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MOLECULAR EVIDENCE FOR RECENT RADIATION IN SOUTHERN HEMISPHERE MASKED GULLS

Andrew D. Given,[1,2,4] James A. Mills,[3] and Allan J. Baker[1,2]

[1]*Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, Toronto, Ontario M5S 2C6, Canada;*
[2]*Department of Zoology, University of Toronto, Toronto, Ontario M5S 1A1, Canada; and*
[3]*10527A Skyline Drive, Corning, New York 14830, USA*

Abstract.—Masked gulls are believed, on the basis of morphological and recent molecular work, to be a monophyletic group within the Laridae, but relationships of species within the group are not well resolved. We used sequence data from four mitochondrial DNA genes (ND2, ND5, ATPase6, and ATPase 8) totaling >3,600 base pairs to clarify relationships among the species and test competing hypotheses about their origin and biogeography. Monophyly of the masked gulls was confirmed. We also found strong support for a clade including all Southern Hemisphere masked gulls as well as a lone Northern Hemisphere representative, the Black-headed Gull (*Larus ridibundus*). The Australasian taxa form a well-supported clade, in which the Black-billed Gull (*L. bulleri*) is sister to the Red-billed Gull (*L. novaehollandiae scopulinus*) of New Zealand and the Australian Silver Gull (*L. n. novaehollandiae*). Another well-supported clade includes the Black-headed Gull as sister to the South African Hartlaub's Gull (*L. hartlaubii*) and the Gray-hooded Gull (*L. cirrocephalus*) of Africa and South America. The strongly supported position of *L. ridibundus* within the "southern clade" suggests that it originated from a Southern Hemisphere ancestor and recently dispersed into the Northern Hemisphere. Estimates of divergence times using rate-smoothing methods are consistent with those from previous molecular work and suggest that (1) masked gulls diverged from other gulls <2 mya and (2) much of the radiation in the group occurred in the last 600,000 years. *Received 11 August 2003, accepted 23 September 2004.*

Key words: biogeography, *Larus*, masked gulls, mtDNA, phylogenetics.

Preuve moléculaire de la radiation récente chez les mouettes "masquées" de l'hémisphère sud

Résumé.—En se basant sur les travaux morphologiques et moléculaires récents, les mouettes "masquées" sont considérées comme un groupe monophylétique chez les Laridés. Néanmoins, les relations entre les espèces de ce groupe ne sont pas toutes clairement définies. Nous avons utilisé des séquences de données provenant de quatre gènes d'ADN mitochondrial (ND2, ND5, ATPase 6 et ATPase 8) totalisant plus de 3 600 paires de bases pour clarifier les relations parmi les espèces et pour tester les hypothèses concernant leur origine et leur biogéographie. La monophylie des mouettes "masquées" a été confirmée. Nous avons également trouvé des preuves solides allant dans le sens d'un clade qui inclurait toutes les mouettes "masquées" de l'hémisphère sud ainsi qu'un seul représentant de l'hémisphère nord, la Mouette rieuse (*Larus ridibundus*). Les taxons australiens constituent un clade qui reçoit un bon support, au sein duquel la Mouette de Buller (*L. bulleri*) est apparentée avec la Mouette scopuline (*L. novaehollandiae scopulinus*) de Nouvelle-Zélande et la Mouette

[4]E-mail: andrew.given@utoronto.ca

argentée (*L. n. novaehollandiae*) australienne. Un autre clade qui reçoit un bon support inclut la Mouette rieuse comme parente de la Mouette de Hartlaub (*L. hartlaubii*) d'Afrique du Sud et la Mouette à tête grise (*L. cirrocephalus*) d'Afrique et d'Amérique du Sud. La position fortement confortée de *L. ridibundus* dans le "clade du sud" suggère que cette espèce proviendrait d'un ancêtre de l'hémisphère sud qui ce se serait dispersé dans l'hémisphère nord. Les estimations des durées de divergence, par l'utilisation de méthodes "rate-smoothing", confortent celles issues de travaux moléculaires précédents et suggèrent (1) que les mouettes "masqués" divergeaient des autres mouettes de moins de 2 millions d'années et (2) que la majeure partie de la radiation au sein du groupe s'est déroulée au cours des 600,000 dernières années.

Studies of the systematics of gulls have been hindered by a high incidence of hybridization (Pierotti 1987, Grant and Grant 1992, Bell 1996), remarkably similar morphology (Crochet et al. 2002), and relatively low levels of sequence divergence among many taxa (Crochet et al. 2000). Furthermore, observation of unusually slow rates of evolution in certain regions of mitochondrial DNA (mtDNA) of gulls makes systematic studies of recently diverged taxa more difficult (Crochet and Desmarais 2000). Although some studies investigating phylogenetic relationships among gulls have included almost all presently known taxa (e.g. Chu 1998), most have been concerned with placement of major clades and testing of monophyly, rather than with determining species relationships in tip clades.

We concentrated on one of those tip clades, commonly referred to as the "masked gull species group" (Moynihan 1959), named for the dark hood that is common to many taxa within the group. The masked gull species group, comprising 10 species, is among the largest within gulls (Crochet et al. 2000) and also one of the most geographically widespread, with representatives in all continents except Antarctica. Species included in the group are *Larus genei* (Slender-billed Gull), *L. philadelphia* (Bonaparte's Gull), *L. ridibundus* (Black-headed Gull), *L. brunnicephalus* (Brown-headed Gull), *L. maculipennis* (Brown-hooded Gull), *L. serranus* (Andean Gull), *L. cirrocephalus* (Gray-hooded Gull), *L. hartlaubii* (Hartlaub's Gull), *L. novaehollandiae* (Silver Gull), and *L. bulleri* (Black-billed Gull).

Almost all the studies that have included taxa belonging to the masked gull group have been concerned primarily with investigating their relationships to other clades of gulls. Consequently, those studies have sampled only a small subset of the masked species. For example, Crochet et al. (2000) included only 6 of the 10 species belonging to the group (Fig. 1). Johnstone (1982) included *L. novaehollandiae* and the two African taxa, *L. hartlaubii* and *L. cirrocephalus*, in a study of morphological variation (Fig. 1). Incomplete taxon-sampling was exacerbated by uncertainty in species limits. *Larus hartlaubii* was treated as a separate species in several studies (e.g. Johnstone 1982, Chu 1998), but in others it was relegated to a subspecies of *L. novaehollandiae* (e.g. Dwight 1925, Moynihan 1959, Schnell 1970a).

Another common feature of previous systematic work (e.g. Moynihan 1959, Chu 1998, Crochet et al. 2000) is that, beyond establishing the monophyly of the group, there has been only poor resolution of species relationships (Fig. 1). However, two studies using morphological characters (Dwight 1925; Schnell 1970a, b) grouped a number of other taxa with the masked species (Fig. 1). Both placed *L. pipixcan* (Franklin's Gull), *L. saundersi* (Saunders's Gull), and *L. melanocephalus* (Mediterranean Gull) with the masked species; but Dwight (1925) also included *L. atricilla* (Laughing Gull) and *L. minutus* (Little Gull) in a clade made up predominantly of masked species. All five taxa have a mask or hood and are most likely examples of convergent evolution; Crochet et al. (2000) placed some of them within the "hooded species group" (*L. pipixcan*, *L. atricilla*) and the "black-headed species group" (*L. melanocephalus*), separate from the masked gulls.

Only two studies used phylogenetic methods to reconstruct the evolutionary history of gulls. Using osteological and integumentary characters, Chu (1998) found only weak support for many of the inferred clades in the shortest tree, but the monophyly of the masked gulls was recovered. Crochet et al. (2000) used 935 base pairs (bp) of mtDNA (cytochrome-*b* and domain

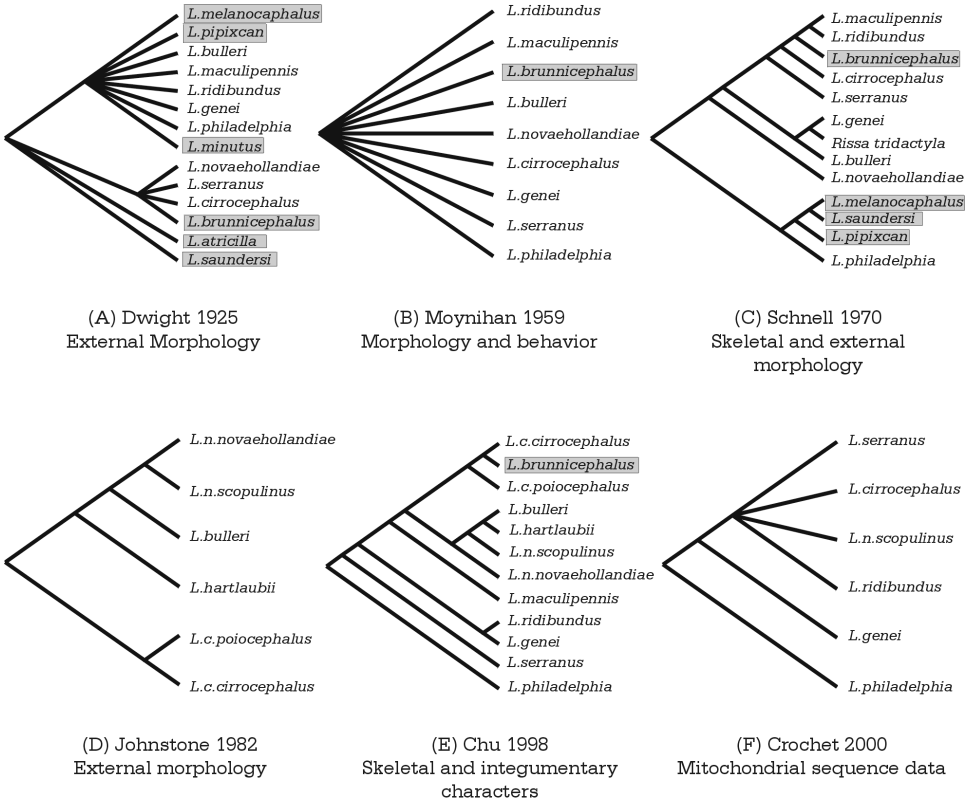

FIG. 1. Trees showing various attempts to resolve relationships within the masked species group: (A) tree from classification of Dwight (1925), (B) clustering of masked gulls from Moynihan (1959), (C) tree from Schnell's (1970a, b) phenetic study of gulls and related groups, (D) relationships among Silver Gulls and related taxa from Johnstone (1982), (E) masked gull relationships in Chu's (1998) shortest tree in a cladistic analysis of skeletal and integumentary characters, and (F) relationships among masked gulls from phylogenetic analysis of mtDNA sequence data (Crochet et al. 2000). Shaded taxa were not sampled in the present study.

II and III of control region) to estimate phylogenetic relationships among 32 representative gull taxa. Despite a lack of resolution for deep nodes within the gulls, several major clades were strongly supported, including the masked species group. The two studies agree in the basal position of *L. philadelphia* within the masked gull clade (Fig. 1), but not in the placement of *L. ridibundus*. Chu (1998) placed it as sister to *L. genei,* whereas Crochet et al. (2000) placed it in a clade with three Southern Hemisphere taxa (Fig. 1).

We attempted to resolve relationships among the masked species group, including 9 of the 10 species regarded as members of the group. Use of large sequence data sets has proved more likely to recover the "correct" trees in phylogenetic studies (e.g. Charleston et al. 1994, Hillis et al. 1994, Cummings et al. 1995, Mindell and Thacker 1996, Russo et al. 1996, Naylor and Brown 1997, Rosenberg and Kumar 2001, Paton et al. 2002). Accordingly, and because of the expected recency of the radiation of this extremely similar group of species, we used sequences from four of the faster-evolving mtDNA genes, totaling >3,600 bp, to reconstruct a phylogeny and estimate divergence dates.

METHODS

Taxon sampling.—We sampled 20 individuals representing 9 species of masked gulls and 2 outgroups, *Sterna eurygnatha* (Cayenne Tern) and *Rissa tridactyla* (Black-legged Kittiwake) (Table 1). Unfortunately, we were not able to

TABLE 1. Scientific names, common names, summer distribution, and collection details of masked gull and outgroup taxa used in the present study (ROM = Royal Ontario Museum, ZM = Copenhagen Zoological Museum).

Scientific name	Common name	Distribution (summer)	Collection number(s)	Locality	Source
Sterna eurygnatha	Cayenne Tern	South America	G12325	Salinas, Brazil	ROM
Rissa tridactyla	Black-legged Kittiwake	Northern Hemisphere	AJB 5531	Avalon Peninsula, Canada	ROM
Larus philadelphia	Bonaparte's Gull	Sub-Arctic northwestern North America	DL413, DL414	Yellowknife, Northwest Territories, Canada	ROM
L. genei	Slender-billed Gull	Mediterranean region	C575-112849	Bahrain	ZM
L. maculipennis	Brown-hooded Gull	Southern South America	N18802	Lagoa do Peixe, Brazil	ROM
			MKP 2440	Punta Arenas, Chile	ROM
L. serranus	Andean Gull	High Andean lakes	C582-112856	Ibarra, Ecuador	ZM
L. ridibundus	Black-headed Gull	Temperate Palearctic	LR 1, LR 3	Tromsø, Norway	ROM
L. cirrocephalus cirrocephalus	Gray-hooded Gull	Warm temperate South America	C373-112647	Guayas, Ecuador	ZM
L. c. poiocephalus	Gray-hooded Gull	Warm temperate Africa	MKP 1477	Durban, South Africa	ROM
L. hartlaubii	Hartlaub's Gull	Southern Africa	MKP 1311, MKP 1313	Muizenburg, South Africa	ROM
L. novaehollandiae novaehollandiae	Silver Gull	Australia	AJB 5612	Perth, Australia	ROM
			JAM 084	Hobart, Australia	ROM
L. n. scopulinus	Red-billed Gull	New Zealand and sub-Antarctic islands	JAM 155	Rotorua, New Zealand	ROM
			R 16	Campbell Island, sub-Antarctic	ROM
L. bulleri	Black-billed Gull	New Zealand	JAM 259, JAM 262	Lochiel, New Zealand	ROM

obtain samples of *L. brunnicephalus.* Where possible, two individuals per taxon were sequenced to confirm the accuracy of the sequences and provide additional support for the phylogenetic relationships obtained. *Rissa tridactyla* was chosen as an outgroup to the masked gulls because it is a representative of the Laridae (Table 1). *Sterna eurygnatha* was used as a more distant outgroup representing the terns (Sternidae), the closest relatives of the Laridae (Sibley and Ahlquist 1990). All tissue samples other than those for *L. genei*, *L. serranus*, and *L. c. cirrocephalus* were obtained from the Royal Ontario Museum tissue collection (Table 1) and were collected from breeding adults between 1987 and 1999. The others were generously provided by the Zoological Museum, University of Copenhagen.

DNA extraction, amplification, and sequencing.—Total genomic DNA was extracted from blood or tissue samples with standard phenol-chloroform extraction techniques (Sambrook et al. 1989). We used primers COIIIRH and LysL to amplify a 926-bp fragment that included the mitochondrial ATPase 6 and 8 genes, and primers MetL2 and AsnH to amplify the 1,012-bp mitochondrial ND2 gene (Table 2). ND5 was amplified in two overlapping fragments, the first using primers LevL and H14205 and the second L14105 and H14910 (Table 2). All primers had M13 fluorescent tails for automated sequencing. Amplification reaction volumes of 25 μL contained 100–200 ng of DNA, 2.5 μL of 10×EH buffer (Hagelberg 1994), 1.0 unit of Taq DNA polymerase (Qiagen, Valencia, California), 10 mM dNTPs, and 5.0 pmoles of each tailed primer. Amplification began with 4 min denaturation at 94°C, followed by 36 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 1 min 30 s, with a final extension of 72°C for 4 min (Perkin Elmer 480 thermal cycler). Amplification products were run on agarose gels to confirm successful amplification, and the appropriate bands were cut out and spun through filter tips to obtain the amplification product (Dean and Greenwald 1995). Sequencing reactions were carried out using the DYEnamic Thermo Sequenase direct-cycle sequencing kit (Amersham-Pharmacia, Upsala, Sweden), and sequencing reactions were run on an LI-COR 4200 bidirectional automated sequencer to simultaneously obtain complementary sequences from both strands. Sequences were edited and aligned using SEQUENCHER, version 4.1 (Gene Codes, Ann Arbor, Michigan).

Sequence analysis.—The program DNASP, version 3 (Rozas and Rozas 1999) was used to calculate the number of variable sites in the sequences, and haplotype and nucleotide diversity. Pairwise genetic distances were calculated using MEGA, version 2.1 (Kumar et al. 2001). The most appropriate model of evolution for the sequence data set was determined using the Akaike Information Criterion (AIC) in MODELTEST, version 3.06 (Posada and Crandell 1998), because that criterion penalizes for over-parameterization of models. The parameters were then input to PAUP*, version 4.0b8 (Swofford 1999) to construct a maximum-likelihood tree. The heuristic search algorithm with 100 replicates was implemented to determine bootstrap support at the nodes of the tree.

Estimating divergence times.—Divergence times for taxa within the masked gull clade were estimated in R8S, version 1.50 using penalized-likelihood rate-smoothing (Sanderson 2002). To calibrate the tree, we used an estimate of 3.3 mya for the split between *Rissa* and *Larus* obtained from a phylogeny of the Charadriiformes

TABLE 2. Sequences of PCR and sequencing primers for mtDNA genes used to investigate phylogenetic relationships among masked gull species.

Primer name	Primer sequence (5′ to 3′) [a]	Target gene
COIIIRH	GTATCGTAGGCCTTTTTGGAC	ATPase 6 and 8
LysL	AGCCTTTTAAGCTAGAGA	ATPase 6 and 8
AsnH	GGGATCGAGGCCCATCTGTCTA	ND2
MetL2	TAAGCTATCGGGCCCATACCCC	ND2
H14205	GGAATGGRGTNCCTATTAGGGC	ND5
LevL	GGARCCANYNAYCYYGGTGCAANTCCA	ND5
H14910	AGTAGNGGGTGGGATTTTCG	ND5
L14105	GCCTTCTCYACATCNAGYCAACTWGGNYTMAT	ND5

[a] All primers are from O. Haddrath (pers. comm.).

(Paton et al. 2003). We felt justified in using that date because divergence times for each node in the charadriiform phylogeny were estimated using a penalized-likelihood rate-smoothing approach with nodes constrained using three independent fossil dates. One hundred nonparametric data sets were generated from the original sequence data set in PHYLIP, version 3.5c (Felsenstein 1993). Trees were constructed from each replicate data set using the appropriate model of substitution, and age of each node for each tree was calculated. Both 90% and 95% confidence intervals (CIs) were then determined for the various nodes, using the estimates obtained from the replicate data sets (Sanderson and Doyle 2001).

Results

Sequence analysis.—A total of 3,661 bp were sequenced for 20 taxa (including outgroups), of which 926 bp were from ATPase 6 and 8, 1,014 bp from ND2, and 1,721 bp from ND5 (GenBank accession numbers AY584112–AY584131, AY590388–AY590427). Of the 3,661 sites, 777 were variable, and each individual had a unique sequence when the four genes were concatenated. For the masked gulls alone, 338 variable sites were observed, with a nucleotide diversity of 0.022 (compared with 0.038 for the total data set).

Uncorrected pairwise genetic distances ranged from 0.03% (between the two *L. ridibundus* individuals) to 14.59% (between *S. eurygnatha* and both *L. serranus* and *L. genei*). Average distances between masked gulls and the two outgroups were 14.46% (vs. *S. eurygnatha*) and 6.41% (vs. *R. tridactyla*). Distances among the masked gull species ranged from 0.03% to 4.81% (*L. genei* vs. *L. ridibundus*), and average distance between taxa was 2.17%.

Phylogenetic analysis.—Maximum-likelihood analysis was conducted using a *TIM + I + G* model of substitution, where *I* = 0.68 and *G* = 5.15, selected with the AIC. To show the short branch lengths (as compared with the branch to *Sterna*) within the masked gulls, *S. eurygnatha* was removed from the maximum-likelihood tree for display purposes. Monophyly of the masked species was strongly supported by bootstrapping (97%) (Fig. 2), as was the grouping of *Rissa* with the *Larus* species (100%).

Two clades were detected within the masked gulls. The first (100% bootstrap support) included species that are almost exclusively from the Southern Hemisphere (*L. serranus*, *L. cirrocephalus*, *L. hartlaubii*, *L. bulleri*, *L. novaehollandiae*, and *L. maculipennis*) along with the Northern Hemisphere species *L. ridibundus*. The second contained the remaining two Northern Hemisphere species, *L. genei* and *L. philadelphia* (54% bootstrap support).

Within the "southern clade," two subclades were strongly supported (Fig. 2). The first comprised the African taxa (*L. c. poiocephalus* and *L. hartlaubii*), *L. c. cirrocephalus* from South America, and *L. ridibundus,* with the latter sister to the rest. The other subclade consisted of the three Australasian taxa; *L. bulleri* was placed as a sister species to the Australian and New Zealand subspecies of *L. novaehollandiae*. Basal relationships within the southern clade were not well resolved, with only weak support for the basal position of *L. maculipennis*.

Divergence estimates.—Estimates of divergence times for the masked gulls suggested that a relatively recent radiation gave rise to much of the diversity within the group (Fig. 3 and Table 3). The Northern Hemisphere species (*L. philadelphia* and *L. genei*) and the southern clade diverged ~2 mya (estimate = 1.85 mya; 90% CI = 1.49, 2.89 mya), with the split between *L. philadelphia* and *L. genei* occurring soon after (estimate = 1.68 mya; 90% CI = 1.34, 2.84 mya). The nine taxa of the southern clade were estimated to have diverged from a common ancestor ~0.5 mya (estimate = 0.55 mya; 90% CI = 0.44, 1.13 mya).

Within the Australasian group, *L. bulleri* and *L. novaehollandiae* were estimated to have shared a common ancestor ~240,000 years ago (90% CI = 0.19, 0.62 mya), and the two subspecies of *L. novaehollandiae* diverged 130,000 years ago (90% CI = 0.10, 0.49 mya). The split between South African *L. c. poiocephalus* and *L. hartlaubii* appeared to be very recent (estimate = 70,000 years ago; 90% CI = 0.03, 0.16 mya) and they, in turn, diverged from the South American nominate subspecies *L. c. cirrocephalus* ~160,000 years ago (90% CI = 0.10, 0.28 mya).

Discussion

Comparison of results with previous hypotheses.—Although our analysis is the first comprehensive attempt to investigate phylogenetic relationships among the species of masked

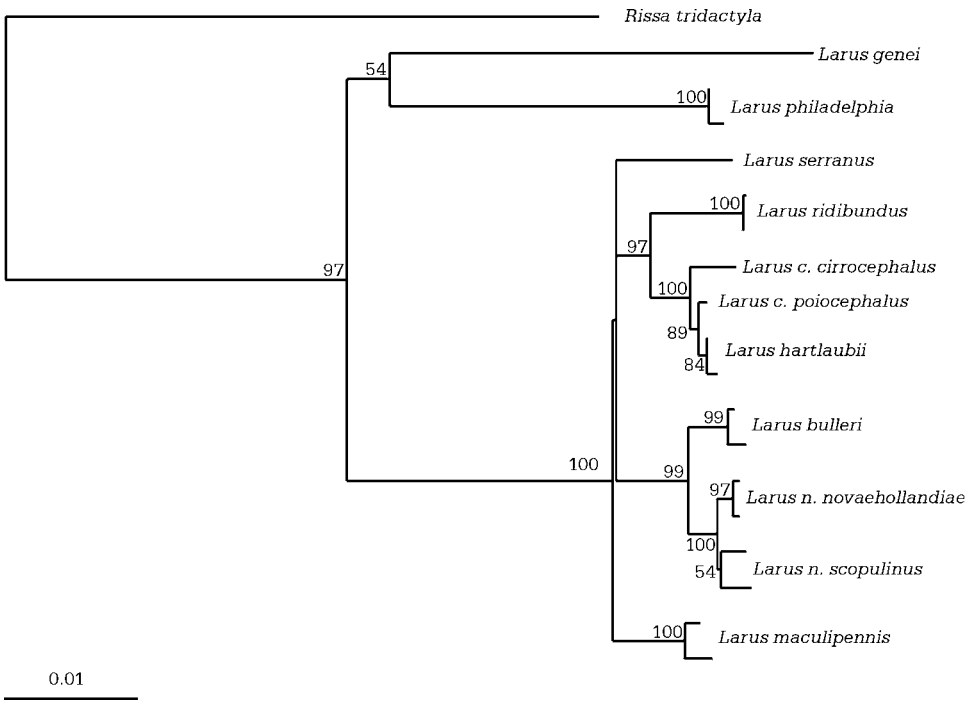

FIG. 2. Maximum-likelihood tree based on 3,661 bp of mtDNA from masked gull and outgroup taxa. Analysis was conducted using a *TIM + I + G* model of substitution with bootstrap support determined using the heuristic search algorithm with 100 replicates. Bootstrap values >50% are shown. For display purposes, the outgroup *Sterna eurygnatha* was removed from the tree to show the short branch lengths (as compared with the branch to *Sterna*) within the masked gulls.

gulls, there have been a number of other hypotheses put forward regarding classification and species relationships within the group. The hypothesis of Crochet et al. (2000) that *L. novaehollandiae*, *L. hartlaubii*, *L. bulleri*, and *L. maculipennis* belong in the masked species group is corroborated. *Larus brunnicephalus* was not sampled in the present study, but is most likely part of the masked species group, as proposed by Moynihan (1959) and Cramp and Simmons (1983). Our results agree with Crochet et al. (2000) in the placement of the two Northern Hemisphere taxa (*L. genei* and *L. philadelphia*) in the basal position with respect to the rest of the masked gulls. Using independent morphological characters, both Schnell (1970b) and Chu (1998) also placed *L. philadelphia* basal in the masked gull clade, providing further support for this arrangement.

We found strong support for a "southern clade" made up of the remaining taxa, including only one Northern Hemisphere representative, *L. ridibundus* (*L. c. poiocephalus* also breeds north of the equator in South America, but is largely a Southern Hemisphere species). Crochet et al. (2000) also found a strongly supported internal clade within the masked gulls, made up of four southern-clade taxa. Because of their smaller mtDNA sequence data set (935 bp) and use of relatively slowly evolving mitochondrial genes, Crochet et al. (2000) were uanble to resolve the relationships among those four taxa. Using a larger sequence data set, as well as increased sampling of taxa, we were able to provide greater resolution within that clade.

One of the most surprising results was the position of *L. ridibundus*, the only Northern Hemisphere species placed within the southern clade. The morphological similarity of *L. ridibundus* to a number of other taxa within the masked gull group has led to its being grouped with species such as *L. maculipennis*, *L. philadelphia*, and *L. genei* (e.g. Dwight 1925, Chu 1998). The sequence data revealed that it is instead most closely related to *L. cirrocephalus* and *L. hartlaubii*, which together form a strongly supported clade.

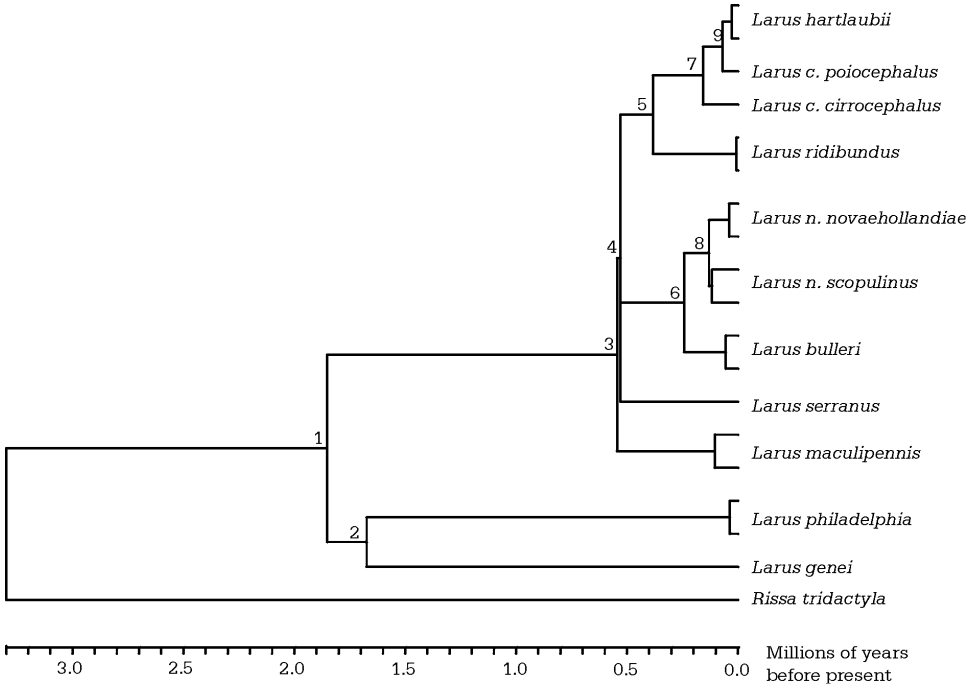

FIG. 3. Chronogram showing divergence times of masked gull and outgroup taxa computed in the program R8S for 3,661 bp of mtDNA. Branch lengths are proportional to time, with a time scale shown below the tree. Numbers at nodes are the same as those used in Table 3.

TABLE 3. Divergence times (millions of years before present) among masked gull species, estimated using penalized-likelihood rate-smoothing in the program R8S. For each point estimate, 90% and 95% confidence intervals (CIs) are also shown. Node numbers are the same as in Figure 3.

Node number	Node	Estimate (mya)	90% CI	95% CI
1	(*L. philadelphia*, *L. genei*) (other masked gulls)	1.85	1.49–2.89	1.33–3.30
2	(*L. philadelphia*) (*L. genei*)	1.68	1.34–2.84	1.23–3.20
3	(*L. maculipennis*) (Southern masked gulls)	0.55	0.44–1.13	0.41–1.39
4	(*L. serranus*) (*L. ridibundus* group) (Australasian taxa)	0.53	0.43–1.13	0.39–1.73
5	(*L. ridibundus*) (*L.cirrocephalus*, *L. hartlaubii*)	0.38	0.30–0.70	0.27–1.02
6	(*L. bulleri*) (*L. novaehollandiae*)	0.24	0.19–0.62	0.18–0.82
7	(*L. c. cirrocephalus*) (southern African taxa)	0.16	0.10–0.28	0.09–0.42
8	(*L. n. novaehollandiae*) (*L. n. scopulinus*)	0.13	0.10–0.49	0.09–0.63
9	(*L. c. poiocephalus*) (*L. hartlaubii*)	0.07	0.03–0.16	0.02–0.25

Monophyly of the Australasian masked gulls *L. n. scopulinus*, *L. n. novaehollandiae*, and *L. bulleri* is strongly supported, but the relationships among the taxa are inconsistent with most previous hypotheses. Dwight (1925) placed *L. bulleri* and *L. novaehollandiae* in separate subgenera on the basis of their morphological differences. Moynihan (1959) recognized that the two species were probably closely related, but no more so than some of the other masked gulls. Only in Johnstone's (1982) study of external morphology was *L. bulleri* placed as sister to *L. novaehollandiae*. However, the *L. cirrocephalus* species group as described by Johnstone (1982) is not a natural group. He included *L. novaehollandiae* from Australia and New Zealand, *L. bulleri* from New Zealand, *L. hartlaubii* from South Africa, and *L. cirrocephalus* from South America

and Africa in a species complex. Whereas the Australasian taxa form a strongly supported clade, our phylogenetic analysis shows that *L. hartlaubii* and *L. cirrocephalus* are more closely related to the Northern Hemisphere *L. ridibundus*, rather than to the Australasian gulls. Two studies have included both *L. n. novaehollandiae* and *L. n. scopulinus* (Johnstone 1982, Chu 1998), but only Johnstone (1982) recovered the sister relationship of those two subspecies.

Classification of taxa.—Within the masked gulls, there has been disagreement on the most appropriate classification of some taxa. In particular, treatment of the South African Hartlaub's Gull has been problematic, resulting in the use of various classifications, including *L. n. hartlaubii* (Dwight 1925, Sibley and Monroe 1990) and *L. hartlaubii* (Johnstone 1982). The sequence data show that inclusion of Hartlaub's Gull within *L. novaehollandiae* is not valid, because it is clearly most closely related to *L. ridibundus* and *L. cirrocephalus*; therefore, full species status as *L. hartlaubii* is warranted. Although *L. hartlaubii* and *L. novaehollandiae* are similar morphologically, in several characters *L. hartlaubii* is more like *L. cirrocephalus*, particularly in terms of plumage patterns around the head (Johnstone 1982). Interbreeding between *L. c. poiocephalus* and *L. hartlaubii* has been observed in South Africa (Sinclair 1977) and may, in part, explain the grouping of *L. c. poiocephalus* with *L. hartlaubii* rather than with the conspecific *L. c. cirrocephalus* from South America. Hybridization between the two species has possibly led to extensive mixing of their gene pools in South Africa. Sequencing of a larger number of *L. c. poiocephalus* and *L. hartlaubii* individuals supports that hypothesis (A. D. Given unpubl. data), with a number of haplotypes shared between the two species.

Recently diverged taxon pairs, such as *L. hartlaubii–L. cirrocephalus* and *L. n. novaehollandiae–L. n. scopulinus,* have evolved distinctive morphological differences despite occasional hybridization events. The latter pair are isolated in Australia and New Zealand, respectively, but the two forms differ in size (especially in tarsus length) and in wing-feather markings. However, because they otherwise look so similar and in the breeding season have striking red color to their external soft parts, they are treated merely as subspecies of *L. novaehollandiae*. Given that they clearly have independent evolutionary histories and thus qualify as phylogenetic species, we recommend formally raising each to full species status as *L. novaehollandiae* (Silver Gull of Australia and New Caledonia) and *L. scopulinus* (Red-billed Gull of New Zealand).

Biogeography of masked gulls.—The basal position of the Northern Hemisphere clade containing *L. genei* and *L. philadelphia* is consistent with a Palearctic origin of the masked gull group (as suggested by Crochet et al. 2000). However, the present-day concentration of southern-clade masked gulls, including basal species, in South America indicates that the clade may have originated in that region. We hypothesize, therefore, that current diversity in the southern clade is the result of speciation within South America (giving rise to *L. maculipennis*, *L. serranus*, and *L. cirrocephalus*), as well as dispersal to and radiation in Africa (leading to the *L. ridibundus–L. c. poiocephalus–L. hartlaubii* group) and Australasia (giving rise to *L. novaehollandiae* and *L. bulleri*) (Fig. 4). The southern clade includes seven species, only one of which is now restricted to the Northern Hemisphere. The strongly supported position of *L. ridibundus* within the southern clade suggests that it originated from a Southern Hemisphere ancestor and recently dispersed into the Northern Hemisphere.

To explain the evolution of the gulls in the "*L. cirrocephalus* species complex," Johnstone (1982) hypothesized the following steps: (1) ancestral *L. cirrocephalus* from South America invaded Africa and speciated into *L. hartlaubii*; (2) that new stock then invaded Australia, where it speciated into *L. novaehollandiae* in Australia and *L. bulleri* in New Zealand; (3) a second invasion into New Zealand occurred, this time of Australian *L. novaehollandiae,* which gave rise to *L. n. scopulinus*; and finally, (4) South American *L. cirrocephalus* reinvaded Africa again and evolved into *L. c. poiocephalus*. Although Johnstone's (1982) grouping of the African and Australasian taxa together is not supported, it is possible that his hypothesis is correct in regard to the radiation of the South African gulls. A very recent re-invasion of South American *L. cirrocephalus* into Africa (after an earlier invasion gave rise to *L. hartlaubii*) could account for extant *L. c. poiocephalus*, with subsequent hybridization of the two African taxa resulting in the present-day genetic similarity of *L. c. poiocephalus* and *L. hartlaubii*.

Contrary to previous suggestions that *L. bulleri* represents a Holarctic element (Falla

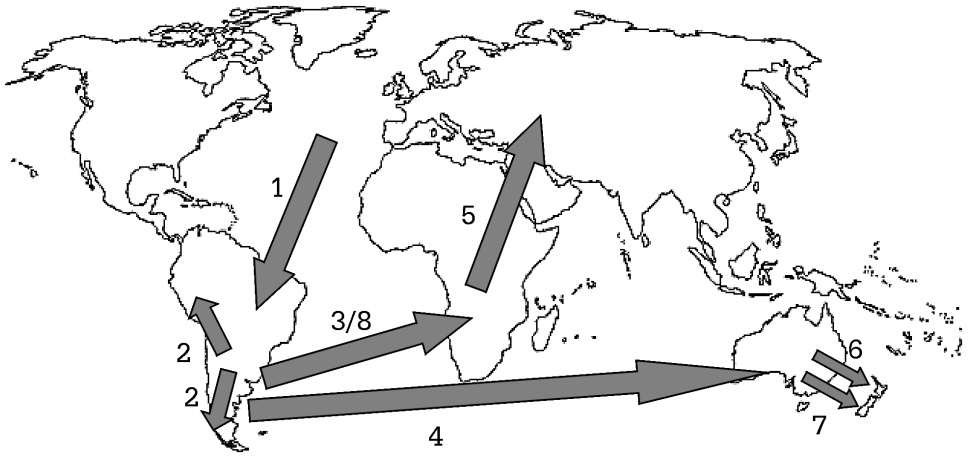

FIG. 4. Hypothesis for the evolution of the southern clade of masked gulls. (1) Invasion of South America by a Northern Hemisphere ancestor. (2) Radiation within South America, giving rise to current forms (e.g. *L. serranus*, *L. maculipennis*, *L. cirrocephalus*). Invasion of (3) Africa and (4) Australasia, giving rise to the *L. cirrocephalus–L.hartlaubii–L. ridibundus* clade and the Australasian clade. (5) Possible re-invasion of the Northern Hemisphere from Africa, giving rise to *L. ridibundus*. (6) Invasion from Australia of Silver Gull ancestor, giving rise to *L. bulleri*, and (7) a second later invasion, giving rise to *L. n. scopulinus*. (8) A second invasion of Africa, this time by *L. c. cirrocephalus*, giving rise to *L. c. poiocephalus*.

1953; Fleming 1962a, b), the DNA sequences clearly show that it is the sister species to the *L. novaehollandiae* gulls (*scopulinus* in New Zealand and *novaehollandiae* in Australia), with the three taxa sharing a very recent common ancestor. Although the evolutionary history of the Australasian group cannot be determined conclusively from the data presented here, there is little evidence to support Tinbergen's (1963) suggestion that the *L. novaehollandiae* gulls arose through dispersal of the Northern Hemisphere *L. ridibundus* into the Southern Hemisphere. Our results are instead consistent with a double invasion into New Zealand from an Australian ancestor, with the first invasion giving rise to *L. bulleri* and a more recent invasion leading to *L. n. scopulinus* (as suggested by Johnstone 1982). Other examples of double invasions of Australian species into New Zealand have been illustrated previously (Baker 1990), with hybridization relatively common between the resulting taxa, as appears to be the case with *L. bulleri* and *L. n. scopulinus* (Gurr 1967).

Evolution of the mask.—Presence of a dark hood or mask is well established as the ancestral state in gulls (Chu 1998, Crochet et al. 2000). Crochet et al. (2000) showed evidence of repeated modification of head coloration among gulls and suggested that the state of the mask was of little use in determining species relationships. Previous efforts to reconstruct the phylogeny of gulls placed significant weight on presence or absence of the mask (e.g. Dwight 1925, Moynihan 1959), no doubt contributing to some of the conflict in the early classification of gulls. The phylogenetic relationships revealed by the present study illustrate why the use of the mask as a character in determining relationships is confusing. *Larus ridibundus*, which possesses a dark mask, has been grouped previously with other masked taxa (e.g. *L. maculipennis*; Schnell 1970b), but is clearly most closely related to *L. cirrocephalus* and *L. hartlaubii*, which have only a faint or pale mask. Within the masked species group, the mask has been retained in some species, has been lost in all three Australasian taxa and *L. genei*, is pale gray in *L. cirrocephalus*, and is faint in *L. hartlaubii* (Johnstone 1982). Judging from our phylogeny, and assuming that the presence of the hood is the ancestral state, the mask has been lost at least twice in the masked gulls and may be in the process of being lost in the *L. cirrocephalus–L. hartlaubii* group.

Divergence time estimates and speciation of masked gulls.—Although the estimates of divergence times for the various taxa should be treated with caution, as indicated by the relatively wide CIs associated with the dates, they illustrate clearly that speciation within the masked group has occurred very recently. Seven of the nine species sampled within the group appear to share a common ancestor <600,000 years ago, a result consistent with the suggestion of Crochet et al. (2000) that most extant gull species originated within their species group during the last million years. However, the deepest divergence dates within the masked group date back to ~2.0 mya, which suggests that the split between Northern and Southern Hemisphere masked gulls (with the exception of *L. ridibundus*) may be relatively deep, with radiation within the Southern Hemisphere a much more recent event.

There have been few attempts to estimate divergence dates for species of gulls using molecular data. Our approach is the first to use a rate-smoothing method in estimating divergence dates, and the results are similar to those obtained using alternative methods in closely related gull species. The *Rissa–Larus* divergence time of 3.3 mya (Paton et al. 2003) fits in well with the estimate of 2.6–5.9 mya for the first split within Larini (Crochet et al. 2000) and, therefore, seems appropriate as a calibration point in this study.

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