-resolution X-ray CT imagery datasets are compared in three exemplar species, *Branta canadensis*, *Chen caerulescens*, and *Aythya americana* (TMM M-12684, TMM M-12680, and TMM M-12045, respectively). Heads of *B. canadensis* and *A. americana* were fixed with 10 % Neutral Buffered Formalin (NBF) solution for ~2 months and then transferred to the 7 % (w/v) I$_2$KI solution (Metscher 2009; Jeffery et al. 2011) for staining. The incubation period lasted ~2 months with solution replacement before the high-resolution X-ray (CT) scans were made. The staining effect obtained here enabled the delicate hyolingual muscular fibers to be clearly distinguished even in the relatively large-sized *B. canadensis*. Another protocol was applied for *C. caerulescens*; after the similar fixation using NBF solution, the specimen was dehydrated in ethanol (>99 %) for 2 weeks and then was transferred to 1 % elemental iodine solution (e.g., 1 g iodine per 100 ml pure ethanol) for staining about a month before the scan.

All samples were scanned using a custom instrument built by North Star Imaging (Rogers, MN) and operated at the University of Texas High-Resolution X-ray CT facility (UTCT). Images obtained were 1024 × 1024 16-bit Tiff format. Total slices were 2439 slices for *B. canadensis*, 1815 slices for *C. caerulescens*, and 1675 slices for *A.*

Fig. 3 Three coronal sections selected from the contrast-enhanced ▶ X-ray CT images for anatids detailed in this study. **a–c** *Branta canadensis*, **d–f** *Chen caerulescens*, and **g–i** *Aythya americana*. The individual hyoid muscles are identified and labeled by *different colors* on one side in comparison of the original contrasts in another side. The three sections are selected from the nasal capsula, middle orbit and the middle of forebrain anteroposteriorly. Anatomical abbreviation: *Bh* basihyal, *Cb* ceratobranchial, *Mbm* M. branchiomandibularis, *Mcg* M. ceratoglossus, *Mic* M. interceratobranchialis, *Mid* M. intermandibularis dorsalis, *Miv* M. intermandibularis ventralis, *Mss* M. stylohyoideus and M. serpihyoideus, *Mst* M. stylohyoideus

americana (Fig. 3). Scan parameters for *B. canadensis* were 180 kV, 0.3 mA, an interslice spacing of 0.07283 mm and a field of reconstruction of 69 mm; scan parameters for *C. caerulescens* were 125 kV, 0.15 mA with a voxel size of 0.0768 mm; scan parameters for *A. americana* were 200 kV, 0.22 mA, an interslice spacing of 0.07144 mm, and a field of reconstruction of 68 mm. Hyolingual bones and the associated muscles were clearly identified from the CT imagery dataset (Fig. 3); individual hyoid elements and muscles were manually segmented and digitally rendered using 'Surfacegen' in Avizo 6.1 (FEI Visualization Sciences Group). Individual materials (e.g., hyoid muscles) were further measured using 'Material-Statistics' in Avizo 6.1 (Table 1) for volume comparison. The original muscle volumes were scaled to head volume to remove the effect of body size differences. These scanned anatids specimens are deposited at TMM Collections at The University of Texas Vertebrate Paleontology Laboratory.

Results

Morphometric Analyses

The first three principal component axes (PCs) accounted for over 90 % of the total shape variation (Appendix 3 of ESM). Most explained variance is primarily distributed within the first two axes (Fig. 4). The first PC axis (PC 1) accounts for about 86 % of the total variance. All variables were loaded negatively for PC 1, and this was most pronounced for skull and mandible lengths (SKL and MDL). The second axis (PC 2) reflects variation in the length of the hyolingual apparatus and SKH, both of which received high positive loading (e.g., EP, BHU, BH, CB, SKH; see Fig. 2 for abbreviation; Appendix 3 of ESM).

Clear separation of species classified by different feeding modes can be discerned from the plots of first two PC axes, especially for grazers and filter-feeding specialists (e.g., shoveler ducks; Fig. 4). These two groups are mostly separated from each other along the PC 1, indicating major differences in their SKL and MDL. Grazers are characterized by having a relatively shorter skull but relatively
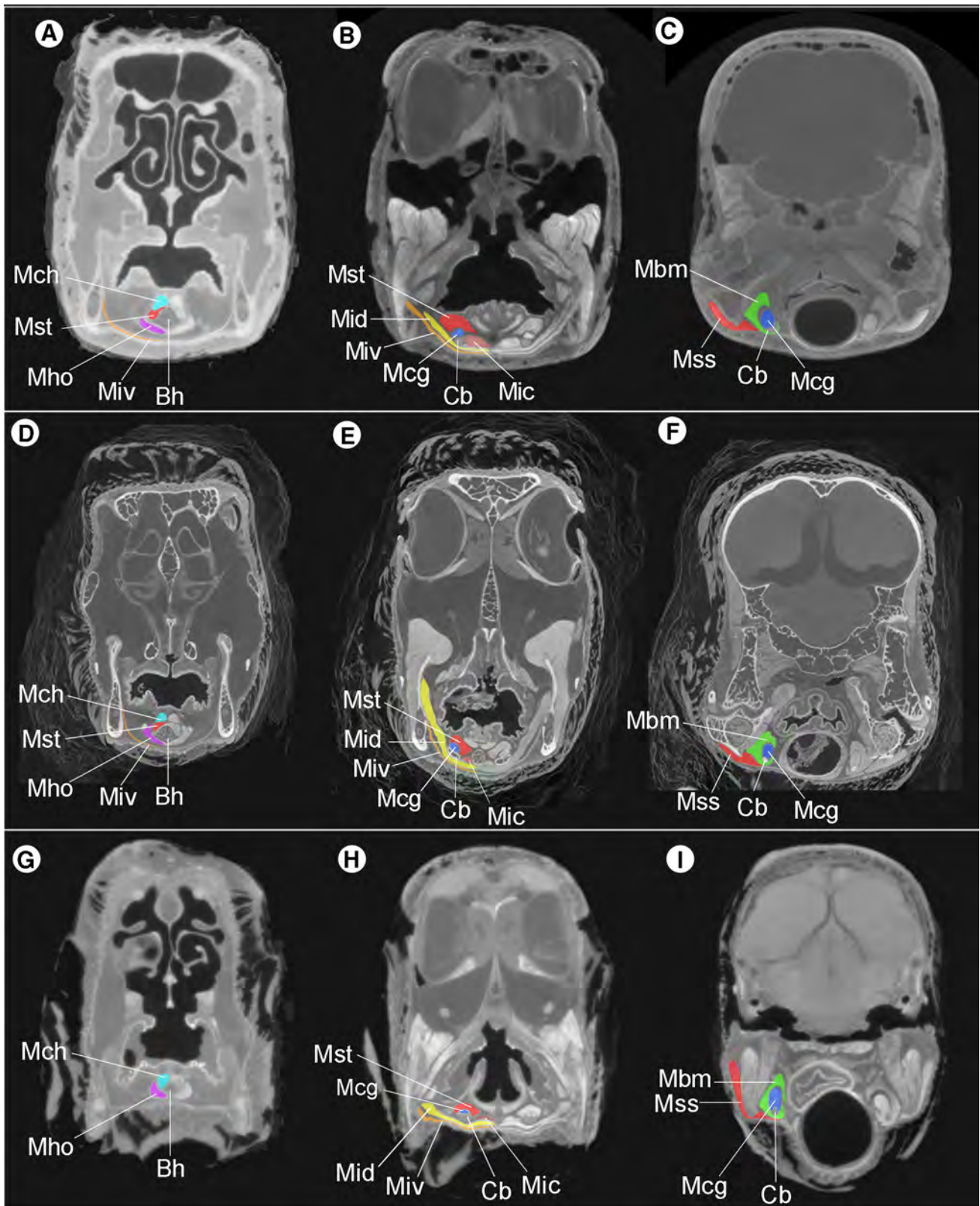
Table 1 Relative volumes of the major intrinsic and extrinsic hyolingual muscles in Canada Goose (CG), Snow Goose (SG), and Redhead Duck (Rd)

Material	Proposed function (Zweers et al. 1977)	Canada goose (CG) (mm³)	Snow goose (SG) (mm³)	Redhead (Rd) (mm³)	Comparative volumes (CG/Rd) before and after size correction		Comparative volumes (SG/Rd) before and after size correction	
1. M. stylohyoideus and M. serpihyoideus	Retraction of the tongue	697.7	460.0	155.2	**4.5**	**1.50**	**3.0**	**1.4**
2. M. branchiomandibularis	Protraction of the tongue	1022.0	675.5	301.1	3.4	1.1	2.2	1.1
3. M. intermandibularis ventralis	Hold the lingual cushion against roof of the mouth during protraction	117.8	83.9	26.5	**4.5**	**1.5**	**3.2**	**1.5**
4. M. intermandibularis dorsalis	Hold the lingual cushion against roof of the mouth during protraction	165.0	216.0	53.0	3.1	1.0	4.1	1.9
5. M. interceratobranchialis	Elevation of lingual bulge	113.4	48.9	26.0	**4.4**	**1.5**	**1.9**	**0.9**
6. M. hyoglossus obliquus	Elevation of lingual bulge	77.3	60.9	17.6	**4.4**	**1.5**	**3.5**	**1.6**
7. M. hyoglossus anterior	Depression of lingual tip	35.2	42.5	30.4	**1.2**	**0.4**	**1.4**	**0.7**
8. M. ceratoglossus	Depression of lingual bulge	174.9	153.3	116.1	**1.5**	**0.5**	**1.3**	**0.6**
9. M. cricohyoideus	Pull the glottal bulge rostrally	94.6	48.5	33.9	**2.8**	**0.9**	**1.4**	**0.7**

Muscles identified to be significantly different in Canada Goose (*Branta canadensis*), Snow Goose (*Chen caerulescens*) and Redhead Duck (*Aythya americana*) are shown in boldface type. The size correction was conducted by multiple a ratio (larger head volume/smaller head volume) to the original muscle volume of the smaller species to remove the size affection
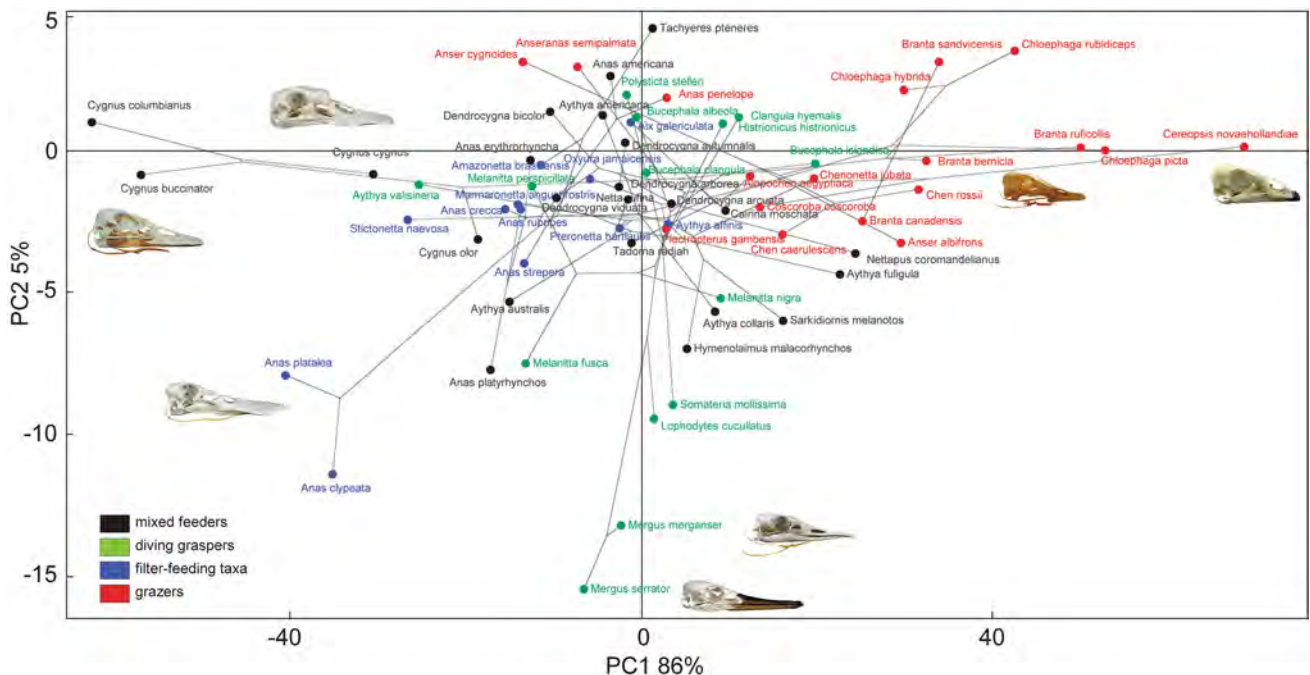

Fig. 4 Phylo.PCA results and phylomorphospace projection showing PC 1 and PC 2. The most heavily weighted variables on PC 1 are skull and mandible length and for PC 2, the epibranchial and basi-urohyal length. Taxa are colored by feeding mode: grazing, *red*; filter-feeding, *blue*; diving grasping, *green*; mixed feeding, *black*. See Appendix 3 of ESM for details on loadings and percent variance explained

longer hyolingual elements (BHU, CB, and EP). Filter-feeding taxa are characterized by having a relatively longer skull with shorter hyolingual elements. Grazing specialists

('true geese') are distant from filter-feeding specialists (e.g., shoveler ducks; Fig. 4) in the morphospace. A few mixed feeders, e.g., whistling ducks (*Dendrocygna*) and

Muscovy duck (*Carina moschata*), occupy a transitional area near some filter-feeding taxa that lie close to the morphospace occupied by grazers. These mixed feeders are considered to use both grazing and filter feeding (Goodman and Fisher 1962).

By contrast, diving, prey-grasping species are widely distributed along PC 2; SKL is less variable than the SKH in these species (Fig. 4). Rather than the SKL, a shallower cranial height (SKH) and the reduction or the shortening of several hyolingual elements (BHU, CB, and EP) appear to be the most significant variables associated with acquiring food by grasping while diving. Modification of cranial and lingual shape has been explained by the hydrodynamics of foraging underwater (Lovvorn et al. 2001). Anatids that are specialized for grasping are the group least utilizing the hyolingual apparatus for feeding, a pattern most pronounced in piscivorous taxa (e.g., *Mergus*), which have reduced bony elements and fleshy tongue. Mixed feeders are separate from grazers but cannot be distinguished from either the diving graspers or other filter-feeding species.

The large-bodied flightless duck from the Hawaiian Islands, *Thambetochen*, was proposed to be a browser in an environment in which other large, herbivorous vertebrates were absent (James and Burney 1997; Sorenson et al. 1999). Here, it is recovered in the cluster of grazing taxa in the PCA morphospace. Its location in cranio-lingual morphospace is far from the dabbling ducks (Fig. 5) to which it

is closely related (Sorenson et al. 1999). The strikingly short mandible combined with relatively longer and robust hyolingual elements distinguish *T. chauliodous* from other diving or filter-feeding species. A browsing behavior has been inferred from analysis of coprolites that revealed ferns comprised a major part of diet of *T. chauliodous* (James and Burney 1997). However, no extant waterfowl adopt browsing as a major feeding mode (Kear 2005). A terrestrial browsing or grazing ecology was also inferred from paleovegetation data for these large-bodied extinct waterfowl that are found on isolated islands (Givnish et al. 1994). Our data are consistent with a grazing terrestrial ecology similar to that of other extant anatids such as geese and do not indicate modifications for a unique feeding mode (e.g., browsing) for *T. chauliodous*. However, for consuming ferns or the unique browsing within Anatidae, cranial and hyolingual morphologies relative to outgroups are similar to those observed in grazing taxa. Given that this species is only estimated to diverge from other dabbling ducks approximately 25,000 years ago (Olson and James 1991; Sorenson et al. 1999), rapid convergent evolution on a more goose-like skull shape and hyolingual morphology is indicated.

The long-necked wading taxon *Presbyornis* has a low, elongate duck-like skull assumed to function in filter feeding (Olson and Feduccia 1980). It has been recovered previously as the sister taxa of Anatidae or to Anseriformes
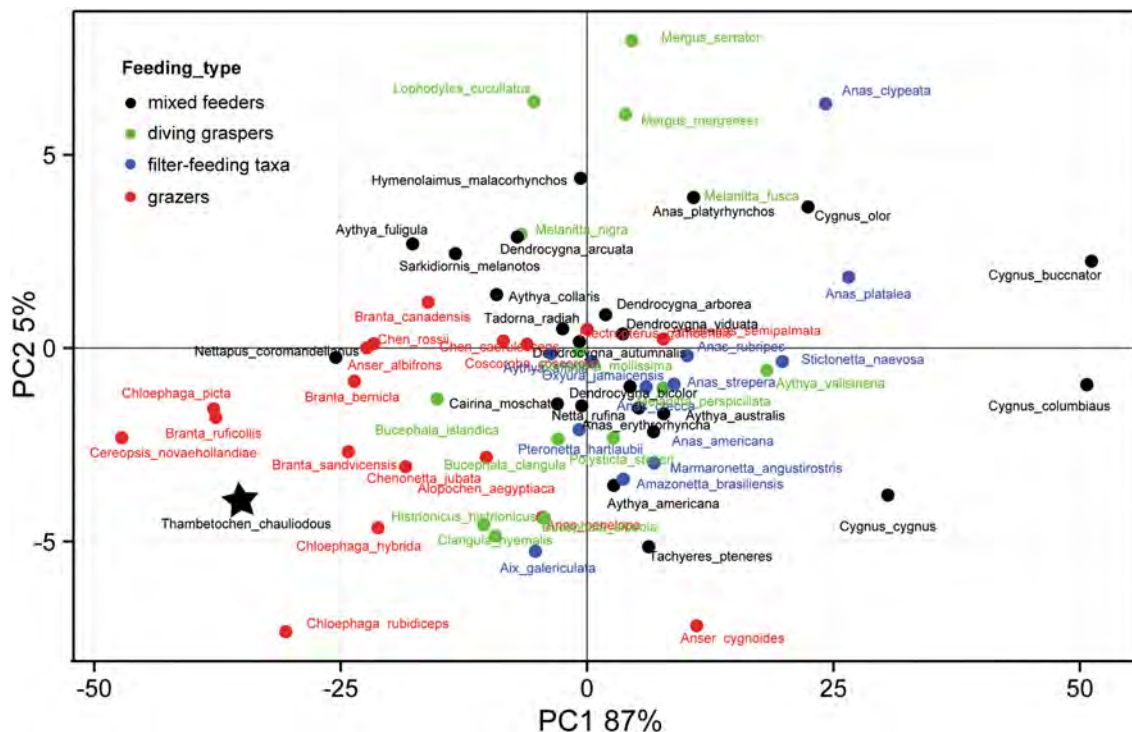

Fig. 5 Phylo.PCA plots of the first two principle components including *Thambetochen chauliodous* indicated by the *star*. The most heavily weighted variables on PC 1 are mandible and paraglossal length, and for PC 2 are basihyal, basi-urohyal, and ceratobranchial length

(Olson and Feduccia 1980; Erickson 1997; Livezey 1997a, b). Here it is recovered in a part of morphospace relatively close to a few mixed feeders (e.g., swans; *Cygnus olor*) and several specialized filter-feeding taxa (e.g., freckled duck, *Stictonetta naevosa*; Fig. 6). In both cranial and lingual proportions, it is most similar to these two taxa. The elongated neck in *Presbyornis* would have enabled this species to reach a considerable depth for grazing on aquatic plants in a lacustrine environment; in this respect, its feeding mode would be similar to species of long-necked *Cygnus*, which is almost entirely herbivorous (Owen and Black 1990). The shallow skull of *Presbyornis* has a marked upwardly recurved rostrum similar to that of the filter-feeding *Stictonetta*. We conclude that both underwater grazing and filter feeding may have been employed by *Presbyornis* but caution that the morphology of this species has no analogue among extant anatids and has not been identified as part of that clade but closely related to it.

Musculoskeletal Variation in the Anatid Hyoid Apparatus

Epithelial structures of the tongue in waterfowl have been found to be specialized for distinct feeding ecologies at both the macroscale and microscale (Kooloos et al. 1989; Iwasaki et al. 1997; Van Der Leeuw et al. 2003; Jackowiak et al. 2011). These findings are consistent with our morphometric analyses, in which the measurements of the bony elements distinguish distinct grazing and filter-feeding

groups (Fig. 4). We examined the discrete myology characters as well as the skeletal correlates in the hyolingual structure of *B. canadensis*, *C. caerulescens*, and *A. americana* through contrast-enhanced CT-mediated imaging to track the relationship between differences observed in bony morphology of the hyoid and differential development and placement of tongue muscles. Muscular arrangements among the three species are generally similar. Six muscles showed significant variation in size and/or attachment sites, including M. intermandibularis ventralis, M. hyoglossus anterior and obliquus, M. ceratoglossus, M. stylohyoideus, and M. serpihyoideus. As discussed below, all of these muscles have an important role in waterfowl feeding (Goodman and Fisher 1962; Zweers 1974).

M. intermandibularis ventralis

The thin sheet-like M. intermandibularis ventralis originates from the medial surfaces of the mandibles. Its two parts extend medially and meet at the midline of the buccal floor in a ventral raphe (Figs. 3, 7). The whole muscle is hammock-like, extending below the larynx and connecting the mandibular rami near their midpoint. The anterior extension of this muscle only reaches urohyal/basihyal junction in *Aythya*, but passes below the basihyal in *Branta* and *Chen*. The longer extension of M. intermandibularis ventralis in geese (e.g., *Branta* and *Chen*) explains its relatively larger volume in these taxa compared to the duck.
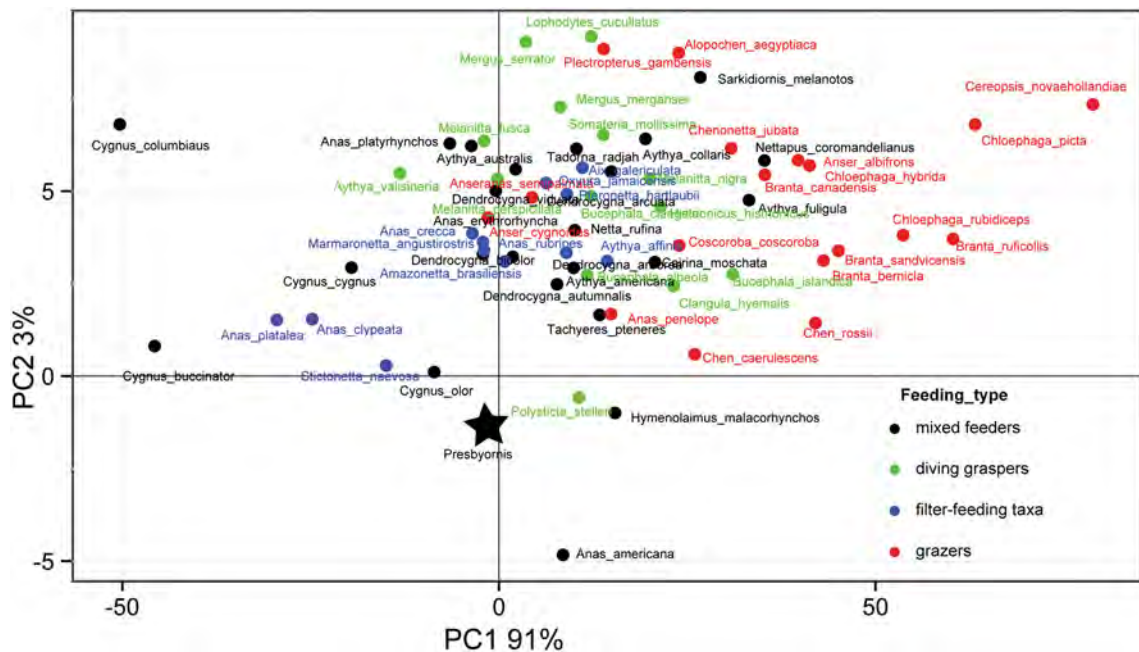

Fig. 6 Phylo.PCA plots of the first two principle components including *Presbyornis* sp. indicated by the *star*. The most heavily weighted variables on PC 1 are mandible and skull length and for PC 2 are basi-urohyal and paraglossal length
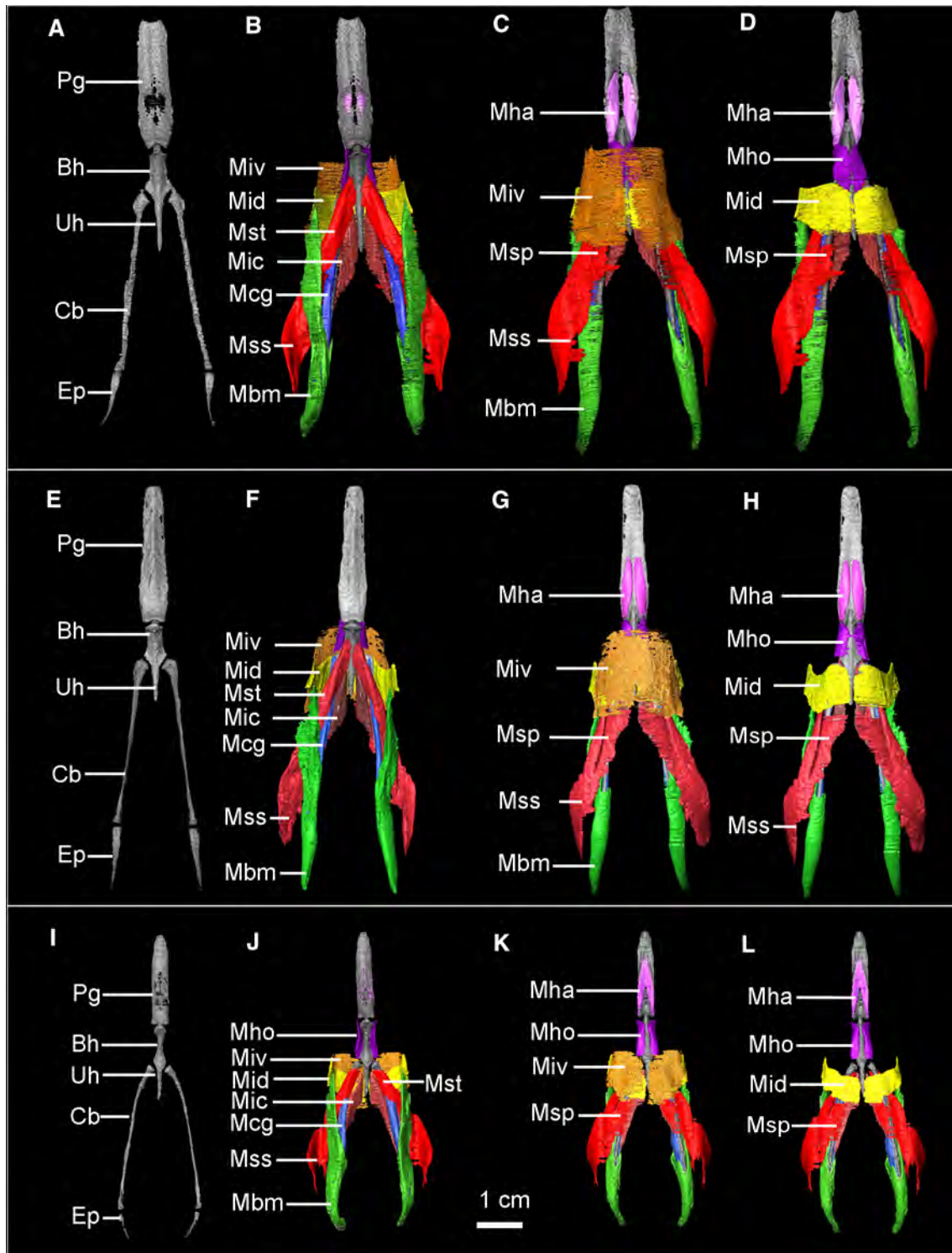
Fig. 7 Reconstructions of hyolingual myology in Anatidae from contrast-enhanced CT-mediated data. **a–d** *Branta canadensis*, **e–h** *Chen caerulescens*, and **i–l** *Aythya americana*. **a**, **b**, **e**, **f**, **i**, **j** *dorsal view*; **c**, **d**, **g**, **h**, **k**, **l** *ventral view*. Anatomical abbreviation: *Bh* basihyal, *Cb* ceratobranchial, *Pg* paraglossal, *Uh* urohyal, *Mbm* M. branchiomandibularis, *Mcg* M. ceratoglossus, *Mha* M. hyoglossus anterior, *Mho* M. hyoglossus obliquus, *Mic* M. interceratobranchialis, *Mid* M. intermandibularis dorsalis, *Miv* M. intermandibularis ventralis, *Msp* M. serpihyoideus, *Mss* M. stylohyoideus and M. serpihyoideus, *Mst* M. stylohyoideus

M. stylohyoideus and M. serpihyoideus

Both M. stylohyoideus and M. serpihyoideus originate on the lateral and ventral aspects of the caudal mandible (primarily on the retroarticular process) and insert on the hyolingual apparatus anteriorly (Vanden Berge and Zweers 1993). As the major external hyolingual muscles, they link the hyoid to the mandible (Figs. 7, 8), and function as the major lingual retractor (Vanden Berge and Zweers 1993). They share a close attachment to M. mandibular depressor but lie in a more lateral position on the posterior tip of the mandible.

M. stylohyoideus and M. serpihyoideus are not separate from each other at their origins. They are distinguishable at the point that they extend ventrally to the mandibular rami as they pass anteriorly toward ceratobranchials and urohyal (Fig. 7). M. serpihyoideus runs farther anteriorly and dorsally than M. serpihyoideus. In *B. canadensis* and *A. americana*, M. serpihyoideus inserts on a median raphe associated with the ventral surface of the urohyal. The insertion of M. stylohyoideus differs markedly between *A. americana*, *B. canadensis* and *C. caerulescens*; it inserts on the dorsal surface of the basihyal in *B. canadensis*, leaving a significant scar (Figs. 7, 8), but inserts on the proximal-most portion of the ceratobranchial in *A. americana* and

other ducks (Figs. 7, 8; Zweers 1974). The insertion observed here in *B. canadensis* differs from that reported in a previous study, in which it was incorrectly proposed to insert on the ventral surface of basihyal (Goodman and Fisher 1962).

M. hyoglossus obliquus and anterior

M. hyoglossus obliquus and anterior are the two major intrinsic hyolingual muscles in birds, which are mainly associated basihyal and paraglossal. The paired muscles of M. hyoglossus obliquus and anterior are both well developed in geese and ducks with similar arrangements. M. hyoglossus obliquus attaches to the lateroventral surface of basihyal, sandwiching the bone mediolaterally. M. hyoglossus anterior is rather anteriorly positioned relative to M. hyoglossus obliquus; it origins from the ventrolateral corner of the paraglossal and fills the concaved groove on the ventral side of paraglossal in ducks and geese. The paired M. hyoglossus anterior in ducks appears to contact each other in front from left and right side, but remain separated in geese. The extension of fibers is relatively longer in ducks, over half the length of its paraglossal but shorter in geese, which is less than the half length of their paraglossal.

Fig. 8 Proposed skeletal correlates of M. stylohyoideus insertion and the relative development of the M. intermandibularis in geese and ducks. **a**, **b**, **e**, **g** *Aythya americana*; **c, f** *Chen caerulescens*, **d, h** *Branta canadensis*, scars related to the attachments of M. intermandibularis dorsalis and M. stylohyoideus are labeled. Anatomical abbreviation: *dep_Mid* the mandibular depression associated with the origin of M. intermandibularis dorsalis, *inser_Mst* the insertion of M. stylohyoideus, *scar_Mst* depressions associated with M. stylohyoideus attachment. Other abbreviations are the same as in Fig. 7. Skeletal and scanned specimens are all from TMM Collections at The University of Texas Vertebrate Paleontology Laboratory
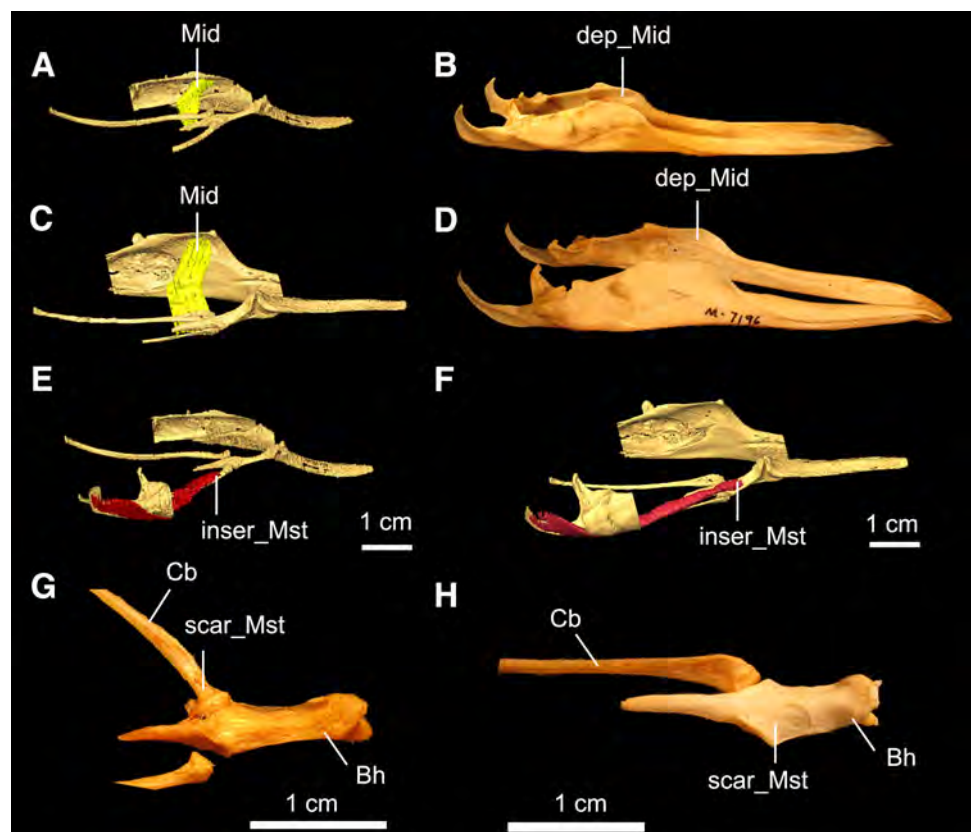
M. ceratoglossus

The origin and insertion of M. ceratoglossus is derived in geese, ducks and screamer (e.g., *Chauna*) compared to Galliformes. The name for this muscle is misleading for geese and ducks in that rather than passing from the cer- atobranchial to the paraglossal, the muscle originates from the proximal end of the epibranchial as well as the distal end of the ceratobranchial; it extends along the dorsal surface of the ceratobranchial and inserts on proximal region of the ceratobranchial. The muscle covers almost the entire dorsal surface of the ceratobranchial but not its proximal-most tip.

Hyolingual Muscle Mass and Volume in Filter-Feeding and Grazing Taxa

Comparison of hyoid muscle volumes reveals significant differences among the *Branta*, *Chen* and *Aythya* exemplars (Table 1). All individual hyoid muscles are significantly larger in *B. canadensis* and in *C. caerulescens*, about 1.3–4.5 the times of same muscle in *A. americana*. The examination of individual muscles in *C. caerulescens* and in *B. canadensis* indicates the majority are around 2–4 times the size of the same muscle in *A. americana*. These observed differences could be ascribed to differences in body size and head size variation or indicate differences in the mass and force of the muscle capable of generating. We therefore preformed further analyses to investigate allo- metric scaling.

Multiple analyses have recovered a negative allometry for jaw muscle mass and head volume relative to body mass in Anseriformes (Goodman and Fisher 1962; Van der Meij and Bout 2004). We assumed that it was more likely that hyolingual muscle volumes would scale proportionally to the head volume as the jaw muscles (Van der Meij and Bout 2004) than overall body mass. Therefore, we adopted cranial volume to rescale the hyoid muscle volumes (cra- nial volume measured: *A. americana*—70 cm^3, *C. caer- ulescens*—150 cm^3, *B. canadensis*—210 cm^3). After size correction, the M. branchiomandibularis volume is about the same for the three species (Table 1); the major function of M. branchiomandibularis is related to lingual protraction during food ingestion (Goodman and Fisher 1962). The rest of the hyolingual muscles compared are either notably larger in *B. canadensis* and *C. caerulescens*, or notably larger in *A. americana*. This variation in individual hyoid muscle volumes, observed after correcting for cranial size differences (as volumes) may reflect difference in function.

The majority of the extrinsic hyolingual muscles (e.g., M. stylohyoideus and M. serpihyoideus, and M. inter- mandibularis ventralis) and only one intrinsic hyolingual muscle (i.e., M. hyoglossus obliquus) are better developed

in *B. canadensis* and *C. caerulescens* (Table 1). Several of these muscles play a key role in raising the lingual bulge (i.e., M. hyoglossus obliquus and M. intermandibularis) suggesting this movement may play a bigger role in graz- ing (Goodman and Fisher 1962). By contrast, two intrinsic hyoid muscles (i.e., M. hyoglossus anterior and M. cer- atoglossus) are relatively larger in the *A. americana*; both function in depressing the tongue (Table 1). We propose that the enlargement of these specific intrinsic hyoid muscles in *A. americana* is consistent with a proposed major role for intrinsic tongue motion in filter feeding.

Discussion

Species distribution in phylomorphospace appears largely explained by feeding mode as indicated by the distinct clusters of filter-feeding, grazing and the more heteroge- neous mixed-feeding taxa (Figs. 4, 5, 6). Convergent evolution of 'grazer-like' cranial and hyolingual mor- phologies is seen in multiple lineages of anatids, including 'true geese,' sheldgeese, Maned Goose and *Thambetochen* (Figs. 4, 5). That some major changes in morphology, from those associated with filter feeding to those associated with grazing, occurred rapidly (i.e., ~25,000 years in *Tham- betochen*) may speak to striking plasticity in avian cranial form and function and/or the strength of certain selective regimes. Different selective pressures on cranial and hyolingual morphology in response to filter feeding, diving grasping, and grazing are inferred from repeated shifts into these areas of morphospace by distinct clades.

Discrete myological characters, such as muscle origin and insertion sites, have been proposed to be less variable than osteological characters in fishes and primates (George and Berger 1966; Gibbs et al. 2000; Diogo 2004). Simi- larly, bony attachment sites for cranial and hyolingual muscles in birds have been reported to be highly conserved (Köntges and Lumsden 1996). Therefore, we consider observed differences in attachment location and type in relatively closely related geese and ducks noteworthy and potentially indicative of differences in hyolingual function. In the case of the lingual retractors, the attachment of the M. stylohyoideus in well developed paired dorsolateral depressions on the basihyal surface in geese is consistent with a role in lingual retraction (George and Berger 1966; Figs. 7, 8). However, the smaller and more posterior insertion of the M. stylohyoideus on the distal end of the ceratobranchial (as opposed to the basihyal) in filter-feed- ing ducks makes it less effective for lingual retraction (Fig. 8). The contraction of the well-developed M. inter- mandibularis (dorsalis and ventralis) muscles raises the tongue from the buccal floor (Zweers 1974). A depression on the medial mandible serves as the origination of the M.

intermandibularis dorsalis; it is significantly larger in all evaluated grazers compared to filter-feeding taxa (Fig. 8). An extended study of these features in other Anatidae (Li, pers. obs.) supports these broader patterns. The type of bone-marking strong attachment of the M. intermandibularis and difference in the location of the M. stylohyoideus attachment are proposed to indicate a more effective role of this muscle in raising the tongue, key to the movement of food within the mouth associated in grazing (Van Der Leeuw et al. 2003).

Data on relative muscle volume provide other means of evaluating the morphological differences between filter-feeding and grazing species (Table 1). Relatively weak development (i.e., smaller relative volume) of extrinsic hyoid muscles in *Aythya* (compared to *Branta* and *Chen*) may indicate that protraction and retraction of the tongue may be less important for filter-feeding taxa than for grazers. The better developed extrinsic muscles in *Branta* and *Chen* as well as relatively larger bony elements of the hyoid are consistent with the greater demands of transferring larger food items over the oropharyngeal region (Kooloos and Zweers 1991). Rather than lingual retraction, lingual depression in filter-feeding taxa plays a key role in moving water beneath the tongue within the mouth. It is associated with a more delicate hyoid apparatus and relative enlargement of two intrinsic hyoid muscles (M. hyoglossus anterior and M. ceratoglossus) (Kooloos et al. 1989; Zweers 1974).

In extinct taxa, muscle development and attachment sites can only be inferred from skeletal correlates and phylogenetic data (Witmer 1995). In *T. chauliodous* paired dorsal lateral depressions are present on the basihyal, although there appears to be limited infraspecific variation in this character (Fig. 8). As noted above these are only seen in grazing taxa with a well-developed lingual retractor (M. stylohyoideus) attachment to the basihyal. Similarly a well developed M. intermandibularis depression on the medial surface of the mandible ventral to the coronoid process (Fig. 9) indicates that these lingual elevators are well developed as in extant anatid grazers. The data from these discrete features are consistent with the estimation of the feeding mode from morphometric analyses.

Conclusions

Our findings support a strong functional relationship in Anatidae between feeding mode and both cranial-lingual morphology. Morphometric data, controlled for body-size and phylogenetic relatedness, recovered separate clusters of terrestrial grazers and filter-feeding taxa. However, diving graspers overlap with mixed-feeders and occupy a large area of morphospace. The estimation of feeding
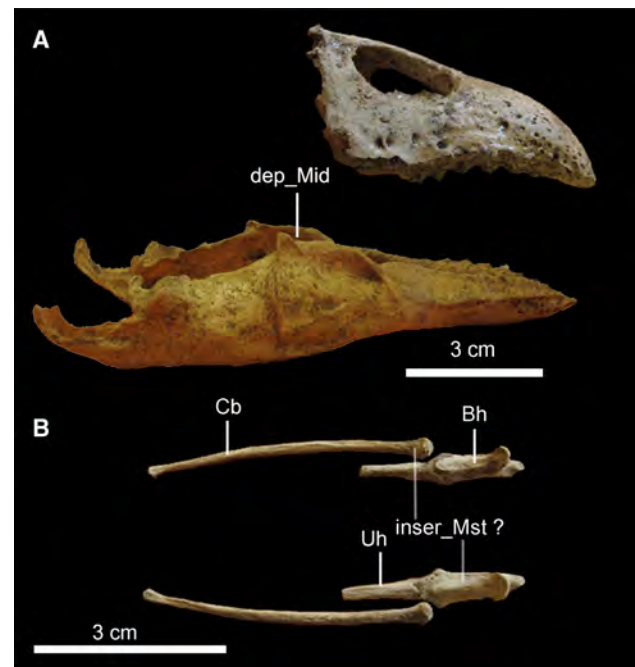

Fig. 9 Skull and hyoid elements of *Thambetochen chauliodous* (USNM uncatalogued). **a** Partial upper rostrum and the mandible, **b** basihyal, urohyal and ceratobranchial, which are placed approximately as in life positions. *inser_Mst ?* the two possible positions for the insertion of M. stylohyoideus; one on basihyal is supported by the skeletal correlates discussed in the text. *dep_Mid* the depression inferred to be associated with the origin of M. intermandibularis dorsalis. Other abbreviations are the same as in Fig. 7

ecology for *T. chauliodous* from both morphometric and skeletal correlates is largely consistent with previous hypotheses from direct dietary information and the island flora (Givnish et al. 1994; James and Burney 1997). In contrast to other dabbling ducks, *T. chauliodous* is quite derived in having a terrestrial browsing or grazing feeding ecology. For *Presbyornis*, ambiguous reconstruction of its feeding mode is obtained either due to missing data or an actual reflection of a mixed feeding mode. Although it was proposed to be a specialized filter-feeding taxon (Feduccia 1999), interestingly morphometric data place it in a cluster of mixed feeders near the only other taxa with elongate cervical series, the swans. Swans deploy both filter feeding and underwater grazing (Kear 2005).

The integrative approach taken here, utilizing contrast-enhanced CT enabled anatomical study of muscle variation in exemplar species and morphometric data, should offer additional rigor in the assessment of form–function in extant taxa and potentially the inference of behavior in extinct taxa. Morphometric analyses reveal that grazing taxa differ from filter-feeding taxa primarily in skull and mandible length, an intuitive result. However, digital dissection of the fine hyolingual muscles and comparison of estimated muscle volumes revealed that these more terrestrial taxa are also characterized by comparatively better

developed extrinsic hyolingual muscles. Overall grazing appears characterized by larger muscles involved in retraction of the tongue as well as those involved in moving food in the mouth by lifting the tongue from the buccal floor. By contrast, filter-feeding taxa show greater development of intrinsic hyolingual muscles and differ in the attachment of one of the major tongue retractors. Several of these muscles are involved in depression of the hyoid to move water ventrally during filter feeding. Newly identified skeletal correlates, on the basihyal and medial mandible, offer promise for further considering feeding mode in some extinct taxa. Certain feeding ecologies (mixed feeders and taxa that consume more invertebrates and fish) were not recovered from the morphometric data. For these taxa, new anatomical data and potential skeletal correlates will be needed to address whether their feeding modes may be characterized by distinct hyolingual myology despite similar skull and hyoid measurements.

Compliance with Ethical Standards

Conflict of interest The authors declare no competing financial interests.

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