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Osteological Histology of the Pan-Alcidae (Aves, Charadriiformes): Correlates of Wing-Propelled Diving and Flightlessness

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ABSTRACT

Although studies of osteological morphology, gross myology, myological histology, neuroanatomy, and wing-scaling have all documented anatomical modifications associated with wing-propelled diving, the osteohistological study of this highly derived method of locomotion has been limited to penguins. Herein we present the first osteohistological study of the derived forelimbs and hind limbs of wing-propelled diving Pan-Alcidae (Aves, Charadriiformes). In addition to detailing differences between wing-propelled diving charadriiforms and nondiving charadriiforms, microstructural modifications to the humeri, ulnae and femora of extinct flightless pan-alcids are contrasted with those of volant alcids. Histological thin-sections of four species of pan-alcids (*Alca torda*, †*Alca grandis*, †*Pinguinus impennis*, †*Mancalla cedrosensis*) and one outgroup charadriiform (*Stercorarius longicaudus*) were compared. The forelimb bones of wing-propelled diving charadriiforms were found to have significantly thicker (~22%) cortical bone walls. Additionally, as in penguins, the forelimbs of flightless pan-alcids are found to be osteosclerotic. However, unlike the pattern documented in penguins that display thickened cortices in both forelimbs and hind limbs, the forelimb and hind limb elements of pan-alcids display contrasting microstructural morphologies with thickened forelimb cortices and relatively thinner femoral cortices. Additionally, the identification of medullary bone in the sampled †*Pinguinus impennis* specimen suggests that further osteohistological investigation could provide an answer to longstanding questions regarding sexual dimorphism of Great Auks. Finally, these results suggest that it is possible to discern volant from flightless wing-propelled divers from fragmentary fossil remains. *Anat Rec*, 297:188–199, 2014. © 2013 Wiley Periodicals, Inc.

Key words: avian cortical bone; bone microstructure; Haversian remodeling; flightlessness; Great Auk; medullary bone; osteosclerosis; pachyostosis

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Bone microstructure can provide valuable insight into a variety of life history traits including ecology, behavior (including locomotive strategy), reproductive biology, and growth strategy (Amprino, 1947; de Burriel and Mazin, 1989; Curry, 1999; de Ricqlès et al., 2001; Erickson et al., 2001; Erickson, 2005; Schweitzer et al., 2005; Ponton et al., 2007; Simons and O'Conner, 2012). Moreover, analyses of bone microstructure provide a means of making comparisons between extinct and extant taxa with known ecological and ethological attributes. Previous studies of the bone histology of birds have largely focused on differences between non-avian dinosaurs and basal birds (i.e., avialan taxa outside of Neornithes such as †*Hesperornis*) and the anatomical and associated physiological transition to more modern avian forms, whereas clades within the crown of Aves have received comparatively little attention (though see, Houde, 1987; Chinsamy et al., 1995, 1998; Padian et al., 2001; Starck and Chinsamy, 2002; Chinsamy-Turan, 2005; Clarke et al., 2005; Erickson et al., 2009).

The loss of aerial flight in some species of Pan-Alcidae (i.e., †*Pinguinus* and †*Mancallinae*) and Sphenisciformes, which have coopted the flight-stroke for wing-propelled diving, must be regarded as fundamentally distinct from the loss of flight in large-bodied, terrestrial birds (e.g., ratites) that no longer employ their wings for locomotion. Wing-propelled diving is a relatively rare form of locomotion among birds, restricted to ~50 of ~10,000 species of extant birds. It is seen in pan-alcids (Charadriiformes), penguins (Sphenisciformes), diving petrels and some shearwaters (Procellariiformes) and dippers (Passeriformes). Studies of osteological morphology, gross myology, neuroanatomy and biomechanics have all documented anatomical modifications associated with wing-propelled diving (Stettenheim, 1959; Storer, 1960; Spring, 1971; Schreiweis, 1982; Pennycuik, 1987; Baldwin, 1988; Livezey, 1988; Raikow et al., 1988; Bannasch, 1994; Habib and Ruff, 2008; Habib, 2010; Smith, 2011a; Smith and Clarke, 2012). However, histological investigations of the derived locomotor strategies of wing-propelled diving birds are quite limited. Histological studies of flight muscles in wing-propelled divers are restricted to penguins and a single species of alcid, the Atlantic Puffin *Fratercula arctica* (Baldwin, 1988; Kovacs and Meyers, 2000). Furthermore, published data on the osteohistology of wing-propelled diving birds is limited to penguins (Meister, 1962; Chinsamy et al., 1998; de Maragerie et al., 2004; Ksepka, 2007) and the furcula of one species of alcid, the Razorbill Auk *Alca torda* (Ponton et al., 2007). Herein we present the first osteohistological data pertaining to the humerus, ulna, and femur of Charadriiformes, which includes the wing-propelled diving Pan-Alcidae, a clade of pelagic seabirds comprising the auks, auklets, puffins, murres, murrelets, and guillemots (del Hoyo et al., 1996; Hackett et al., 2008). Because penguins and pan-alcids are not closely related (Hackett et al. 2008), anatomical comparisons between these two clades of pelagic birds facilitate new insights into the potential relationship between osteohistological morphology and the evolution of wing-propelled diving more generally.

Charadriiformes represent a unique opportunity to investigate closely related species that span a range of locomotor strategies including nondiving skimmers, aerial and terrestrial foragers, as well as volant and

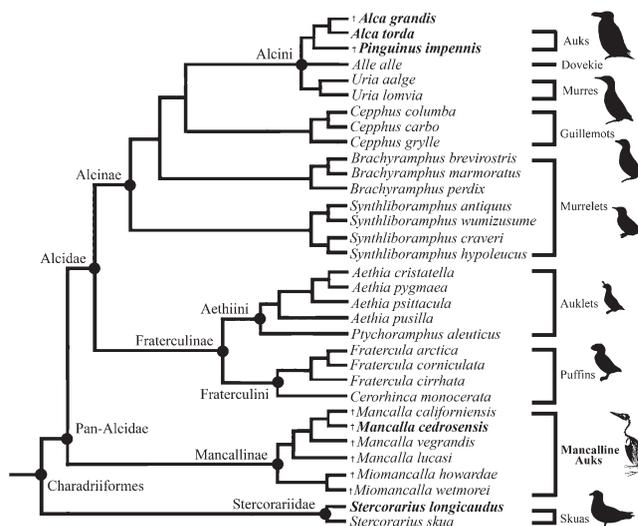


Fig. 1. Systematic position of histologically sampled taxa (shown in bold font; topology based on trees modified from Smith 2011b, Fig. 15; Smith and Clarke, 2011, Fig. 6).

flightless wing-propelled divers. Strongly supported hypotheses of charadriiform relationships provide phylogenetic context for observations regarding the clade (Smith, 2011a,b; Fig. 1). Sampling of extant and extinct charadriiform taxa that include volant non-divers, volant wing-propelled divers and flightless wing-propelled divers permits phylogenetically contextualized within-clade comparisons of bone microstructure, and may elucidate if, and what type of microstructural modifications are associated with the initial loss of aerial flight in wing-propelled diving birds. Whereas stem lineage penguins from the Paleocene (~61 Ma) already possessed much of the derived osteological morphology that characterizes the penguin crown clade (Ksepka and Clarke, 2010), at least one clade of pan-alcid charadriiform, †*Pinguinus*, is considered to have transitioned to flightlessness relatively recently in geological time. The oldest fossils of †*Pinguinus* are from the early Pliocene (~4.4 Ma; Olson, 1977), and divergence estimation suggests that †*Pinguinus* diverged from its sister taxon *Alca* sometime in the Miocene (~16 Ma; Smith, 2011a).

Variation in the gross osteological morphology, morphometry and endocranial anatomy between Pan-Alcidae and other Charadriiformes has been documented (Livezey, 1988; Smith and Clarke, 2011, 2012; Smith, 2011a,b, 2013a,b). Moreover, the cross-sectional shape of forelimb bones varies greatly among Pan-Alcidae, and between Pan-Alcidae and other Charadriiformes (see Smith, 2013b, Fig. 3). The thickened cortices and compressed cross-sectional shape of the forelimb bones of pan-alcids (e.g., *Alca*) contributes to the structural strength and ballast required for flapping “flight” in a medium as dense as water (Habib and Ruff, 2008; Habib, 2010) and potentially associated microstructural modifications remain uncharacterized in Pan-Alcidae. Furthermore, described differences in anatomy of flightless and volant pan-alcids (Smith, 2011b; Smith and Clarke, 2012) suggest that potential differences in osteological microstructure associated with different locomotor modes may be indicated.

Herein we document the osteohistological (i.e., microstructural) anatomy of the forelimbs and hind limbs of Pan-Alcidae and its nearest outgroup taxon, the Stercorariidae. By making comparisons between those taxa and other diving birds we address the three following questions: (1) are there microstructural differences in the osteology of the forelimbs and hind limbs of the nondiving outgroup to Pan-Alcidae and the wing-propelled diving pan-alcids?; (2) are there microstructural differences in the osteology of the forelimbs and hind limbs of flightless and volant pan-alcids?; (3) how does the microstructural anatomy of pan-alcid forelimbs and hind limbs compare with that documented in other flightless wing-propelled divers (i.e., penguins)?

MATERIALS AND METHODS

Sampling Strategy

Taxa were sampled to represent the transition from nonwing-propelled diving Charadriiformes to wing-propelled diving Pan-Alcidae and to represent the transition from volant charadriiforms (including volant alcids) to secondarily flightless clades in Pan-Alcidae (Fig. 1). The immediate outgroup to Pan-Alcidae in Charadriiformes is represented by the Long-tailed Skua *S. longicaudus*. Phylogenetic analyses of molecular sequence data and combined data (i.e., analyses also including morphological characters) support the Stercorariidae as the sister taxon to Pan-Alcidae (Baker et al., 2007; Smith, 2011b). †*Mancalla cedrosensis* was chosen to represent the extinct stem lineage of crown clade Alcidae (i.e., †Mancallinae) because the flightless mancalline auks have been recovered as the sister taxon to other Pan-Alcidae in phylogenetic analyses of morphological and combined data (Chandler, 1990; Smith, 2011a,b; Fig. 1). Within Alcidae, the flightless Great Auk †*Pinguinus impennis* and two species of *Alca* (extinct †*A. grandis* and extant Razorbill Auk *A. torda*), which represent the volant sister clade to †*Pinguinus*, were sampled. The sister taxon relationship between the volant *Alca* lineage and the flightless †*Pinguinus* clade is supported by phylogenetic analyses of morphological, molecular sequence, and combined data (Strauch, 1985; Chandler, 1990; Baker et al., 2007; Pereira and Baker, 2008; Smith and Clarke, 2011; Smith, 2011a,b; Fig. 1). The names of extinct taxa are preceded by "†" throughout.

Destructive sampling of extinct taxa was limited to species that are represented by abundant fossil remains; thus, minimizing the loss of scientifically valuable specimen data. All fossil specimens were measured, photographed, molded and cast prior to destructive sampling. The remaining portions of specimens, molds, casts, histological thin-sections and associated data are archived at contributing museums (North Carolina Museum of Natural Sciences, San Diego Natural History Museum, National Museum of Natural History). Remains of the extinct species †*A. grandis* are among the most abundant avian fossils from the Early Pliocene (~4.4 Ma) Yorktown Formation exposed at the Potash Corporation of Saskatchewan Phosphate Mine in Aurora, North Carolina, with ~8,000 specimens referred to *Alca* from that location (Olson and Rasmussen, 2001; Smith and Clarke, 2011). Remains referred to the coeval (with respect to †*A. grandis*) Pliocene species †*Pinguinus alfrednewtoni* number only ~20 isolated and largely fragmentary speci-

mens (Olson and Rasmussen, 2001; Smith and Clarke, 2011). Because of the rarity of †*P. alfrednewtoni* fossils, as well as the lack of ecological and ethological data available for that species, specimens for histological study were drawn from the relatively more abundant Holocene fossil remains of its sister taxon †*P. impennis*. Histological thin-sections were also prepared from the remains of the flightless species †*M. cedrosensis* from the Early Pliocene (3.6–5.0 Ma) Almejas Formation of Baja California. Although ~4,000 specimens have been referred to the clade Mancallinae (Smith, 2011a,b) the remains of †*M. cedrosensis*, †*Mancalla lucasi*, and †*Mancalla vegrandis* are more common than those of other species in the clade (e.g., †*Mancalla californiensis*, †*Miomancalla howardae*; Smith, 2011b).

The forelimbs are highly modified in all targeted pan-alcid species and thus, offer insight into how microstructural features might be correlated with functionally significant modifications such as shaft-flattening (see Habib, 2010 for discussion of mechanical stress and its relation to cross-sectional geometry in pan-alcids and other diving birds). The forelimbs of volant pan-alcids experience biomechanical stresses as a direct result of both aerial and subaqueous locomotion. The different types of propulsion employed by volant and flightless pan-alcids impose different selective pressures on bone structure. The evolutionary history of strong selective forces acting on this skeletal element in flightless auks, coupled with opportunity to compare the same element in closely related volant species, makes the humerus and ulna well-suited candidates for histological study. Humeri and ulnae were evaluated for potential histological differences associated with different locomotive strategies (Table 1)—wing-propelled diving in Pan-Alcidae versus a volant non-diving charadriiform with a generalist foraging ecology, *S. longicaudus*.

Histological comparison of forelimb and hind limb elements may provide details of how the microstructure of forelimbs has evolved relative to the hind limbs. Lines of arrested growth (LAG) were reported only in the femora of penguins (i.e., not in forelimb elements; Castanet, 2006). The morphology of the hind limb generally, and the femur in particular, is much less variable among pan-alcid species (e.g., the isolated femora of extinct *Alca* species are difficult to distinguish from one another on any basis other than size; Smith, 2011a). We sampled femora from the Great Auk †*Pinguinus impennis*, its closest volant relative, the extant Razorbill Auk *A. torda*, and the immediate outgroup to Pan-Alcidae represented by the Long-tailed Skua *S. longicaudus* (Table 1). Available material for sampling was limited in part because many Mancallinae femora were part of named holotypes.

The sex of all specimens was unknown prior to histological examination except in the case of *S. longicaudus* (NCSM 10269), which was identified as a female at the time of salvage. Because charadriiforms in general, and alcids in particular, are not characterized by substantial degrees of sexual dimorphism (del Hoyo et al., 1996), lack of sex determination is not expected to be a significant source of bias in the histological study of this clade. All specimens were sampled from adult individuals. Ontogeny was assessed based on plumage for extant salvaged specimens and on degree of longbone ossification for extant and extinct specimens (Chapman, 1965).

TABLE 1. Taxonomic sampling, specimen numbers, body mass and relative bone wall thickness (RBT; i.e., % thickness) of cortex relative to medullary cavity (in parentheses following specimen numbers) of sampled humeri, ulnae and femora

Taxa	Humeri	Ulnae	Femora	Body mass (g)
<i>Stercorarius longicaudus</i>	NCSM 10269 (10.8%)	NS	NCSM 10269 (9.7%)	337.9
† <i>Alca grandis</i>	NCSM 8886 (24.2%)	NCSM 8854 (28.9%)	NS	U
<i>Alca torda</i>	NS	NS	NCSM 20058 (13.2%)	726.0
† <i>Pinguinus impennis</i>	USNM 623456 (37.5%)	USNM 623442 (29.8%)	USNM 623458 (19.1%)	5000*
† <i>Mancalla cedrosensis</i>	SDSNH 42535 (40.4%)	SDSNH 59048 (35.4%)	NS	2400*

Extinct species denoted by "†", elements not sampled denoted by "NS" and unknown values denoted by "U". Body mass data are averages from Dunning (2008), except for †*P. impennis*, and †*M. cedrosensis*, which are estimates (denoted by "*") from Livezey (1988). The estimate used for †*M. cedrosensis* corresponds with the "intermediate †*Mancalla*" of Livezey (1988, table 8:692) as †*M. cedrosensis* is intermediate in size between the smallest †*Mancallinae*, †*M. vegrandis*, and larger taxa including †*M. californiensis* and †*M. lucasi*.

Histological Methods

Prior to sectioning, all specimens were hardened with cyanoacrylate (Paleobond Penetrant and Stabilizer—PB 002). Portions of bone measuring ~0.5 cm were excised from the mid-shaft region of each specimen and embedded in clear epoxy resin (Silmar—Sil95BA-41) after marks used to monitor orientation of the specimens throughout the process were made to the proximal and dorsal surfaces with a fine-tipped permanent ink pen. Mid-shaft sections were used to avoid variation in muscle attachment between species that might result in more variable microstructure than at the mid-shaft, which is relatively free of muscle insertion or origination in the taxa we examined (Smith, pers. obs.). After the epoxy was fully cured (~24 hr, refrigerated at ~45°F) a 1.5-mm thick wafer was cut from the billet in the transverse plane (i.e., cross-sectional orientation) with a lapidary saw (Buehler Isomet 1000), mounted to glass microscope slides with clear epoxy glue (Devcon 2-Ton Epoxy), ground to a thickness of ~30 µm using a combination of diamond impregnated grinding disks (60, 120, 320, 600, 4,000 grades) on a rotary grinder (Buehler Eco-Met 4000 variable speed polisher/grinder), polished with fine-grade diamond grit against a glass plate and photographed (Zeiss Axioscop with AxioCam). Section thickness was monitored throughout the process with a micrometer (accurate to 0.01 µm). Photomicrographs were taken using transmitted and polarized light microscopy ranging from 10 to 40× magnification and composites of the thin-section images were assembled using the photomerge tool in Adobe Photoshop.

Assessment of Cortical Bone Thickness

To evaluate the relative contributions of cortical bone and medullary cavity to the overall diameter of the sampled mid-shaft sections, measurements of the cortical and medullary portions of the sections were taken in the dorsoventral plane (i.e., the plane of least diameter in compressed pan-alcid forelimb bones). Data are presented as % thickness of cortex (Table 1) and were calculated as follows: $(C_D + C_V / (D/100))/2 = \text{RBT}$, where C_D = thickness of dorsal cortex; C_V = thickness of ventral cortex; D = dorsoventral diameter at mid-shaft; RBT = relative bone thickness (i.e., % thickness of cortical bone at mid-shaft). Thus, RBT values are an average of the dorsal and ventral cortical bone walls, which varied slightly in all specimens examined.

Comparative Data Utilized in Descriptions

Additional comparisons were made with published data from Chinsamy et al. (1998), Ksepka (2007) and Simons and O'Conner (2012) for penguins (Sphenisciformes; humeri and femora), foot-propelled divers including the Red-necked Loon *Gavia stellata* (femur) and the Double-crested Cormorant *Phalacrocorax auritus* (humerus), and two plunge-divers, the Brown Pelican *Pelecanus occidentalis* (humerus), and the Northern Gannet *Morus bassanus* (humerus).

Institutional Abbreviations

NCSM, North Carolina Museum of Natural Sciences, Raleigh, North Carolina, USA; SDSNH, San Diego Natural History Museum, San Diego, CA, USA; USNM, Smithsonian National Museum of Natural History, Washington, DC, USA.

HISTOLOGICAL RESULTS

S. longicaudus

Humerus (NCSM 10269; extant salvaged specimen). Indicative of an individual that has reached adulthood, (Fig. 2A) a relatively thick, well defined inner circumferential layer (ICL; endosteally formed perimedullary bone) and an outer circumferential layer (OCL; primary periosteal bone) are present and border a fibrolamellar layer (FBL) with minimal secondary remodeling (i.e., Haversian remodeling; not shown in Fig. 2A). Note, that we follow Chinsamy-Turan (2005) in the usage of circumferential layers, rather than the more traditional use of circumferential lamellae (sensu Ham, 1953; Enlow and Brown, 1957) because these inner and outer layers are composed of lamellar bone in most extant birds, not lamellated bone (i.e., periosteal bone with fine laminations). The OCL and FBL are well vascularized and azonal (i.e., lacks distinct lamellae). Longitudinal (perpendicular to the transverse plane of the sections; appear as rounded dots) and oblique (Volkmann's canals) primary (non-Haversian) vascular canals are predominant in the sample; however, circular (parallel to transverse plane of section and outer cortex) and radial (orthogonal to outer cortex) canals are present in relatively small quantities. The large, rounded medullary cavity and thin cortical bone walls that characterize the humerus of *S. longicaudus* (RBT = 10.8%; Table 1)

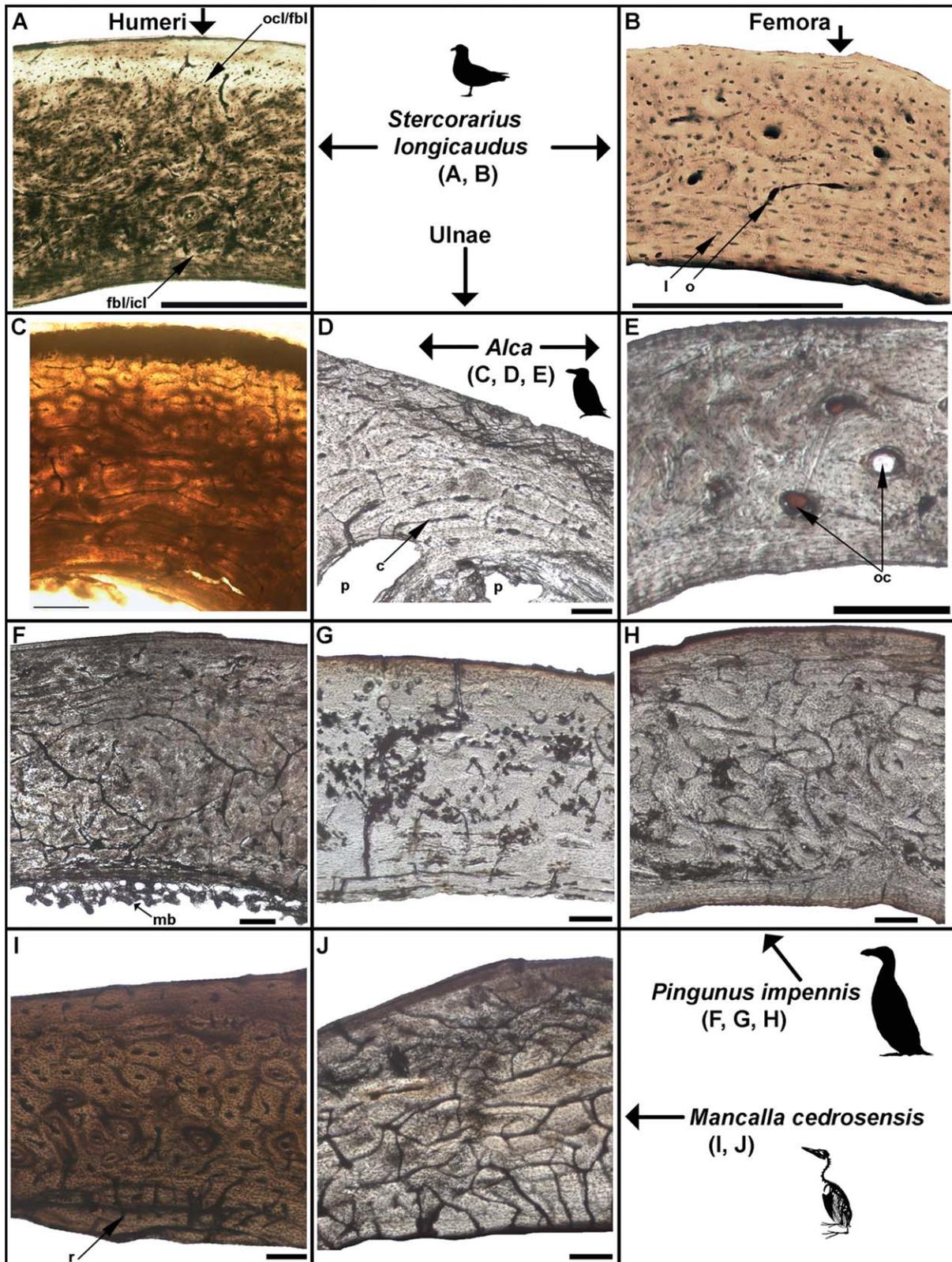
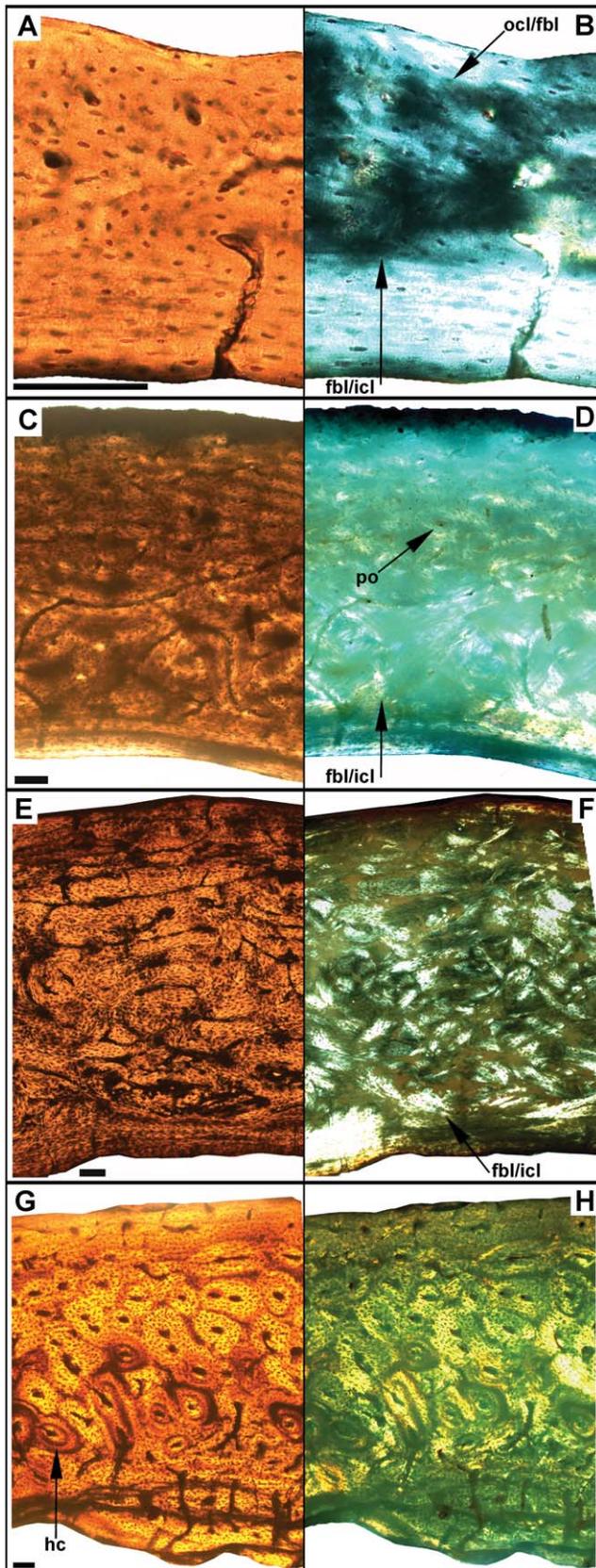


Fig. 2. Transverse mid-shaft thin-sections of cortical bone from the humeri (left column), ulnae (middle column) and femora (right column) of *S. longicaudus* (A, B), †*A. grandis* (C, D), *A. torda* (E), †*P. impennis* (F–H) and †*M. cedrosensis* (I, J). External surfaces of cortical bone are oriented towards the top of the page in all thin-section images and all scale bars = 250 μ m. Anatomical abbreviations: c, circular canal; fbl/icl, boundary between the fibro-lamellar layer and the inner circumferential layer; l, longitudinal canal; mb, medullary bone; o, oblique (Volkman's)

canal; p, porosity; ocl/fbl, boundary between the outer circumferential layer and the fibro-lamellar layer; r, radial canal. Note that dark linear features and masses and in the humerus, ulna and femur of *Pinguinus* (F–H) are taphonomic artifacts of preservation, not histological features. Complete, high-resolution images of thin-sections are available from the corresponding author on request and digital photographs have also been deposited along with the sampled specimens at NCSM, SDSNH, and USNM.



contrast starkly with the relatively smaller medullary cavities and thicker cortices of sampled Pan-Alcidae described below, in which the forelimbs are dorsoventrally compressed. In this respect, the cross-sectional geometry of *S. longicaudus* is more like that of other divers such as *Pelecanus occidentalis* and *Phalacrocorax auritus* (see Simons and O'Connor, 2012) than that of species of Pan-Alcidae.

Femur (NCSM 10269; extant salvaged specimen). The OCL and ICL are relatively thicker than in the humerus of this individual (Figs. 2B and 3A,B). The femur is azonal and no lines of arrested growth (LAG) are present in this sample. Haversian remodeling (i.e., presence of secondary osteons demarcated by cement lines; not shown) is minimal. The ICL is relatively thicker than the OCL. The relative thickness of the tripartite anatomy (i.e., ICL, FBL, ICL) common to most extant birds is most evident in polarized light (Fig. 3). As in the humerus, the FBL of the femur is well vascularized. Primary vascular canals are dominated by those with longitudinal and oblique orientation; however, the abundance of circular and radial canals is greater than in the humerus of this species. As in the humerus of *S. longicaudus* the cortex of the femur is quite thin (RBT = 9.7%; Table 1).

†*A. grandis*

Humerus (NCSM 8886; fossil). In comparison with the humerus of *S. longicaudus*, the ICL and OCL are relatively thin in the sampled humerus of †*A. grandis* (Figs. 2C and 3B,C). The relatively thick FBL is characterized by minimal Haversian remodeling (i.e., predominance of primary osteons) and dominated by circular and oblique vascular canals with smaller quantities of longitudinal and radial canals. Distinct lamellae are present in the ICL. In comparison with the medullary cavity of *S. longicaudus*, that of †*A. grandis* is relatively small and surrounded by relatively thick cortical bone walls (RBT = 24.2%; Table 1).

Ulna (NCSM 8854; fossil). As in the humerus of †*A. grandis*, the ulna of this species is characterized by a relatively thin ICL (Fig. 2D). A single, relatively large endosteal porosity is present within the ICL posterior to the medullary cavity. The OCL of this specimen is relatively thick; however, fine microstructural details are difficult to discern owing to abundant micro-fractures in this region of the specimen (likely due to weathering). The bone is azonal and no Haversian remodeling is

Fig. 3. Comparisons of transverse mid-shaft thin-sections of under transmitted (left column) and polarized light (right column). Specimens shown include: femur of *S. longicaudus* (A,B); humerus of †*A. grandis* (C,D); femur of †*P. impennis* (E,F); and ulna of †*M. cedrosensis* (G,H). Note the greater birefringence of the lamellar bone that characterizes the ICL and OCL as compared to the relatively darker appearance of the FBL (e.g., *S. longicaudus*). External surfaces of cortical bone are oriented towards the top of the page in all thin-section images and all scale bars = 100 μ m. Anatomical abbreviations: hc, Haversian canal; fbl/icl, boundary between the fibro-lamellar layer and the inner circumferential layer; ocl/fbl, boundary between the outer circumferential layer and the fibro-lamellar layer; po, primary osteon.



Fig. 4. Complete transverse mid-shaft thin-sections of cortical bone from the humeri of †*P. impennis* (A) and †*M. cedrosensis* (B). Note relatively small medullary cavities in both specimens, medullary bone along endocortical surface in †*P. impennis* and extensive Haversian remodeling in †*M. cedrosensis*. The dorsal surfaces are oriented towards the right in both specimens and the scale bar = 500 μ m. Anatomical abbreviations: hc, Haversian canal; mb, medullary bone.

present. The ulna is highly vascularized. Longitudinal and circular canals are most abundant in this sample. The cortical bone wall is significantly thicker than that of *S. longicaudus* (RBT = 28.9%; Table 1).

A. torda

Femur (NCSM 20058; extant salvaged specimen). The bone is azonal and no LAG are present (Fig. 2E). Haversian remodeling is present, albeit minimal. The open canals in this sample suggest that this was a young individual. Open canals such as these have been recorded in other taxa, and are later filled by primary osteons (Chinsamy-Turan, 2005). This presence of open canals is, however, contrary to the presence of Haversian remodeling, the fully ossified epiphyses of the longbones and the adult plumage of this specimen, all of which indicate that this individual had reached maturity. The ICL and OCL are well defined and relatively thick in comparison with that of the non-wing-propelled diver, *S. longicaudus*. Oblique vascular canals predominate, with smaller relative amounts of longitudinal canals and minimal quantities of circular and radial canals. The cortex is slightly thicker than that of *S. longicaudus*, the only non-diving species sampled herein (RBT = 13.2%; Table 1), and is similar in thickness to the foot-propelled diving Red-throated Loon *Gavia stellata* (RBT = 15%; Chinsamy et al., 1998).

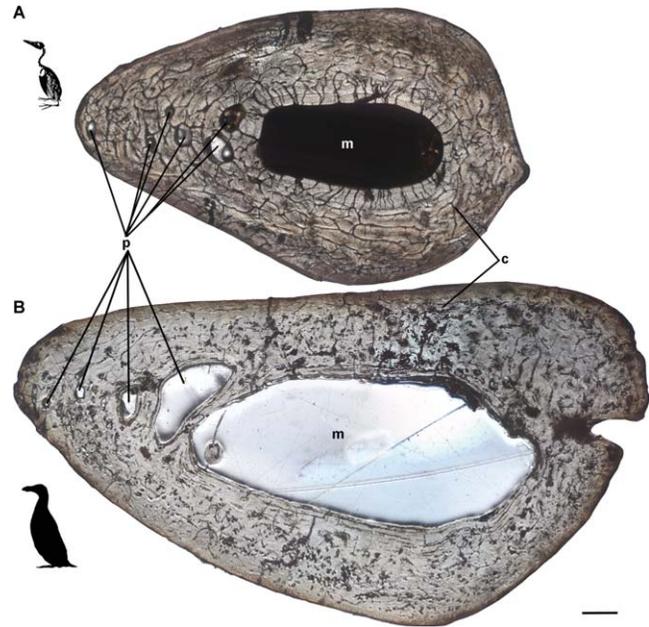


Fig. 5. Complete transverse mid-shaft thin-sections of cortical bone from the ulnae of †*M. cedrosensis* (A) and †*P. impennis* (B) showing multiple porosities posterior to the medullary cavity. Dorsal margins are oriented toward the top of the page (image of †*M. cedrosensis* reversed for comparison) and the scale bar = 500 μ m. Anatomical abbreviations: c, cortical bone; m, medullary cavity; p, porosity.

†*P. impennis*

Humerus (USNM 623456; fossil). As in the humerus of †*A. grandis*, the ICL and OCL are relatively thin in the sampled humerus of †*P. impennis* (Figs. 2F and 4A). The humerus is azonal and lacks secondary Haversian remodeling. The relatively thick FBL is primarily characterized by longitudinal and oblique vascular canals, with less abundant circular and radial canals. As in the quickly growing young of extant alcids (del Hoyo et al., 1996), the fibro-lamellar texture of the bone is indicative of a typically avian (i.e., relatively fast) rate of growth. Medullary bone is present along the perimedullary surface (i.e., endocortical surface), indicating that the specimen represents a female of reproductive age (Figs. 2F and 4A). Based on the minimal amount of medullary bone present, it is unclear whether this represents an early stage in the deposition of medullary bone (i.e., active ovulation) or a later stage in the resorption of that tissue. The medullary cavity is relatively small and the cortical bone walls are quite thick in comparison with that of †*A. grandis* (RBT = 37.5%; Table 1). The humerus of †*P. impennis* can be considered osteosclerotic (i.e., significantly thickened cortices associated with aquatic lifestyle; (de Burr enil and Mazin, 1989)), a condition previously documented in penguins (Chinsamy et al., 1998; Ksepka, 2007). Although the term pachyostosis has also been applied to the thickened cortices in birds including penguins, osteosclerosis is used to describe that condition herein because it does not involve for example, hyperplasia of periosteal bone but rather, involves extension of the cortex into the medullary cavity (discussed by Ksepka, 2007). The term sclerosis is not intended to reference a pathological

condition, as it is more traditionally used in the medical sciences. Based on the reduced medullary cavities of flightless pan-alcids, osteosclerosis is a better descriptor of the condition observed in †*Pinguinus* and †*Mancallinae* (see below).

Ulna (USNM 623442; fossil). The OCL and ICL are relatively thick and minimally vascularized (Figs. 2G and 4B). The OCL and FBL are azonal, whereas the ICL displays distinct lamellae. No Haversian remodeling is present in this sample. The orientation of primary vascular canals is predominantly longitudinal, with oblique, circular and radial canals present in successively less abundant quantities. As in the humerus of †*P. impennis*, the ulna is also osteosclerotic (RBT = 29.8%; Table 1). There is a large porosity posterior to the medullary cavity as in †*A. grandis* and additional porosities are present nearer the posterior periosteal margin (Fig. 5B). These additional porosities vary in size, with the smallest areas positioned nearest the internal border of the OCL. The largest of these porosities are partially bounded by lamellar layers, suggesting secondary formation of these structures—after a period of resorption. Given the randomness of the present sample, the presence of this feature in all three sampled pan-alcid species (see description of †*M. cedrosensis* ulna below) suggests a functional role (i.e., biomechanic or structural). Furthermore, the struts of ICL that are created by the largest of these porosities angle toward the dorsal surface of the bone in all three sampled species, albeit less so in †*M. cedrosensis*. Evaluation of the biomechanical properties of these structures is beyond the scope of the present study, but warrants further investigation.

Femur (USNM 623458; fossil). Overall the femur of †*P. impennis* is quite similar to that of *A. torda* (Figs. 2H and 3E,F). As in *A. torda*, this femoral specimen of †*P. impennis* is azonal, no LAG are present, and Haversian remodeling is minimal and localized near the OCL. The lamellar ICL and OCL are well defined and relatively thick in comparison with that of the nonwing-propelled diver *S. longicaudus*. The FBL is highly vascularized with abundant oblique, longitudinal, and circular canals. The cortex of the femur of †*P. impennis* is 5.9% thicker than in *A. torda* (RBT = 19.1%; Table 1) but does not approach the osteosclerotic condition observed in the forelimbs of †*P. impennis* or the femora of penguins (e.g., Emperor Penguin *Aptenodytes forsteri* femoral RBT = 33%; Chinsamy et al., 1998).

†*M. cedrosensis*

Humerus (SDSNH 42535; fossil). In contrast to the humerus of †*P. impennis*, the ICL and OCL are relatively thick in the sampled humerus of †*M. cedrosensis* (Figs. 2I, 3G,H, and 4B). The ICL is relatively thicker than in any other taxon sampled herein. There is extensive Haversian remodeling throughout the FBL (Fig. 4B). The FBL is primarily characterized by longitudinal and oblique vascular canals, whereas the ICL contains abundant radial canals. The humerus of †*M. cedrosensis* is osteosclerotic, with a relatively smaller medullary cavity than in †*P. impennis* (RBT = 40.4%; Table 1).

Ulna (SDSNH 59048; fossil). As in the ulna of †*P. impennis*, the OCL and ICL are relatively thick and

minimally vascularized in comparison with the highly vascularized FBL (Figs. 2J and 4A). Lamellae are present in the OCL and ICL. In contrast with the sampled humerus from this species, no Haversian remodeling is present in this sample. The orientation of primary vascular canals in the OCL and FBL is predominantly oblique, with longitudinal and circular canals in smaller quantities. As in the humerus of this species, radial canals dominate the ICL. Although less extensive than in †*P. impennis* and †*A. grandis*, porosities are present posterior to the medullary cavity in the ulna of †*M. cedrosensis* (Fig. 5A). As in the humerus of †*M. cedrosensis*, the ulna of this species is osteosclerotic, (RBT = 35.4%; Table 1).

DISCUSSION

Correlates of the Transition to Wing Propelled Diving in Pan-Alcidae

From an osteohistological perspective, a not altogether unexpected suite of characters is associated with the anatomical transition to wing-propelled diving in Pan-Alcidae. The cortices are relatively thicker and the medullary cavities are relatively smaller than that of the closely related non-diving charadriiform *S. longicaudus*. The marked increase in RBT observed in the forelimbs of pan-alcids (RBT ~30%) parallels that seen in penguins. However, pan-alcids do not possess the osteosclerotic femora that characterize penguins (e.g., *A. forsteri* femoral RBT = 33%; see Chinsamy et al., 1998; Chinsamy-Turan, 2005; Ksepka, 2007; Habib, 2010). The cortical bone walls of the femora of sampled pan-alcids are also not significantly thicker than that of nondiving charadriiforms (Table 1). Although the RBT value for the femur of †*P. impennis* (19.2%; Table 1) is nearly double that of *S. longicaudus* (9.7%), the RBT value of the femur of *A. torda* is only 13.2%. Osteohistological samples of loons, including †*Polarornis* and *Gavia*, are all femora; thus, comparisons with charadriiform forelimb RBT cannot be made herein.

With respect to relative bone wall thickness (RBT) of both forelimbs and hind limbs, values for the two volant alcids (*A. torda* and †*A. grandis*) are intermediate between the only sampled non-diver (*S. longicaudus*) and the flightless wing-propelled divers (†*Mancalla* and †*Pinguinus*; Table 1). However, volant pan-alcids may be derived in their own right. Storer (1960) proposed that their relatively short wing-lengths and fast, straight-line style of flight (see Pennycuik, 1987) are functional limitations associated with competency for wing-propelled diving in alcids. Conversely, it has been suggested that the flight of volant pan-alcids such as *A. torda* is an adaptation that enables them to traverse long distances between foraging and nesting locations (Kovacs and Meyers, 2000). Therefore, volant pan-alcids should not be viewed as an evolutionary stage along a trajectory towards flightlessness.

Other aspects of bone microstructure in pan-alcids such as type and orientation of vascular canals, predominance of secondary osteons, and lack of LAGs are fairly consistent among pan-alcids and are similar to the condition in the nondiving charadriiform outgroup. However, denser taxonomic sampling across Charadriiformes may identify additional variation and would facilitate

evaluation of the phylogenetic distribution of identified histological characters. Furthermore, sampling of additional individuals from each species examined would also provide a more detailed understanding of intraspecies osteohistological variation.

Osteohistological Correlates of Flightlessness in Pan-Alcidae

As in penguins, the flightless pan-alcids †*Pinguinus* and †*Mancalla* possess osteosclerotic forelimb bones, albeit to a lesser degree. The RBT values of flightless Pan-Alcidae humeri are, on average, ~57% lower than those of penguins (Habib, 2010; Table 1). In alcids and penguins this trait has been proposed to be linked to mechanical strength of humeri and resistance of neutral buoyancy during dives (Habib and Ruff, 2008). However, degree of osteosclerosis does not appear to be related to dive depth or the correlated variable of body mass (Table 1). Available data for other pan-alcid taxa corroborate these findings. The smallest of flightless pan-alcids, †*M. vegrandis*, has forelimb bones that are diminutive in comparison with *A. torda* (see Smith, 2011a for measurements), yet its humeri have thickened cortices and medullary cavities of reduced size (i.e., osteosclerosis; see Smith, 2013b, Fig. 3). Furthermore, the humeri of the second largest and deepest diving extant alcid, the Thick-billed Murre *Uria lomvia* (964 g, up to 210 m; Croll et al., 1992; Dunning, 2008) do not have thickened humeral cortices relative to the taxa sampled herein (Smith, personal observations). Additional sampling of volant and flightless pan-alcids of relatively small size (e.g., †*M. vegrandis* and *Alle alle*) may inform the relative contributions of body mass and biomechanical stress that factor in to the thickened forelimb cortices that are apparently associated with flightlessness.

Increased RBT may be associated with increased stiffening the forelimb, regardless of body mass or depth of dives. The potential need for increased mechanical strength in a flipper-like wing may be a factor (Habib, 2010). Among Pan-Alcidae volant species have lower forelimb RBT values (*A. torda* forelimb RBT = ~26%) and higher values of wing flexion (see Storer, 1960, Fig. 4; Raikow et al., 1988; Smith, 2011b, Fig. 16). Compared to its volant sister taxon *Alca* (485g; Table 1), †*Pinguinus* is characterized by reduced wing flexion, and average forelimb RBT value of ~34% and an estimated body mass of ~5,000 g (Table 1). †Mancallinae (†*Mancalla* and †*Miomancalla*) display the least amount of wing flexion among Pan-Alcidae (Smith, 2011b). Similarly, †*M. cedrosensis* shows the highest RBT values recovered herein, despite having an estimated body mass of less than half of that of †*P. impennis* (humeral RBT = 40.4%, mass = ~2,400 g; Table 1). Furthermore, penguins have a very rigid, flipper-like wing and even relatively small penguins such as *Spheniscus magellanicus* (4,120 g; Dunning, 2008) have forelimb RBT values greater than flightless pan-alcids (humeral RBT = 96%; Habib and Ruff, 2008; Habib, 2010). These data suggest that decreased wing flexion and increased cortical thickness of forelimbs are somehow correlated and are associated with the removal of constraints related to aerial flight.

Whereas wing-propelled diving may provide an explanation for the increase in forelimb RBT, the reason(s) for

the contrasting femoral and forelimb RBT values of pan-alcids and the increase in femoral RBT of †*P. impennis* are unclear. Extant penguins show thickened femora (RBT ~32%; Chinsamy-Turan, 2005) in comparison with volant pan-alcids. Flight loss could release a constraint on bone density. However, observed patterns across diving taxa are complex; the hind limbs of at least two species of Eocene penguins (flightless) and the extinct loon †*Polarornis* (proposed to be volant) have relatively thicker hind limb cortices than that of any extant penguin or loon (Chinsamy-Turan, 2005; Ksepka, 2007). Further limiting inference about the pattern observed in pan-alcids, little is known of the natural history of †*Pinguinus* or its form of terrestrial locomotion because these birds were never the focus of detailed ornithological study while extant (Fuller, 1999). The contrasting trends of increasing and decreasing degrees of femoral osteosclerosis in pan-alcids, penguins and loons warrant additional scrutiny.

Medullary Bone in the Great Auk

Medullary bone is only naturally produced by ovulating female birds and other dinosaurs. It is strictly associated with calcium production for the formation of eggs and has been previously used to identify the gender of fossil specimens (Bloom et al., 1941; Schweitzer et al., 2005; Lee and Werning, 2008; Chinsamy et al., 2013). The presence of medullary bone in the humerus of †*P. impennis* (USNM 623456; Fig. 2F) is congruent with the slaughter of Great Auks on Funk Island (i.e., the location where the fossil we sampled was collected) during their breeding season when these birds were accessible to hunters in large quantities. Previous studies of potential sexual dimorphism with respect to the size of †*P. impennis* have been complicated by the lack of sexed specimens to be used as a reference and by the isolated preservation of Great Auk skeletal remains (i.e., disassociated skeletal elements; Lucas, 1890; Hufthammer, 1982; Burness and Montevecchi, 1992). The identification of medullary bone in †*P. impennis* fossils suggests that histological methods could be employed to identify the remains of multiple females representing that species, and that those data could be used to address previously raised questions regarding sexual dimorphism in the Great Auk.

Osteohistological Evidence of Growth Strategies in Pan-Alcidae

Although values of RBT are higher, the relative proportions of OCL, ICL, FBL, and degree and types of vascularity of flightless pan-alcids are similar to those of volant taxa sampled. No LAGs were observed, and lack of extensive secondary remodeling in most sampled elements makes it unlikely that LAG were once present but have been subsequently lost. Such growth marks are known in crown clade birds including: the extant parrot *Amazona*; the recently extinct moa †*Dinornis*; the giant extinct Eocene anseriform †*Gastornis*; and one species of extant penguin, the King Penguin *Aptenodytes patagonicus* (Chinsamy et al., 1995; de Ricqlès et al., 2001; Starck and Chinsamy, 2002; Turvey et al., 2005; Castanet, 2006). However, the LAGs identified in penguins were attributed to possible stalls or slowing of fast

growth rates during the breeding season (de Margerie et al., 2004) and detailed ontogenetic data are not available for extinct pan-alcids.

Albeit somewhat speculative, some evidence suggests that the chicks of †*P. impennis* were among the most precocial of pan-alcid species; however, little consensus exists on this issue (Bengston, 1984; Gaskell, 2004; Houston et al., 2010). The fibrolamellar texture of the FBL in all sampled pan-alcid species is indicative of the fast growth rates that are common to Aves, and there is little data to suggest that large flightless pan-alcids grew in different ways or at different rates than their volant counterparts. Growth rates are absolutely higher in altricial birds than in precocial birds and both histological investigations and evaluation of growth curves based on body mass of extant birds suggest that both extant and extinct avian taxa of large adult size typically grow at rates absolutely higher than related smaller taxa (Ricklefs, 1973; Erickson et al., 2001; Padian et al., 2001). Furthermore, penguins show absolutely higher growth rates than most other birds and it has been suggested that large flightless pan-alcids may have adopted a similar growth strategy (Bengston, 1984; de Margerie et al., 2004). The present study does not indicate absolutely higher rates in †*Pinguinus* and †*Mancallinae*. In comparison with penguins and other avian taxa with higher growth rates that are characterized by bones with coarse radial structure indicative of very rapid growth (de Margerie et al., 2004), the bones of flightless pan-alcids are not as highly vascularized overall and are characterized by predominantly longitudinal and oblique canals. Identification of an ontogenetic series representing †*P. impennis*, the only flightless pan-alcid taxon for which there is sufficient material identified to the species level from which such a series is likely to be identified, is required to further assess the growth rates of extinct flightless pan-alcids.

The degree of Haversian remodeling in the humerus of the flightless taxon †*Mancalla* is similar to that documented in the gannet *M. bassanus*, a volant plunge diver (Simons and O'Conner, 2012) and was not observed in any of the other taxa sampled. It exceeds that observed in the forelimbs of penguins, and has not been documented in other diving birds (e.g., *Phalacrocorax auritus*, *Pelecanus occidentalis*; Chinsamy et al., 1998; Ksepka, 2007; Simons and O'Conner, 2012). Extensive Haversian remodeling of avian bones is commonly attributed to advanced age or mechanical stress (Currey, 1984, 2003; Lee et al., 2002; Chinsamy-Turan, 2005). Determination of the cause(s) of the extensive Haversian remodeling in †*Mancalla* would likely require additional sampling (i.e., an ontogenetic series).

Validation of the conclusions reached as a result of this study awaits evaluation with larger sample sizes and increased taxon sampling. However, characterization of pan-alcid osteohistology facilitated comparisons between penguins and pan-alcids, builds on the insights gained from previous studies of the transition to wing-propelled diving, and provides a broader base of knowledge for the future investigation of evolutionary strategies in other wing-propelled divers such as plotopterids, diving petrels and dippers.

The cortical bone walls of the forelimbs of wing-propelled diving pan-alcids are characteristically thicker (RBT = ~33%) than those of non-diving charadriiforms

(RBT = ~10%). However, the hind limbs and microstructural characteristics (e.g., vascularity and absence of LAG) of pan-alcids are relatively unchanged from those of other charadriiforms (i.e., femoral RBT = 10–19%). The forelimbs of flightless pan-alcids are differentiated from those of volant pan-alcids by the presence of osteosclerosis (average RBT = 36%), a characteristic shared with penguins. However, the hind limbs of pan-alcids (femoral RBT = 13–19%) and penguins differ in the degree of cortical bone wall thickening (penguin femoral RBT = ~32%; Chinsamy-Turan, 2005), consistent with differences in the physiology, life histories and locomotor strategies exhibited in these taxa. Additionally, the degree of increased forelimb cortical bone wall thickness (i.e., osteosclerosis) in flightless wing-propelled divers appears to correlate with the degree of decreased range of flexion associated with anatomical modifications for wing-propelled propulsion in these taxa. Subsequently, knowledge of the shared osteosclerotic condition of penguins and pan-alcids suggests that flightless wing-propelled divers can be identified based upon the degree of osteosclerosis of isolated and fragmentary forelimb remains. Finally, the identification of medullary bone in a specimen of the Great Auk provides a tantalizing new direction for future research focused on resolving the question of sexual dimorphism in that enigmatic species.

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