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Phylogenetic afýnities of the New Zealand blue duck (*Hymenolaimus malacorhynchos*)

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Abstract We investigate the phylogenetic affinites of the New Zealand blue duck (*Hymenolaimus malacorhynchos*), a riverine specialist of uncertain relationships, using 2613 bp of DNA sequence data from 3 mitochondrial genes. *Hymenolaimus* has variously been considered an aberrant *Anas* species, or an ancient taxa in the tribe Anatini. Presently, it is placed in a highly-derived clade (Tribe Merganettini) with the shelducks. Our findings show that *Hymenolaimus* forms a monophyletic clade, and does not fit within any of the other duck tribes around the world. Our study also confirms convergent evolution among duck species that inhabit fast flowing rivers.

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Keywords blue duck; Anseriformes; cytochrome *b* gene; control region gene; ND2 gene; mitochondrial DNA; phylogenetics

INTRODUCTION

A number of phylogenies for the Anatidae have been proposed (*e.g.*, Delacour & Mayr 1945; Del Hoyo *et al.* 1992; Livezey 1986, 1997) and a consensus appears to be emerging (*e.g.*, Donne-Goussé *et al.* 2002; Gonzalez *et al.* 2009; Bulgarella *et al.* 2010), yet uncertainty remains about the affinities of some monotypic genera. The endangered blue duck (*Hymenolaimus malacorhynchos*) of New Zealand, an endemic riverine specialist (Marchant & Higgins 1990; Robertson *et al.* 2007), is one such taxa of uncertain placement. *Hymenolaimus* inhabits mountain rivers and shares ecological adaptations in common with other river ducks (*e.g.*, torrent duck , *ÌÙÎÈÔÌÙÈ*ËÙÔÈÚÈ and Salvadori's duck *Salvadornia waigiuensis*), which has contributed to speculation on the shared phylogenetic affinities of these species (see Kear 2005). Delacour and Mayr (1945), however, refuted any suggestion that blue ducks were similar to either , *ÌÙÎÈÔÌÙÈ* or *Salvadornia*.

Studies of morphology, behaviour and DNA have consistently placed *Hymenolaimus* in the Family Anatidae (Gill *et al.* 2010). However, the taxon has variously been suggested to be: (i) an aberrant *Anas* species in the dabbling ducks (Tribe Anatini: *sensu* Delacour & Mayr 1945); (ii) an ancient Anatini with no close relatives due to affinities with both the perching ducks (Tribe Cairinini) and

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the Tribe Anatini (Kear 1972; Kear 2005); or (iii) a member of a highly derived clade, along with *Tachyeres* (steamer-ducks) and , *ÌÙÎÈÕÌÙÈ* within the shelducks (Tribe Tadornini, subtribe Merganetteae: Livezey 1986). The presently accepted taxonomy (*e.g.*, Kear 2005) has *Hymenolaimus* placed in a *'àÔÌÕÕÔÈÐÔÜÚı , ÌÙÎÈÔÌÙÈı3ÈÊÏàÌÙÌÚ* clade with the shelducks (Tribe Merganettini: *sensu* Livezey 1997). To date, the only phylogenetic analysis of *Hymenolaimus* involved the species as an outgroup species in an investigation of South American duck genera (Bulgarella *et al*. 2010). *Hymenolaimus* was placed in a clade with an Australian (*"ÏÌÕÕÔÌÙÈ jubata*) and an Old World/Neotropical (*Sarkidiornis melanotos*) taxon.

Here we use DNA sequences from 3 mitochondrial regions (cytochrome b gene, ND2 & control region sequence), plus a large number of taxa, to examine the phylogenetic affinities of the blue duck in a wider phylogeny of Anseriformes. Based on this new analysis and the available literature, we evaluate the historic placement of *Hymenolaimus*.

METHODS

Hymenolaimus samples were collected and genomic DNA was extracted as described in Robertson *et al.* (2007). Cytochrome b gene was amplified from 3 blue duck samples, one each from the Manganuiateao River (WG118), Clinton River (L36927) and the Cleddau River (L38360) (see Robertson *et al.* 2007) using PCR primers L14841 (Kocher *et al.* 1989) and H16064 (Sorenson *et al.* 1999). The complete cytochrome b gene was amplified in a 25 μl reaction volume containing 50 ng genomic DNA, 1.0 pmol of each primer, 200 μM each of dATP, dGTP, dTTP, and dCTP, 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$ and 0.5 unit of Taq polymerase (Bioline USA, Inc, Randolph MA 02368-4800). The thermal cycling parameters were an initial 2 min denaturation at 94°C, followed by 35 cycles at 94°C/30 sec, 58°C/45 sec and 72°C/90 sec, and finally 72°C/5 mins. PCR reactions were purified with Millipore Montage PCR96 Multiscreen filter plates (Biolab, New Zealand). Sequence was generated using a Big Dye v. 3.1 sequencing kit (Applied Biosystems, Foster City, CA) as per the manufacturer's instructions using the L14841 and H16064 primers.

Control region sequences obtained by Robertson *et al.* (2007) were used in this study, in combination with sequences from GenBank for all 3 genes (Table A1). In particular, GenBank sequences from previous molecular phylogenetic studies of Anseriformes were used (Donne-Goussé *et al.* 2002; Gonzalez *et al.* 2009; Bulgarella *et al.* 2010). We were unable to source DNA from Salavadori's duck *Salvadornia waigiuensis* to fully examine putative convergent evolution among the riverine ducks. A multiple alignment of all sequences for each gene was achieved using default parameters in ClustalX (Thompson *et al.* 1997), and all variable sites and ambiguous sections were confirmed by visual inspection in BIOEDIT version 5.0.6 (Hall 1997).

Phylogenetic analysis

Maximum likelihood and Bayesian Probability methods were used to test the phylogenetic relationship of *Hymenolaimus* with other Anseriformes taxa. A partial fragment of the mtDNA control region was analysed separately from the concatenated ND2 (954 base pair, bp) and cytochrome b (1018bp) genes. For control regions sequence, domains II and III were aligned as per Donne-Goussé *et al.* (2002), with gaps excluded. Major gaps of 30 - 100bp were observed only in outgroup taxa and so were deleted as these groups were not the focus of the study. Monophyly of outgroups was maintained despite this exclusion. The remaining sequences were reviewed for substitution/saturation, with no asymptote observed for transitions or transvertions, which was also observed when these data were published previously (*i.e.*, Donne-Goussé *et al*. 2002). Domain I was not used due to its hypervariable nature and the absence of sequence available for this region for many taxa. We did not combine the 3 genes for mixed analyses (*cf.* Donne-Goussé *et al.* 2002), as there are few taxa that have been sequenced for both the control region and cytochrome b genes, due to the different foci of previous studies. For both datasets, we determined the best fit evolutionary model using Modeltest v. 3.06 (Posada & Crandall 1998) following the Akaike Information Criterion (AIC: Sakamoto *et al.* 1986).

Maximum likelihood analysis was performed with 100 bootstrap replicates using PAUP*4.0b10 (Swofford 1998) for the control region data only. The genetic distance among taxa was also determined using maximum likelihood parameters. Bayesian analysis was conducted for 1,000,000 generations, sampled every 100 generations (25% burnin) in Mr Bayes (Ronquist & Huelsenbeck 2003), using likelihood parameters determined by Modeltest: 4by4 nucleotide model with substitution type 6 (GTR) for all sequence alignments and among-site rate variation estimation for invariable sites and the gamma distribution.

RESULTS

The 2 sequence alignments used in this study consisted of 641bp of control region fragment and 1972bp of ND2 and cytochrome b sequence. The control region dataset included 46 Anseriformes taxa and the ND2/cytochrome b dataset included 82 taxa from the Anatinae sub-family (Table 1). Although

Table 1. Scientific name, region of mtDNA and accession number of relevant sequences.

Species	Sample code	Control Region	ND2	Cytb
Aix galericulata		AY112953*	-	-
Aix sponsa	2	-	EU585668~	AF059053^
Alopochen aegyptiacus	7	AY112964*	HM063564^	^
ÔÈáÖÕÌỤÈ₪ÉÙÈÚÐÐÌÕÚÐÚ	17	-	AF059115`	AF059054^
Anas acuta	29	AY112939	-	AF059055`
Anas americana	30	-	AF059163`	AF059103^
Anas aucklandica	31		AF059117`	AF059059
Anas bahamensis	32	AY112940*	AF059120`	AF059058`
Anas bernieri	33	-	AF059121`	AF059060`
Anas capensis	34	-	AF059165`	AF059105`
Anas castanea	35	-	AF059125`	AF059065`
Anas chlorotis	36	-	AF059122`	AF059061`
Anas c. carolinensis	13	-	AF059123`	AF059063`
Anas c. crecca	14	AY112942*	EU585670~	AF059064`
Anas c. cyanoptera	37	-	AF059127`	AF059067`
Anas clypeata	12	AY112941*	AF059174`	AF059062`
Anas diazi	38	-	AF059129`	AF059069`
Anas discors	39	-	AF059128`	AF059068`
Anas erythrorhyncha	40	-	AF059130`	AF059070`
Anas falcata	41	-	AF059166`	AF059106`
ÕÈÚ₪Í₪ŘÈÝÐÙÖÚÛÙÐÚ	42	-	AF059131`	AF059071`
Anas f. oxyptera	43	-	AF059132`	AF059072`
Anas formosa	44	-	AF059133`	AF059073`
Anas fulvigula	46	-	AF059134`	AF059074`
Anas g. spinicauda	45	-	AF059135`	AF059075`
Anas g. gracilis	47	-	AF059136`	AF059076`
ÕÈÚ₪ÏÖỤÌÖÚÔÚÈ	48	-	AF059137`	AF059077`
Anas laysanensis	49	-	AF059138`	AF059078`
Anas luzonica	50	-	AF059139`	AF059079`
Anas melleri	51	-	AF059140`	AF059080`
Anas penelope	52	-		AF059107`
Anas platalea	53	-	AF059144`	AF059084`
Anas platyrhynchos	15	AY112938*	EU585672~	AF059081`
Anas poecilorhyncha	54	-	AF059143`	AF059083`
Anas puna	64	-	AF059145`	AF059085`
Anas querquedula	55	-	AF059146`	EU585610~
Anas r. rhynchotis	56	-	AF059147`	AF059087`
Anas rubripes	57	-	AF059148`	AF059088`
Anas sibilatrix	58	AY112943*	AF059168`	AF059108`
Anas smithii	59	-	AF059149`	AF059089`
Anas sparsa	60	-	AF059151`	AF059091`

Table 1. Continued.

Anas strepera	61	AY112944*	AF059169`	AF059109`
Anas s. rogersi	62	-	AF059152`	AF059092`
Anas undulata	63	-	AF059153`	AF059093`
Anas versicolor	65	-	AF059154`	AF059094`
Anser albifrons		AY112967*	-	-
Anser anser		AY112966*	-	-
Anser caerulescens		AY112968*	-	-
Anser canagicus		AY112969*	-	-
Anser erythropus		AY112970*	-	-
Anser indicus		AY112971*	-	-
Anser rosii		AY112972*	-	-
Asarcornis scutulata	9	-	AF059159	AF059099^
àÛÏàÈ‰ÈŠÕÞÚ	67	-	EU585684~	EU585621~
Aythya americana		AY112946*		AF090337^
Aythya australis	66	-	EU585685~	EU585622~
Aythya ferina	68	-	EU585686~	EU585623~
Aythya fuligula	69	-	EU585687~	EU585624~
Aythya marila	70	AY112947*	EU585688~	EU585625~-
Aythya nycora	71	AY112948*	EU585689~	-EU585626~
Branta bernicla	1	AY112973*	EU585691~	EU585628~
Branta canadensis		AY112974*	-	-
Branta leucopsis		AY112975*	-	-
!ÙÈÕÛÈ‰ÙÜƒÊÕÚÞÚ		AY112976*	-	-
Bucephala albeola	72	-	EU585696~	EU585633~
Bucephala clangula	24	AY112959*	EU585697~	AF515261*
Bucephala islandica	74	-	EU585698~	EU585635~
Cairina moschata	75	AY112952*	AF059158`	AF059098`
"ÈÚÚÕÕÎÙÈ‰ÚÎÜÊÕ×ÏÙàÚ	27	AY112960*	AF059157`	AF059097^
Cereopsis novaehollandiae		AY112977*	-	-
Chauna torquata		AY112982*	-	-
"ÏÌÒÕÔÎÙÈ‰ÙÜÉÈÙÈ	22	AY112951*	AF059160`	AF059100`
Chloephaga picta	4	AY112965*	AF515266*	AF515262*
Clangula hyemalis	77	AY112954*	EU585701~	-EU585638~
Coscoroba coscoroba		AY112979*	-	-
Cyanochen cyanopterus	11	-	AF059161`	AF059101^
Cygnus atratus		AY112978*	-	-
Cygnus olor		-	-	
Dendrocygna bicolour		AY112980*	-	-
Dendrocygna eytoni		AY112981*	-	-
Hymenolaimus malacorhynchos	28	EF395946/955>	>	#
Lophodytes cucullatus	78	-	EU585650~	EU585713~
+Õ×ÏÕÔÎÙÈ‰Ú×ÌÊÜÚÈÙÖÞËÌÚ	16	AY112945*	AF059162`	AF059102`

Table 1. Continued.

Malacorhynchus membranaceus	79	-	EU585714~	EU585651~
, ÈÙÔÈÙÖÕÌÙÈ№ÈÕÎÜÚÛÞÙÖÚÛÞÚ	8	AY112950*	AF059164`	AF059104`
, ÌÚÈÕÞÙÈ№ÕÞÎÙÈ	25	-	AF515267*	AF515263*
, ÌÛÈÕÞÙÈ№×ÌÙÚ×ÞÊÐÓÚÈÛÈ	80	-	EU585715~	EU585652~
, ÌÙÎÈÖÌÙÈ№ÈÙÔÈÛÈ	3	-	HM063566^	^
Mergus albellus	81	AY112957*	EU585716~	-EU585653~
Mergus cucullatus		AY112958*	-	-
Mergus m. merganser	82	-	EU585717~	EU585654~
Mergus serrator		AY112956*	-	-
Neochen jubata	5	-	HM063564^	^
- ÌÙÈ№×Ì×ÕÚÊÈÈ	83	-	EU585719~	EU585656~
- ÌÙÈ№ÙÜ┌ÕÈ		AY112949*	-	-
/ÛÌÙÖÕÌÙÈ№ÏÈÙÚÚÈÜÉÐ	10	-	AF059170`	AF059110^
Sarkidironis melanotos	23	-	EU585723~	AF059111^
Somateria mollissima	26	AY112955*	EU585724~	AF515264*
Somateria spectabilis	84	-	Eu585725~	EU585662~
Speculanas specularis	18	-	AF059150`	AF059090^
Tachyeres bachypterus	20	-	HM063559^	^
Tachyeres leucocephalus	19	-	HM063560^	^
Tachyeres pteneres	21	-	AF059172`	AF059112^
Tadorna cana	85	-	EU585726~	EU585663~
Tadorna ferruginea	87	-	EU585727~	EU585664~
Tadorna radjah	86	-	EU585728~	EU585665~
Tadorna tadorna	6	AY112962*	AF059173`	AF059113`
Tadorna tadornoides	88	AY112963*	EU585729~	-EU585666~

Note: symbols represent the respective publications in which the sequences are published. ^ Bulgarella et al. 2010. * Donne-Goussé et al. 2002, ` Johnson & Sorenson 1998, ~ Gonzalez et al. 2009, > Robertson *et al*. 2007, # present study. – shows where no sequence was available.

the 2 datasets were not directly comparable, due to the different taxa available for each of the genes, consistencies did exist in the relationships among major taxonomic units (Figs. 1 & 2). For example, the placement of the Tadornini, Aythyini, Anatini, and Mergini tribes were consistent for each dataset, despite bootstrap and Bayesian support for the short control region fragment being low for these clades (Fig. 1).

Hymenolaimus was placed firmly within the Anatinae sub-family for both datasets, but maximum likelihood and Bayesian analyses were unable to resolve many of the relationships within the Anatinae (Figs. 1 & 2). For both analyses, *Hymenolaimus* was not placed within any of the existing tribes, nor was it associated with any of the historical placements (*e.g.*, in the Anatini or in the Merganettini with torrent ducks and steamer ducks; Fig. 2). The concatenated ND2/cytochrome b duck phylogeny placed the *Hymenolaimus* in a clade with *S. melanotos*, that did not include *C. jubata*.

The genetic distances between *Hymenolaimus* and all other Anatinae taxa supports its distinct placement. Genetic distances ranged from 7% - 13% for the control region (Table 2) and 8% - 12% for the cytochrome b gene (Table 3). Genetic differentiation within *Hymenolaimus* was negligible in comparison, at 0 – 2% for the control region and 0% for the cytochrome b gene.

DISCUSSION

Although the precise placement of the New Zealand blue duck is unclear, it appears to be a unique entity within the Anatinae sub-family. Our results are consistent with the major Anseriformes clades of Donne-Goussé *et al.* (2002) and mostly consistent with the Anatinae clades identified by Bulgarella *et al.* (2010) in their phylogenetic study of South

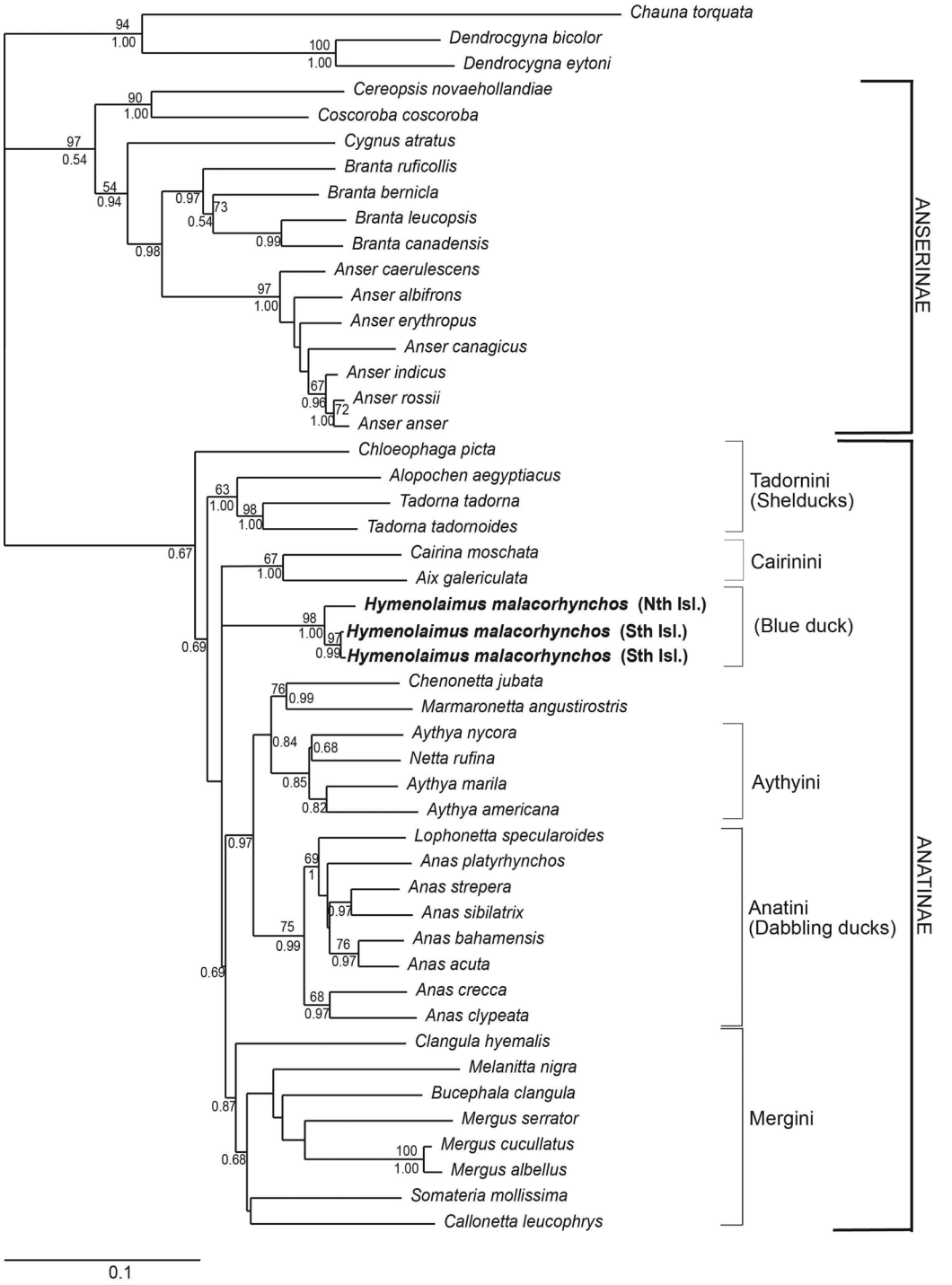

Fig. 1. A 50% majority-rule phylogenetic tree of the partial mitochondrial control region gene. Bootstrap (above branches) and Bayesian probability (below branches) support are shown. *Hymenolaimus* sequences are highlighted and the historically derived tribes and common names for the major tribes are shown. The scale bar represents the branch lengths as the number of substitutions per site.

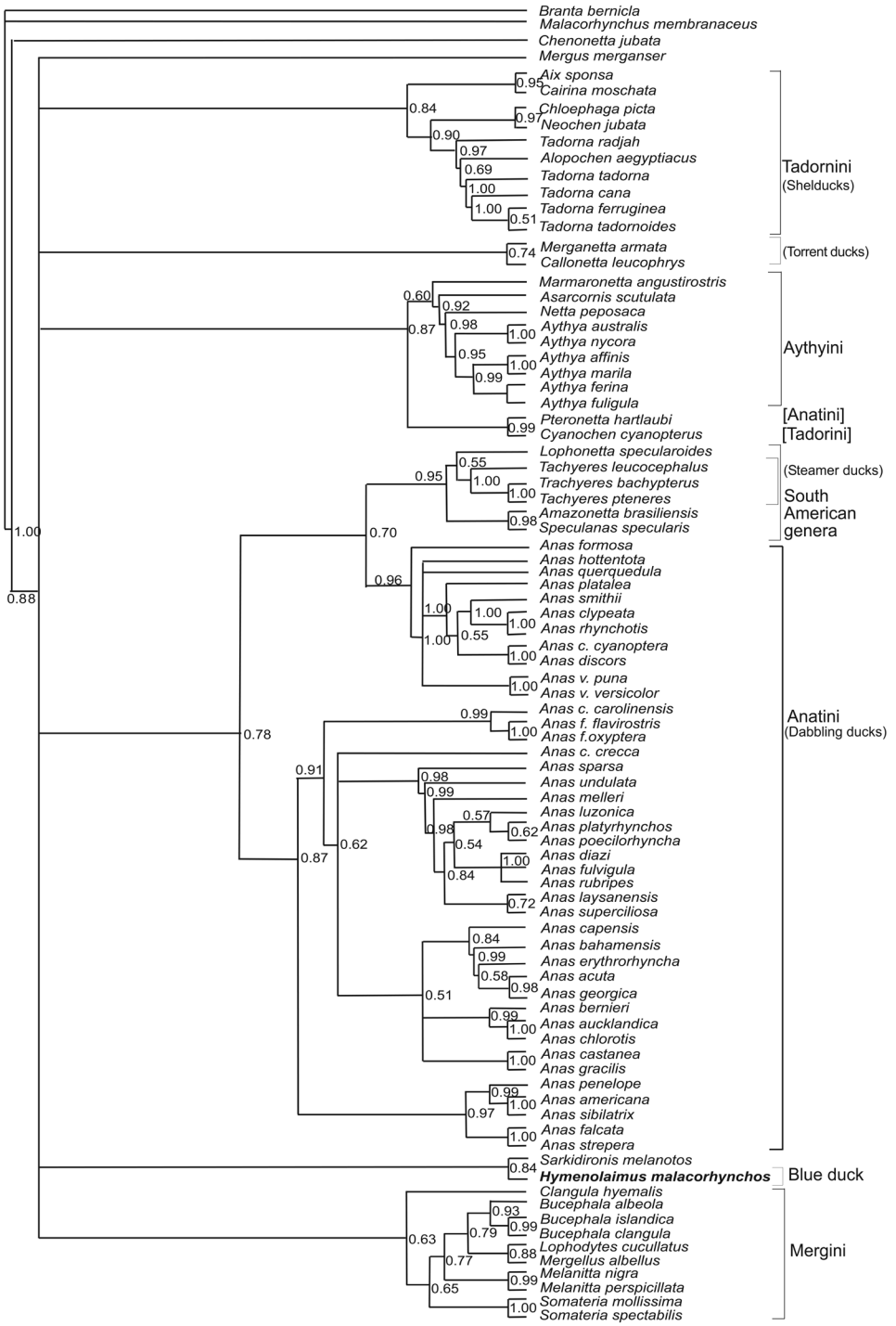

Fig. 2. A 50% majority-rule phylogenetic tree of the concatenated partial mitochondrial cytochrome *b* and ND2 genes. Bayesian probability (below branches) support is shown. *Hymenolaimus* sequences are highlighted and the historically derived tribes and common names for the major tribes are shown. The scale bar represents the branch lengths as the number of substitutions per site.

Table 2. Genetic distance for the partial mitochondrial DNA control region gene fragment.

	C. leucophrys	*S. mollissima*	*M. cucullatus*	*M. albellus*	*M. serrator*	*B. clangula*	*M. nigra*	*C. hyemalis*	*C. jubata*	*M. angustirostris*	*A. nycora*	*-ãwÙÜ┬ÔÈ*	*A. marila*	*A. strepera*	*A. sibiliatrix*	*A. acuta*	*L. specularoides*	*A. crecca*	*C. moschata*	*A. galericulata*	*H. malacorhynchos*	*T. tadorna*	*T. tadornoides*	*A. aegyptiacus*	*C. picta*
S. mollissima	0.11																								
M. cucullatus	0.10	0.10																							
M. albellus	0.10	0.10	0.01																						
M. serrator	0.13	0.11	0.10	0.10																					
B. clangula	0.12	0.10	0.08	0.09	0.08																				
M. nigra	0.11	0.13	0.10	0.10	0.13	0.12																			
C. hyemalis	0.11	0.09	0.10	0.10	0.11	0.08	0.11																		
C. jubata	0.12	0.10	0.12	0.13	0.14	0.11	0.15	0.11																	
M. angustirostris	0.12	0.11	0.12	0.12	0.12	0.09	0.13	0.10	0.08																
A. nycora	0.11	0.11	0.10	0.11	0.13	0.09	0.14	0.10	0.09	0.08															
-ãwÙÜ┬ÔÈ	0.11	0.10	0.11	0.12	0.12	0.10	0.14	0.10	0.07	0.07	0.05														
A. marila	0.11	0.09	0.10	0.10	0.11	0.09	0.12	0.09	0.08	0.07	0.05	0.05													
A. strepera	0.12	0.10	0.10	0.11	0.12	0.09	0.14	0.10	0.08	0.09	0.07	0.07	0.06												
A. sibiliatrix	0.11	0.10	0.11	0.11	0.12	0.09	0.15	0.11	0.08	0.09	0.07	0.06	0.07	0.03											
A. acuta	0.12	0.11	0.11	0.12	0.12	0.09	0.14	0.11	0.08	0.09	0.08	0.06	0.07	0.03	0.03										
L. specularoides	0.13	0.11	0.11	0.11	0.12	0.09	0.14	0.10	0.08	0.09	0.07	0.07	0.08	0.04	0.04	0.05									
A. crecca	0.11	0.09	0.11	0.11	0.11	0.08	0.12	0.09	0.08	0.09	0.08	0.06	0.07	0.05	0.05	0.04	0.05								
C. moschata	0.12	0.10	0.09	0.10	0.11	0.09	0.12	0.09	0.10	0.10	0.10	0.09	0.09	0.09	0.09	0.10	0.10	0.08							
A. galericulata	0.12	0.11	0.11	0.11	0.14	0.12	0.15	0.11	0.11	0.11	0.10	0.11	0.10	0.08	0.09	0.10	0.10	0.10	0.08						
H. malacorhynchos	**0.11**	**0.10**	**0.10**	**0.10**	**0.11**	**0.09**	**0.13**	**0.10**	**0.10**	**0.09**	**0.06**	**0.08**	**0.08**	**0.07**	**0.08**	**0.08**	**0.07**	**0.08**	**0.09**	**0.08**					
T. tadorna	0.12	0.11	0.12	0.12	0.13	0.11	0.14	0.11	0.11	0.11	0.10	0.09	0.08	0.09	0.10	0.10	0.10	0.09	0.09	0.11	**0.10**				
T. tadornoides	0.12	0.12	0.10	0.11	0.13	0.11	0.13	0.12	0.12	0.12	0.11	0.11	0.10	0.11	0.11	0.11	0.11	0.09	0.09	0.11	**0.09**	0.07			
A. aegyptiacus	0.11	0.10	0.10	0.11	0.12	0.09	0.13	0.09	0.09	0.09	0.08	0.07	0.07	0.07	0.07	0.07	0.08	0.06	0.07	0.08	**0.08**	0.08	0.08		
C. picta	0.11	0.09	0.11	0.11	0.12	0.11	0.13	0.09	0.10	0.11	0.08	0.08	0.07	0.08	0.07	0.08	0.09	0.08	0.09	0.09	**0.08**	0.08	0.10	0.08	
B. bernicla	0.21	0.16	0.17	0.17	0.17	0.17	0.22	0.17	0.19	0.19	0.18	0.19	0.18	0.17	0.19	0.18	0.19	0.17	0.18	0.19	**0.18**	0.18	0.19	0.18	0.17

Note: A representative species from several clades in Fig. 1 were selected for presentation here. The species not used were nearly identical to other taxa within the clade.

American ducks. Both datasets used in our study show that historic placements of *Hymenolaimus* based on morphology and behaviour (Delacour & Mayr 1945; Kear 1972; Kear 2005; Livezey 1986; 1997) were incorrect. *Hymenolaimus* is not a member of dabbling (Anatini) or perching ducks (Cairinini), and the taxon does not group as a highly derived clade of shelducks (Merganettini), as it displays no phylogenetic affinities with steamer-ducks or the torrent duck. *Hymenolaimus* does not exhibit a close affinity to any other particular genus or tribe.

One major difference with our study was that Bulgarella *et al.* (2010) placed *Hymenolaimus* in a clade with *S. melanotos* and *C. jubata*. Bulgarella *et al.*'s placement of *Hymenolaimus* was a strongly supported grouping based on 3 mtDNA gene regions and 5 nuclear loci. In our study, we sampled 1972bp of cytochrome b and ND2 sequence (*cf.* 500bp in Bulgarella *et al.* 2010), which may account for the change in the relationship between *Hymenolaimus* and *C. jubata*. The additional sites in the cytochrome b fragment obtained in specimens used in our study have potentially increased resolution. The placement of *S. melanotos* and *C. jubata* is not consistent across studies, with some authors finding this relationship (Sorenson *et al.* 1999; Gonzalez *et al.* 2009; Bulgarella *et al.* 2010; present study for cytochrome b), yet others have not (Gonzalez *et al.* 2009; Johnson & Sorenson 1999). "*ÌÌÕÖÕÔÌÙÈ*/*ÑÜÉÈÙÈ* was placed basally in the Anatini by Gonzalez *et al.* (2009).

Many of the deeper phylogenetic clades within Anatinae were lost in our data, which is consistent with low support for these clades shown by Donne-Goussé *et al.* (2002) and Bulgurella *et al*. (2010). Most phylogenetic analyses involving Anseriformes have not been well resolved despite the large amount of nuclear and mitochondrial genetic data that has now been obtained by various studies (*e.g.*, Donne-Goussé *et al.* 2002; Bulgurella *et al.* 2010). Indeed, low resolution in phylogenetic analyses of avian orders is apparently common and most likely due to their rapid and ancient diversification (*e.g.*, Sorenson *et al.* 2003; Bulgarella *et al.* 2010).

Our results on the phylogenetic affinities of *Hymenolaimus* within the Anatinae provide evidence for convergent evolution among the duck species that inhabit fast flowing rivers (Williams & McKinney 1996). Three species, including *Hymenolaimus*, inhabit such rivers (torrent duck *M. armata* & Salavadori's duck *S. waigiuensis*; Kear 2005). While it is clear that the torrent duck is not closely related to *Hymenolaimus*, it does share morphological (Livezey 1986) and behavioural (Kear 1972) similarities. However, *Hymenolaimus* also shares characters in common with some anatinine ducks (*e.g.*, syrinx structure, head-bobbing, patterning of ducklings & duckling calls), cairininine ducks (*e.g.*, patterning of ducklings), tadorninine ducks (preflight signals) and aythyinine ducks (preflight signals; Kear 1972). In contrast, the knob-billed duck *S. melanotus* is described as a pond duck and shares no phenotypic characteristics that would suggest monophyletic affinities with *Hymenolaimus*, despite its apparent close genetic relationship. Livezey (1986) also noted widespread homoplasy among 120 morphological characters in his thorough phylogenetic analysis of the Anseriformes, which has undoubtedly contributed to the challenge of resolving the taxonomy of the group. Resolving the remaining phylogenetic uncertainty in the Anseriformes, and indeed the phylogenetic placement of *Hymenolaimus*, is going to require more comprehensive sampling among the "*ÎÌÕÖÕÔÌÙÈ*/ *Sarkidiornis* taxa and more powerful application of genomics.

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LITERATURE CITED

Bulgarella, M.; Sorenson, M.D.; Peters, J.L.; Wilson, R.E.; McCracken, K.G. 2010. Phylogenetics relationships of *ÔÈáÖÕÌÙÈ*, *Speculanas*, *+Ö×ÏÖÕÌÙÈ*, and *Tachyeres*: Four morphologically divergent duck genera endemic to South America. *Journal of Avian Biology 41*: 186-199.

Del Hoyo, J.; Elliot, A.; Sargatal, J. 1992. *Handbook of the birds of the world*, *vol. 2. New World vultures to guineafowl.* Barcelona: Lynx Edicions.

Delacour, J.; Mayr, E. 1945. The family Anatidae. *Wilson Bulletin 57*, 4-54.

Donne-Goussé, C.; Laudet, V.; Hänni, C. 2002. A molecular phylogeny of anseriformes based on mitochondrial DNA analysis. *Molecular Phylogenetics and Evolution 23:* 339-356.

Gill, B.J. (Convener) 2010. *Checklist of the Ornithological Society of New Zealand . Checklist of the Birds of New Zealand*, *Norfolk and Macquarie Islands*, *and the Ross Dependency*, *Antarctica.* 4th edition. Wellington: Te Papa Press in association with OSNZ.

Gonzalez, J.; Düttmann, H.; Wink, M. 2009. Phylogenetic relationships based on two mitochondrial genes and hybridization patterns in Anatidae. *Journal of Zoology 279*: 310-318.

Kear, J. 1972. The blue duck of New Zealand. *Living Bird 11*: 175-192.

Kear, J. 2005. *Ducks*, *geese and swans (Volume 1).* Oxford: Oxford University Press.

Kocher, T.D.; Thomas, W.K.; Meyer, A.; Edwards, S. .; Pääbo, S.; Villablanca, F.X.; Ailson, A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences U.S.A. 86*: 6196–6200.

Table 3. Genetic distance for the partial fragment of mitochondrial DNA cytochrome b gene.

	S. mollissima	*C. picta*	*T. tadorna*	*M. angustirostris*	*B. clangula*	*M. nigra*	*C. moschata*	*A. acuta*	*A. sibilatrix*	*L. specularoides*	*C. jubata*	*H. malacorhynchos*	*T. bachypterus*	*T. pteneres*	*A. brasiliensis*	*S. specularis*	*A. c. carolinensis*	*S. melanotos*	*A. sponsa*	*A. scutulata*	*P. hartlaubi*	*C. cyanopterus*	*C. leucoocephalus*	*A. aegyptiacus*	*M. armata*	*C. olor*
C. picta	0.09																									
T. tadorna	0.10	0.09																								
M. angustirostris	0.10	0.10	0.09																							
B. clangula	0.12	0.11	0.10	0.11																						
M. nigra	0.11	0.11	0.12	0.11	0.10																					
C. moschata	0.12	0.11	0.11	0.11	0.11	0.12																				
A. acuta	0.11	0.12	0.11	0.12	0.13	0.12	0.14																			
A. sibilatrix	0.12	0.11	0.11	0.12	0.13	0.12	0.12	0.08																		
L. specularoides	0.10	0.11	0.11	0.10	0.11	0.12	0.11	0.08	0.08																	
C. jubata	0.12	0.10	0.10	0.10	0.10	0.11	0.12	0.10	0.10	0.11																
H. malacorhynchos	**0.09**	**0.08**	**0.09**	**0.10**	**0.11**	**0.11**	**0.10**	**0.12**	**0.10**	**0.10**	**0.09**															
T. bachypterus	0.11	0.12	0.13	0.11	0.13	0.12	0.12	0.10	0.10	0.06	0.12	**0.11**														
T. pteneres	0.11	0.12	0.13	0.11	0.13	0.12	0.12	0.10	0.10	0.06	0.12	**0.11**	0.00													
A. brasiliensis	0.10	0.11	0.10	0.10	0.12	0.11	0.12	0.09	0.09	0.06	0.10	**0.09**	0.07	0.07												
S. specularis	0.10	0.12	0.10	0.09	0.12	0.11	0.12	0.09	0.10	0.05	0.10	**0.10**	0.06	0.06	0.05											
A. c. carolinensis	0.11	0.12	0.12	0.12	0.12	0.12	0.13	0.06	0.07	0.08	0.11	**0.11**	0.09	0.09	0.08	0.08										
S. melanotos	0.10	0.10	0.09	0.10	0.10	0.12	0.11	0.10	0.11	0.10	0.09	**0.08**	0.11	0.11	0.10	0.10	0.11									
A. sponsa	0.11	0.09	0.10	0.09	0.12	0.12	0.11	0.13	0.12	0.11	0.10	**0.09**	0.12	0.12	0.12	0.12	0.13	0.10								
A. scutulata	0.10	0.10	0.09	0.09	0.12	0.12	0.12	0.12	0.11	0.10	0.10	**0.09**	0.11	0.11	0.11	0.10	0.11	0.11	0.10							
P. hartlaubi	0.09	0.10	0.11	0.11	0.12	0.11	0.13	0.11	0.11	0.11	0.11	**0.10**	0.11	0.11	0.10	0.11	0.11	0.10	0.12	0.09						
C. cyanopterus	0.10	0.11	0.09	0.09	0.10	0.11	0.13	0.11	0.11	0.11	0.09	**0.10**	0.11	0.11	0.11	0.10	0.12	0.10	0.11	0.09	0.09					
C. leucoocephalus	0.10	0.09	0.10	0.11	0.11	0.10	0.11	0.11	0.11	0.11	0.10	**0.10**	0.11	0.11	0.09	0.11	0.11	0.10	0.12	0.10	0.11	0.10				
A. aegyptiacus	0.10	0.09	0.07	0.12	0.10	0.12	0.12	0.12	0.11	0.11	0.10	**0.10**	0.12	0.12	0.11	0.11	0.12	0.10	0.11	0.11	0.10	0.10	0.09			
M. armata	0.11	0.11	0.11	0.12	0.11	0.12	0.14	0.12	0.13	0.12	0.11	**0.11**	0.13	0.13	0.13	0.12	0.12	0.12	0.14	0.12	0.11	0.11	0.12	0.11		
C. olor	0.13	0.14	0.11	0.12	0.15	0.14	0.15	0.13	0.14	0.14	0.12	**0.13**	0.14	0.14	0.13	0.12	0.13	0.13	0.12	0.13	0.14	0.14	0.14	0.13	0.14	
N. jubata	0.09	0.06	0.08	0.11	0.10	0.09	0.10	0.12	0.11	0.10	0.09	**0.09**	0.11	0.11	0.10	0.10	0.12	0.11	0.10	0.10	0.10	0.10	0.09	0.08	0.12	0.12

Note: A representative species from several clades in Fig. 2 were selected for presentation here. The species not used were nearly identical to other taxa within the clade.

Hall, T. 1997. *BioEdit v. 5.0.6*. North Carolina : North Carolina State University, Department of Microbiology.

Johnson, K.P.; Sorenson, M.D. 1998. Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome b and ND2) in the dabbling ducks (Tribe: Anatini). *Molecular Phylogenetics and Evolution 10*: 82–94.

Livezey, B.C. 1986. A phylogenetic analysis of recent anseriform genera using morphological characters. *Auk 103*: 737-54.

Livezey, B.C. 1997. A phylogenetic classification of waterfowl (Aves: Anseriformes), including selected fossil species. *Annals of the Carnegie Museum* 66: 457–496.

Marchant, S.M.; Higgins, P.J. 1990. *Handbook of Australian, New Zealand and Antarctic birds. Vol 1*. Oxford: Oxford University Press.

Posada, D.; Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Robertson, B.C.; Steeves, T.E.; McBride, K.P.; Goldstien, S.J.; Williams, M.J.; Gemmell, N.J. 2007. Phylogeography of the New Zealand blue duck (*Hymenolaimus malacorhynchos*): implications for translocation and species recovery. *Conservation Genetics 81*: 1431-1440.

Ronquist, F.; Huelsenbeck, J.P. 2003. MR BAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics 19*: 1572-1574.

Sakamoto, Y.; Ishiguro, M.; Kitagawa, G. 1986. *Akaike Information Criterion Statistics*. Dordrecht: D. Reidel Publishing Company.

Sorenson, M.D.; Ast, J.C.; Dimcheff, D.E.; Yuri, T.; Mindel, D.P. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular Phylogenetics and Evolution 12*: 105–114.

Sorenson, M.D.; Oneal, E.; Garcia-Moreno, J.; Mindell, D.P. 2003. More taxa, more characters: the hoatzin problem is still unresolved. *Molecular Biology and Evolution 20*:, 1484-1498.

Swofford, D.L. 1998. *PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4b10*. Sunderland, Massachusetts: Sinauer Associates.

Thompson, J.D.; Gibson, T.J.; Plewniak, F.; Jeanmougin, F.; Higgins, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research 24*: 4876–4882.

Williams, M.; McKinney, F. 1996. Long-term monogamy in a river specialist – the blue duck. In *Partnerships in Birds. The Study of Monogamy*: (Ed J. M. Black). Oxford: Oxford University Press.