THE ROYAL SOCIETY

Relationships of the extinct moa-nalos, flightless Hawaiian waterfowl, based on ancient DNA

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The extinct moa-nalos were very large, flightless waterfowl from the Hawaiian islands. We extracted, amplified and sequenced mitochondrial DNA from fossil moa-nalo bones to determine their systematic relationships and lend insight into their biogeographical history. The closest living relatives of these massive, goose-like birds are the familiar dabbling ducks (tribe Anatini). Moa-nalos, however, are not closely related to any one extant species, but represent an ancient lineage that colonized the Hawaiian islands and evolved flightlessness long before the emergence of the youngest island, Hawaii, from which they are absent. Ancient DNA yields a novel hypothesis for the relationships of these bizarre birds, whereas the evidence of phylogeny in morphological characters was obscured by the evolutionary transformation of a small, volant duck into a giant, terrestrial herbivore.

Keywords: Anseriformes; Anatidae; molecular systematics; mitochondrial DNA; fossils; ducks

1. INTRODUCTION

The moa-nalos or 'lost fowl' were among the most extraordinary of the birds that vanished following human settlement of the Hawaiian islands some 1600 years ago, an event that led to the extinction of over half the endemic avifauna (Olson & James 1982*a*). Known only from recently discovered bones, moa-nalos were ponderous, flightless waterfowl (family Anatidae) with tiny wings, heavy lower bodies and massive beaks (Olson & Wetmore 1976; Olson & James 1982*b*, 1991; James & Olson 1983). Four species have been described: *Chelychelynechen quassus* from Kauai, *Thambetochen xanion* from Oahu, *Thambetochen chauliodous* from Molokai, Maui and Lanai and *Ptaiochen pau* from Maui (figure 1). *Chelychelynechen* (the 'turtle-jawed goose') had a very broad and deep bill reminiscent of a tortoise, while the other two genera had unusual bony pseudoteeth. Apparently adapted to browsing, moa-nalos were the largest herbivores on the islands where they occurred and were the ecological counterparts of giant tortoises on other oceanic islands lacking large mammals. A recent study of the coprolites of *T. chauliodous* suggests that moa-nalos were specialized for hindgut fermentation of plant fibres (James & Burney 1997).

The evolution of flightlessness and terrestrial herbivory altered the morphology of moa-nalos to such an extent that assessment of even their subfamilial relationships has been problematic (Livezey 1996*a*). Many potentially informative osteological characters have been obscured in moa-nalos by the extreme reduction in the wings and pectoral girdle, including loss of the keel from the sternum and the arcocoracoid portion of the coracoid, hypertrophy of the hind limbs and specialization of the skull and bill (Olson & James 1982*b*, 1991; James & Olson 1983; Livezey 1996*a*). Although initially thought to be geese (subfamily Anserinae) (Olson & Wetmore 1976), the discovery of syringeal bullae, which are associated with the skeletons of *Thambetochen* and *Ptaiochen*, in lava tubes on Maui led Olson & James (1991) to suggest that moa-nalos are part of the subfamily Anatinae, a diverse radiation of more than 30 extant genera, including the typical ducks (Johnsgard 1978). The syringeal bulla is an ossified resonating chamber formed by the bronchi where they join the trachea. The simple, asymmetrical bullae of moa-nalos are most similar to those of dabbling ducks (tribe Anatini) and shelducks (Tadornini) but are less elaborate than the highly modified, fenestrated bullae of pochards (Aythyini) and some sea ducks (Mergini) (Livezey 1986*a*; Olson & James 1991). Given that the Hawaiian avifauna includes two endemic dabbling ducks (*Anas laysanensis* and *Anas wyvilliana*), both part of the 'mallard' group (Livezey 1991) and several Holarctic *Anas* species that are winter visitors, Olson & James (1991) speculated that moa-nalos were derived from a mainland dabbling duck, such as the mallard (*Anas platyrhynchos*). In contrast, a recent osteological study (Livezey 1996*a*) questioned the homology of syringeal bullae in moa-nalos and ducks and tentatively

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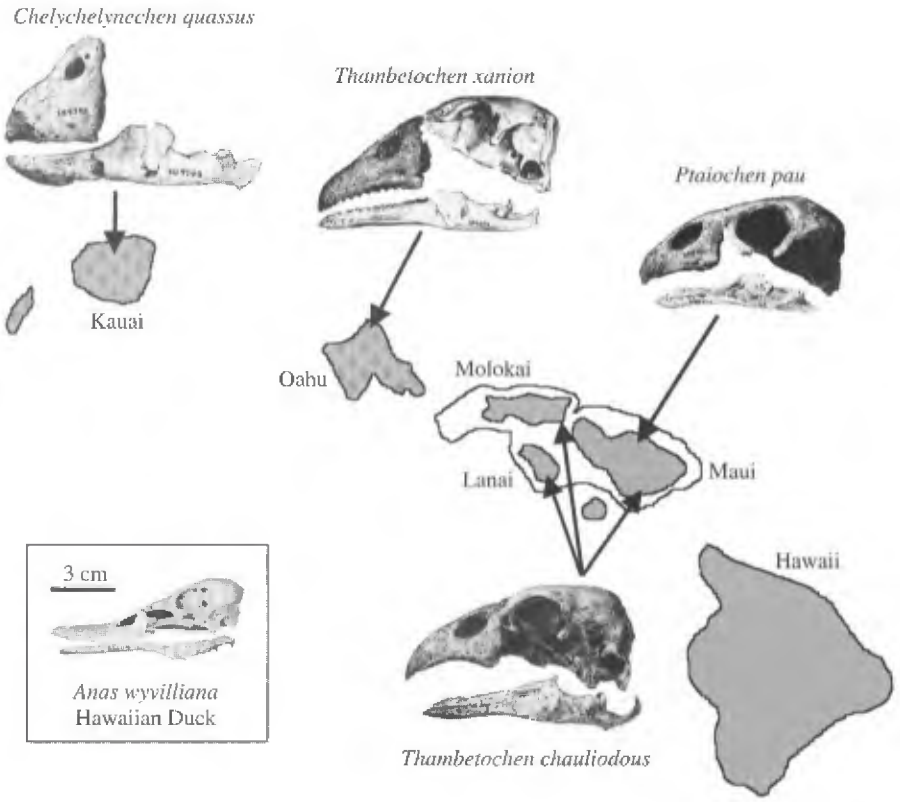

Figure 1. Map of the Hawaiian islands showing the islands on which remains of the four moa-nalo species described have been found. The approximate greatest extent of the Pleistocene island Maui Nui is shown as a line around Maui, Lanai and Molokai. The skull of the Hawaiian duck or koloa, a typical dabbling duck, is shown for comparison.

placed moa-nalos as the sister group of the 'true' geese and swans (Anserinae), which lack syringeal bullae.

Given the limitations of the morphological data for moa-nalos, Livezey (1996*a*) suggested that DNA sequence data might help resolve their uncertain systematic relationships. Our analysis of mitochondrial DNA (mtDNA) sequences obtained from fossil remains provides strong support for the inclusion of moa-nalos in the subfamily Anatinae and leads to a novel conclusion about their placement within this group. The resulting phylogeny also provides a clear explanation for the absence of moa-nalos from the youngest island, Hawaii.

2. MATERIAL AND METHODS

We extracted DNA from the bones of three moa-nalos, all from lava tube caves on Mount Haleakala, Maui. The samples included a pedal phalanx and tibiotarsus of *T. chauliodous* excavated from Puu Naio Cave, a left tibiotarsus of *T.* cf. *chauliodous* from Polipoli Cave and a fibula of *P. pau* from Auwahi Tube (James *et al.* 1987). The museum accession numbers and collection dates for the three sample are USNM 397942 (February 1984), BPBM 1997.009 (January 1997) and USNM 384171 (1982), respectively. Radiocarbon date estimates are not available for these particular samples, but other bones from extinct birds excavated on Maui have been dated from 9000 to 700 years ago and other samples from the same stratigraphic layer as USNM 397942 date to 564–790 years before present (James *et al.* 1987; Olson & James 1991).

DNA was extracted from subfossil bones, amplified and sequenced following published protocols (Cooper *et al.* 1992; Cooper 1994). We first amplified and sequenced around 400 base pairs (bp) of the mitochondrial gene for the small subunit (12S) ribosomal RNA for *T. chauliodous*, *P. pau* and 124 other Anseriformes, including most species and all extant genera. For the moa-nalos, two overlapping fragments were amplified and sequenced using primers 12SA, 12SH, 12SB2 (Cooper 1994) and 12SK (5′-CCTACATACCGCCGTCGCCAG-3′). Primers 12SA and 12SB2 were used for extant taxa.

Because the 12S sequences failed to resolve the position of moa-nalos within the Anatinae (see below), we sequenced three additional mtDNA fragments (cytochrome *b* (306 bp), tRNA-Lys/ATP8 (192–193 bp) and control region/tRNA-Phe (341–365 bp)) for moa-nalos and a sample of 37 extant waterfowl in 25 genera, the choice of which was based on the 12S results. The 37 extant taxa included representatives of all major groups within the Anatinae (see § 3 for taxa included in this subfamily) and all major lineages within the diverse genus *Anas*. The control region fragment was amplified and sequenced with L736 (5′-ATCTAAGCCTGGACACACCTG-3′), L825 (5′-TGACACTGATGCACTTTGACC-3′) or L1117 (5′-TTATTAGAGAAACTCCA GTAC-3′) and H1251 (5′-TGGCAGCTTCAGTGCCATGC-3′), L and H numbers referring to the strand and nucleotide positions of the 3′-end in the chicken sequence (Desjardins & Morais 1990). The highly divergent control region sequences of *Anseranas*, *Dendrocygna*, *Anser*, *Branta* and *Cygnus* were scored as missing because they could not be aligned, except arbitrarily, with sequences of the ingroup taxa. We used L9051 (5′-CAGCACTAGCCTTTTAAG-3′) and H9241 (5′-TTGGTCGA AGAAGCTTAGGTTCA-3′) for the tRNA-Lys/ATP8 fragment and standard primers for cytochrome *b* (Kocher *et al.* 1989). L15104 (5′-CCTCCGTAGCCCACACATG-3′) was used to amplify 174 bp of the cytochrome *b* fragment in *Thambetochen*. An additional fragment in the highly variable 5′-end of the control region (190–199 bp)

was amplified and sequenced for two moa-nalos and nine other taxa with primers C1, C1R, RHN1 and C1R2 (Sorenson & Fleischer 1996). We took requisite precautions to avoid nuclear copies of mtDNA sequences, which included using tissues other than blood for all taxa (see Sorenson & Quinn 1998).

We are confident that the DNA sequences obtained from the bones belong to the moa-nalos and do not reflect modern contamination. In Washington, DNA extractions and polymerase chain reactions (PCRs) for moa-nalos were completed with appropriate negative controls in a separate building from work on extant taxa. In addition, the 12S sequence for *T. chauliodous* was independently replicated in Colorado starting with extraction of DNA from a different bone of the same individual bird and with no discrepancies in the sequences determined by the two laboratories. ATPase subunit 8 (ATP8) and control region sequences for *T. chauliodous* were later replicated from the new sample found in Polipoli Cave in January 1997. Finally, the moa-nalo sequences clearly fall within the waterfowl (family Anatidae), yet differ from each other and from all other species that we sampled.

Due to the difficulty in working with ancient material, the sequence data were incomplete for the three moa-nalos. Nonetheless, 1308 and 846 bp were determined for *Thambetochen* (combining two samples) and *Ptaiochen*, respectively. The *Ptaiochen* sample and one of the *Thambetochen* samples were sequenced for the 12S and ATP8 fragments and two portions of the control region (197 and 94–95 bp). A partial cytochrome *b* sequence (174 bp) was obtained for *Thambetochen* only. The second *Thambetochen* sample was sequenced for ATP8 and one control region fragment (357 bp) and differed at only one position from the first *Thambetochen* sample over 288 bp sequenced for both.

Phylogenetic analyses based on parsimony and maximum likelihood were conducted in PAUP*, v. 4.0d65 (Swofford 1999). At least 100 replicate heuristic searches with random addition of taxa were used in the parsimony analyses. Alignment of the sequences was generally straightforward, although the control region fragments included numerous indels. Alignment gaps were treated as a fifth character state because most indels in our data set involve the addition or loss of a single base. To explore the sensitivity of our results to the alignment parameters, we performed additional analyses using POY (Gladstein & Wheeler 1996), a program that uses direct optimization to minimize alignment and cladogram costs simultaneously (Wheeler 1996). In the latter analyses, we used the 28 anatine taxa for which complete data were available over approximately 1130 alignment positions and ran 30 random addition searches for each of five weighting schemes. These included relative transition, transversion and gap costs of 1:1:1, 1:1:2, 1:2:2, 1:2:4 and 3:4:5. Sequences from the 5′-end of the control region were also analysed using POY. Genetic distances between clades were calculated by the method of Steel *et al.* (1996) and were based on Kimura's (1980) two-parameter model. We excluded positions with alignment gaps for distance calculations.

The sequences used in this study have been deposited in GenBank (accession numbers U83730–33, AF059053–55, 63, 73, 76–77, 81, 86, 90, 98, 100–104 and 109–113, AF090337 and AF173684–815). The alignments used in all analyses can be found in the electronic appendices from this paper on the Royal Society Web site.

3. RESULTS AND DISCUSSION

Phylogenetic analyses of the 12SrDNA sequences placed moa-nalos within the subfamily Anatinae or 'ducks' but left their position within this group unresolved. Parsimony searches based on 416 aligned positions for 126 anseriform taxa and with equal weights for all characters yielded over 10 000 equally parsimonious trees of length 908 (CI = 0.27 and RI = 0.69). All of these trees included a large clade (79 taxa including 35 genera) of relatively recently derived ducks which includes *Thambetochen* and *Ptaiochen*. In the following, we refer to this clade as the subfamily Anatinae (or 'ducks'), a definition that includes most of the taxa in Johnsgard's (1978) Anatinae, and in Livezey's (1997) Tadorninae and Anatinae combined. In contrast to previous treatments, however, our definition excludes the 'stifftails' (tribe Oxyurini: *Heteronetta*, *Nomonyx*, *Oxyura and Biziura*) and the genera *Malacorhynchus* and *Nettapus*. This definition is consistent with other recent molecular analyses (Sraml *et al.* 1996; M. D. Sorenson and K. P. Johnson, unpublished data). Other distantly related taxa included *Stictonetta* and members of the Anserinae (geese and swans) and Dendrocygninae (whistling ducks). The shortest trees in which *Thambetochen* and *Ptaiochen* are excluded from the Anatinae, as defined above, require six additional steps in the 12S data.

Phylogenetic analyses of an expanded set of mtDNA characters for moa-nalos and 37 extant anatids are consistent with the above results and resolve the relationships within the Anatinae (figure 2). We first discuss the subfamilial placement of moa-nalos and then their position within Anatinae. The monophyly of a large clade of 'ducks' (Anatinae) that includes moa-nalos is well supported (decay index = 8 and bootstrap value = 93). Our data strongly reject the alternative hypothesis of a sister relationship between moa-nalos and the 'true' geese and swans (Anserinae). Using the same data as in figure 2, trees in which the moa-nalo clade is sister to Anserinae require at least 14 additional steps, a significant increase in tree length ($p < 0.01$; Kishino & Hasegawa 1989).

In contrast to this clear result from the molecular analysis, the existing morphological data are largely inconclusive. Only two additional steps were required to place moa-nalos as sister to 'ducks' in Livezey's (1996*a*) analysis and these extra steps depend on the 'estimated' 19 or 20 cervical vertebrae in the *Thambetochen* holotype. The holotype was actually found with 14 cervical vertebrae, ten of which were still in articulation, but missing the axis. Smooth articulation of all 14 vertebrae and comparison with the cervical vertebrae of other waterfowl suggest that the most likely alternatives are that *Thambetochen* actually had only 15 vertebrae (one less than in any other waterfowl) or that the specimen is missing one vertebra in addition to the axis and, therefore, had 16 vertebrae as in most ducks. A single modification to Livezey's (1996*a*) data set, scoring *Thambetochen* as having 16 cervical vertebrae, makes trees in which moa-nalos are sister to 'ducks' and sister to Anserinae equally parsimonious.

Among the other morphological characters considered by Livezey (1996*a*), three potentially group moa-nalos with Anserinae. These are an enlarged end of the pubis (the significance of which is debatable given the hypertrophied pelvis of moa-nalos), a larger modal number of synsacral vertebrae, a relatively variable character both within and between waterfowl genera (Woolfenden 1961),

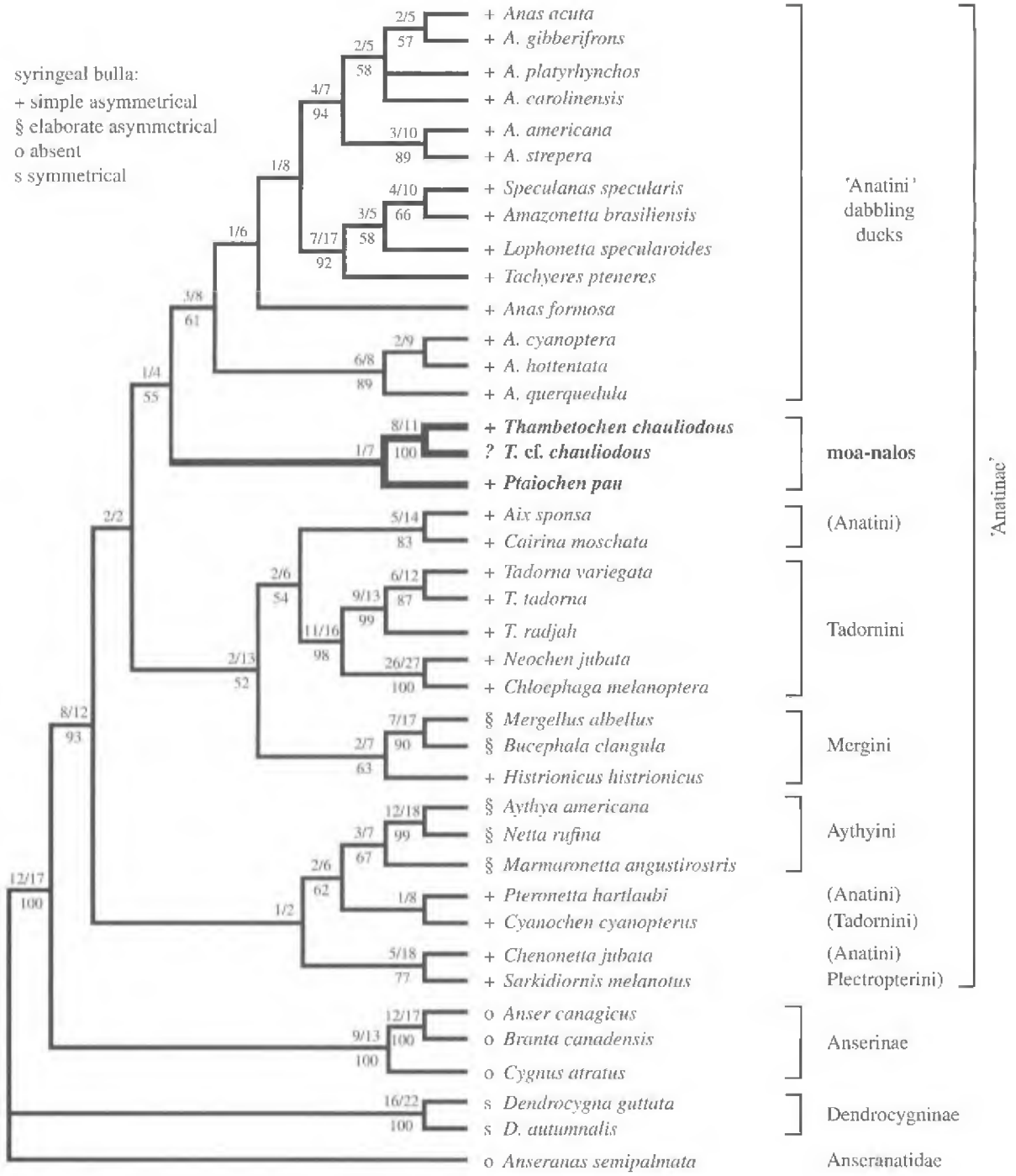

Figure 2. Strict consensus of three shortest trees of length 2089 (CI = 0.39 and RI = 0.48) found in parsimony analysis of all available mtDNA sequence data (1309 alignment positions). Alignment gaps were treated as a fifth character state and all characters and changes were given equal weight. Decay indices (number of additional steps required in the shortest tree without the node in question; Bremer 1988) and minimum branch lengths are given above each branch and bootstrap values for nodes found in greater than 50% of replicates are given below each branch. The tree is rooted with *Anseranas*, most recently placed as a monotypic family (Anseranatidae) sister to Anatidae (Livezey 1997). Groups names correspond to Livezey (1997) except for Anatini and Anatinae, which are defined in the text. *Aix*, *Cairina*, *Pteronetta* and *Chenonetta* were included in Anatini by Livezey (1997) but are excluded from the tribe as defined here. Moa-nalos are shown in bold.

and a depression in the skull for the nasal gland, a character scored as variably present in Anserinae and differing between the two moa-nalo genera. These are countered by three characters consistent with the inclusion of moa-nalos in Anatinae: the number of cervical vertebrae (see above), a reduced extension of the caudal–lateral process of the sternum (also of questionable significance given the highly modified sternum of moa-nalos) and the presence of an asymmetrical syringeal bullae in males. In the context of the molecular tree,

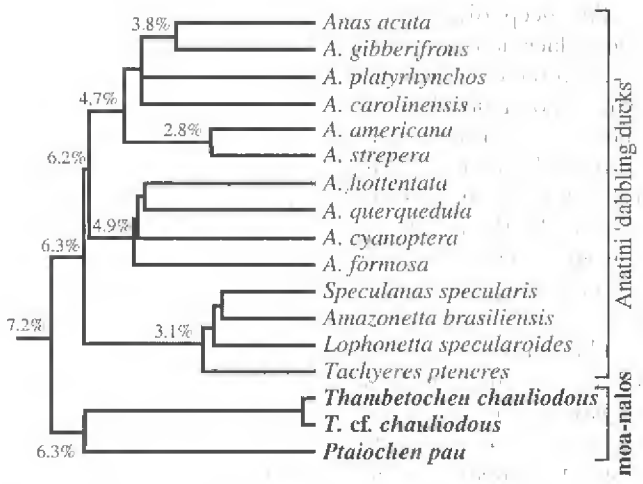

Figure 3. Relationships within the moa-nalo-Anatini clade based on weighted parsimony analysis. In this example, transitions were given a relative weight of 0.2. Five trees of length 868.4 differed only in their relationships between *Anas acuta*, *Anas gibberifrons*, *Anas platyrhynchos* and *Anas carolinensis*. The analysis included all taxa and used the same data set as in figure 2 but only a portion of the tree is shown. The branch lengths are proportional to the genetic distances between clades (Kimura 1980; Steel *et al.* 1996), the values of which are shown for selected nodes. Moa-nalos are shown in bold.

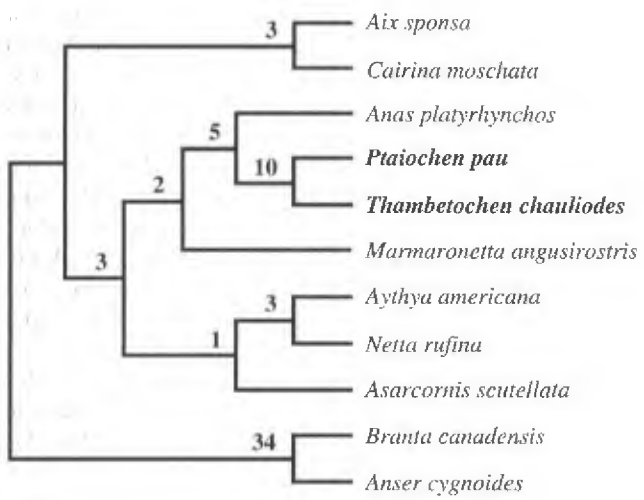

Figure 4. Shortest tree (length 512) resulting from an optimization alignment analysis using POY (Gladstein & Wheeler 1996) of sequences from the 5′- and 3′-ends of the mtDNA control region for 11 anatid taxa (286–297 bp per taxon). The sister relationship between moa-nalos and *Anas* was stable to variation in transversion and gap costs. Decay indices are shown for each node.

asymmetrical syringeal bullae in moa-nalos and other ducks are homologous and this character uniquely diagnoses the subfamily Anatinae (Johnsgard 1961). Placement of moa-nalos with the Anserinae would require the *de novo* appearance of this secondary sexual structure in moa-nalos, a scenario that seems particularly unlikely given that most island waterfowl lack sexual dimorphism (Lack 1970; Weller 1980).

Within Anatinae, the molecular data consistently unite the two moa-nalo genera sampled and place them sister to the dabbling ducks or Anatini (figure 2). For the purpose of this discussion, we define Anatini as including the genera *Anas*, *Speculanas*, *Lophonetta*, *Amazonetta* and *Tachyeres*. Although the moa-nalo–dabbling duck clade is supported by relatively few characters, two unambiguous and unreversed changes are the synapomorphies of this group, including a unique C to A transversion resulting in an amino acid substitution in ATP8. Parsimony analyses with transversions weighted by as little as 1.1:1 over transitions (we also used weights of 2:1, 5:1 and 1:0) yielded trees in which *Anas* is monophyletic and sister to a clade of four South American genera (*Speculanas*; *Lophonetta*; *Amazonetta* and *Tachyeres*) but which retain the sister relationship between moa-nalos and dabbling ducks (figure 3). (The character state reconstruction on the most parsimonious trees found with equal weights indicated an overall transition:transversion ratio of around 5:1.) Maximum-likelihood analyses using default parameter settings in PAUP* and either including or excluding positions with alignment gaps also yielded trees in which *Anas* is monophyletic and moa-nalos are sister to dabbling ducks. This placement of moa-nalos was also stable to variation in the alignment parameters: the best solution for each set of parameters used in POY (Gladstein & Wheeler 1996) included Anatini and *Thambetochen* as sister taxa. Finally, sequences from the highly variable 5′-end of the control region, data that were not included in any of the above analyses, also support a sister relationship between moa-nalos and dabbling ducks (figure 4).

While partially consistent with Olson & James' (1991) suggestion that moa-nalos are derived from a dabbling duck, the molecular data indicate that moa-nalos are not closely related to any one extant species or genus but, instead, represent a relatively early divergence within the subfamily Anatinae. Three unambiguous synapomorphies in the control region/tRNA-Phe fragment, including two unique transversions, support the monophyly of dabbling ducks to the exclusion of moa-nalos. Hypotheses in which moa-nalos are sister to any of the terminal taxa (i.e. individual species) in our analysis are strongly refuted. For example, using equal weights for all characters, 13 additional steps are required when moa-nalos are constrained to be the sister taxon of mallard (*A. platyrhynchos*) and between nine and 54 extra steps are needed to make moa-nalos sister to other terminal taxa in the subfamily Anatinae. A sister relationship with shelducks, another widely distributed group with relatively simple syringeal bullae, is likewise refuted; 32 and 12 additional steps are needed to make moa-nalos sister to *Tadorna* and 'Tadornini' (including *Chloephaga* and *Neochen*), respectively.

The relatively basal position of moa-nalos within Anatinae somewhat limits the conclusions that can be drawn about the morphology and geographical origin of the moa-nalos' ancestor, but it is likely that moa-nalos evolved from a relatively small duck. All of the dabbling ducks, with the exception of *Tachyeres*, are small (0.2–1.5 kg) and vary little in body proportions. Likewise, other members of the subfamily range from 0.3 to 2 kg, except for *Chloephaga*, *Cyanochen*, *Sarkidiornis*, *Plectropterus* and *Alopochen*, in which larger, more 'goose-like' morphologies have evolved independently (figure 2; Livezey 1996*b*). In contrast to dabbling ducks, moa-nalos ranged from 4 to 7.5 kg (based on a regression of avian body masses against tibiotarsus shaft circumference; Campbell & Toni 1983).

The evolution of moa-nalos very probably included a dramatic increase in body size and extensive modification of bill morphology in addition to the extreme reduction in structures associated with flight. Our analysis also includes *Tachyeres* (steamer ducks) as part of the Anatini (dabbling ducks), a result consistent with Woolfenden (1961) but not with more recent classifications (Johnsgard 1978; Livezey 1997). *Tachyeres* represents a more recent, independent example of the evolution of flightlessness and larger body size. Three out of four *Tachyeres* species are flightless, with individuals ranging up to 6 kg as compared to 3 kg for the flighted species (Todd 1996).

Analysis of genetic distances suggests that moa-nalos are ancient denizens of the Hawaiian islands. Using the largest data set for which sequences are complete for *Thambetochen*, *Ptaiochen* and other Anatinae (33 taxa and 625 characters), moa-nalos are on average 7.2% divergent from dabbling ducks and 6.3% divergent from each other. Particularly striking is the deep split between the two moa-nalo genera, which is as large as the deepest divergences between dabbling ducks (figure 3) and which is also evident in sequences from the 5′-end of the control region, in which *Thambetochen* and *Ptaiochen* differ in 23% of 202 aligned positions. We hesitate to translate these values into precise estimates of divergence time because the fossil record lacks the temporal resolution to calibrate the rates of sequence evolution in Anatinae adequately. In addition, the distances based on our subset of 625 characters are generally smaller than those based on 2086 bp of cytochrome *b* and ND2 sequences (Johnson & Sorenson 1998) for 23 of the anatine taxa in our study, such that our estimates of divergence are not necessarily representative of the entire mitochondrial genome. With these caveats, the often-cited figure of 2% sequence divergence per million years (My) for mtDNA (Brown *et al.* 1979; Tarr & Fleischer 1993; Fleischer *et al.* 1998) yields rough point estimates of 3.6 My for the divergence of moa-nalos and Anatini and 3.1 My for the divergence of *Thambetochen* and *Ptaiochen*.

Assuming a single colonization from the mainland, the latter estimate suggests that the ancestor of moa-nalos arrived in the Hawaiian islands relatively early in the histories of Kauai and/or Oahu, the oldest rocks of which are 5.7 and 3.8 My, respectively (Macdonald *et al.* 1983). A detailed account of the diversification of moa-nalos in relation to the geological history of the islands on which they occur would be premature given the lack of genetic data for *Chelychelynechen* and recent discoveries of additional moa-nalo taxa on Kauai (S. L. Olson and H. F. James, unpublished data). This history, however, must have included either successful dispersal by flightless moa-nalos across the open ocean between Kauai and Oahu, an event we consider unlikely or the independent loss of flight in more than one moa-nalo lineage after colonization of the various islands by volant ancestral stock. Ancestral moa-nalos surely passed through a stage of incipient flightlessness during which dispersal between islands might still have been possible and from which convergent evolution of complete flightlessness is very plausible. Examples of such intermediate stages are observed in flying steamer ducks (*Tachyeres patachonius*) (Humphrey & Livezey 1982; Livezey 1986*b*) and extinct geese (*Branta spp.*) from Hawaii (Olson & James 1991).

The deep divergence between moa-nalo genera and their placement as the sister group of dabbling ducks also leads to the conclusion that the continental ancestor from which moa-nalos derived has long been extinct. Although there is no direct evidence of a link with moa-nalos, *Anabernicla*, known from Late Miocene to Late Pleistocene deposits in western North America (Howard 1964; Bickart 1990), is one example of an extinct waterfowl lineage which may have been capable of long distance colonization at the time the ancestor of moa-nalos colonized the Hawaiian islands.

That moa-nalos represent an ancient lineage not closely related to any extant species provides an explanation for their absence from the island of Hawaii. Long before Hawaii emerged from the sea some 500 000 years ago, moa-nalos had evolved flightlessness, limiting their ability to disperse to new islands. Interestingly, the fossil avifauna of Hawaii includes a large, flightless goose that is very closely related to the extant, flighted nene (*Branta sandvicensis*) and the extinct *Branta hylobadistes* (E. Paxinos, H. F. James, S. L. Olson, M. D. Sorenson, A. Cooper and R. C. Fleischer, unpublished data). This group of Hawaiian geese is closely related to the larger subspecies of Canada geese (*Branta canadensis*) still extant on the mainland and is therefore relatively recently derived from a flighted ancestor (E. Paxinos, H. F. James, S. L. Olson, M. D. Sorenson, A. Cooper and R. C. Fleischer, unpublished data). Thus, distantly related waterfowl have at least twice colonized the Hawaiian islands and evolved flightlessness. The striking convergence between moa-nalos and flightless geese in the Hawaiian islands may reflect similar evolutionary responses to the ecological opportunities presented by the absence of mammalian and reptilian herbivores.

The evolution of moa-nalos included an extreme adaptive shift from volant, filter-feeding, aquatic birds to flightless, terrestrial herbivores. When an extinct lineage has undergone such radical change in a new environment, identifying a sufficient number of homologous morphological characters to establish systematic relationships may be difficult, though not necessarily impossible (e.g. Worthy *et al.* 1997). As in other studies of extinct flightless birds (e.g. Cooper *et al.* 1992; Houde *et al.* 1997), ancient DNA from moa-nalos provided a valuable source of characters for phylogenetic analysis. In contrast to the inconclusive evidence available from morphology, molecular data provide a clear resolution of the subfamilial relationships of moa-nalos and a novel hypothesis for their position within the subfamily Anatinae, results consistent with an unusual morphological character, the syringeal bulla.

We thank Mike and Ali Lubbock for providing most of the samples of extant waterfowl, Mark Siddall and two anonymous reviewers for comments on the manuscript and Carl McIntosh and Suzy Brown for assistance in the laboratory. Funding was provided by the Smithsonian Institution Scholarly Studies Program and Walcott Fund awards to R.C.F., S.L.O., H.F.J. and T.W.Q. and by US National Science Foundation grants to M.D.S., T.W.Q. and Robert B. Payne.

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