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The evolution of echolocation in swiftlets

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The abilities of some cave-nesting swiftlets to echolocate has traditionally been used to separate the genus *Aerodramus*, which includes echolocating species, from the genus *Collocalia*, thought to lack echolocation. Here we report the discovery of echolocation in a member of the latter genus, the pygmy swiftlet *Collocalia troglodytes*. We also present a well-supported molecular phylogeny for the swiftlets and their relatives based on DNA sequence data from two mitochondrial genes, which we use to reconstruct the evolution of echolocation. Our data provide strong evidence that the swiftlets are a monophyletic group. This monophyly plus the presence of echolocation in *C. troglodytes* indicate that either (1) echolocation evolved much earlier in the swiftlets than previously thought and has since been lost in most *Collocalia* taxa, or (2) this ability evolved independently in *Aerodramus* and *Collocalia*. Based on our results, echolocation can no longer be considered a useful character for distinguishing these two genera.

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An ability to orient by means of echolocation has evolved in only two groups of birds: the Neotropical oilbird (Steatornithidae) and members of the Paleotropical swiftlets (Apodidae). Both nest in caves and produce clearly audible clicks while flying in dim light or complete darkness (Griffin 1958, Medway 1959). In swiftlets, studies show that individuals use the echoes of these clicks primarily for avoiding obstacles during flight rather than for capturing insect prey as do insectivorous bats (Medway 1962, 1967, Cranbrook and Medway 1965, Griffin and Suthers 1970, Fenton 1975, Griffin and Thompson 1982, Collins and Murphy 1994). Echolocation appears to provide a unique advantage for these birds by permitting them to roost and nest in the dark recesses of caves, free from visually orienting predators or competitors (Fenton 1975, Medway and Pye 1977).

Unlike the oilbird, in which orientation sounds are single discrete pulses (Griffin 1954, Konishi and Knudsen 1979), most echolocating swiftlets emit double clicks during echolocation, each of which consists of two broadband pulses separated by a short pause (Griffin and Suthers 1970, Medway and Pye 1977, Suthers and Hector 1982). The second click of the doublet is typically louder than the first, with acoustic energy primarily between 2 and 8 kHz, and the intraclick pause can vary somewhat within taxa and even within individuals (Suthers and Hector 1982). Only two swiftlet species are known to produce single clicks rather than doublets: the black-nest swiftlet (*Aerodramus maximus*; Medway 1959, Medway and Pye 1977) and the Atiu swiftlet (*A. sawtelli*; Fullard et al. 1993). Whether these single clicks represent an ancestral condition during the evolution of echolocation or a more recent specialization in these species has not been investigated previously.

Swiftlet taxa are well known for their remarkable lack of distinguishing morphological characteristics (Chantler and Driessens 1995, Chantler et al. 2000). Characteristics of their nests also provide little information about phylogeny (Lee et al. 1996). In contrast, the presence of echolocation is considered a relatively informative character and has been used to delineate

the genus *Aerodramus*, which includes echolocating species, from other swiftlet taxa in the genera *Collocalia* and *Hydrochous* (Brooke 1972, Medway and Pye 1977). This scheme largely depends on echolocation first appearing in the immediate ancestors of the *Aerodramus* clade, however, only two of the three species currently recognized as *Collocalia* have been shown to lack this ability (Cranbrook and Medway 1965, Medway 1967, Fenton, 1975). The presence or absence of echolocation in the third species, *Collocalia troglodytes*, has been considered an open question (Chantler and Driessens 1995, Chantler et al. 2000), yet it is crucial for understanding how echolocation evolved in this avian group (Medway and Pye 1977).

Here we report for the first time the presence of echolocation in *C. troglodytes*, the pygmy swiftlet. Recordings of echolocation clicks, as well as tissue for molecular analyses, were obtained from a *C. troglodytes* representative collected directly off its nest in total darkness 30 meters inside a cave (Fig. 1). A previous molecular analysis of the swiftlets by Lee et al. (1996) using partial cytochrome *b* (cyt *b*) sequences suggested that the genera *Collocalia* and *Aerodramus* are not sister clades and, accordingly, that *C. troglodytes* is relatively distantly related to echolocating *Aerodramus* taxa. Given this proposed phylogeny, our finding would suggest that echolocation evolved independently in the two clades, as this ability is unlikely to have arisen once and then been lost repeatedly (Medway and Pye 1977). However, the evidence presented by Lee et al. (1996) against swiftlet monophyly was not strong.

In this study, we construct a molecular phylogeny for the swiftlets using sequence data from two mitochondrial genes, cyt *b* and NADH dehydrogenase subunit 2 (ND2), and use this tree to reconstruct the evolution of echolocation using our new data concerning this ability in the genus *Collocalia*. Our principal objectives are to: (1) test the monophyly of swiftlets, and in so doing confirm the phylogenetic position of *C. troglodytes* relative to the genus *Aerodramus*, and (2) investigate the origin of echolocation and changes in the acoustic structure of orientation sounds (e.g., single clicks versus double clicks) during swiftlet evolution.

Fig. 1. Voucher nest of a pygmy swiftlet *Collocalia troglodytes* located in total darkness approximately 30 meters inside the entrance of Balete cave on Samal Island, Philippines. The nest, which was anchored to a vertical wall with copious amounts of saliva cement, was composed of fine vegetable fibers held together with saliva. It contained two eggs being incubated by a bird removed directly from the nest for positive identification. To our knowledge, this is the first illustration of the nest of the species. Illustration by Sarah Bush.

Methods

Sequencing

DNA was extracted from muscle or blood tissue from 60 individuals representing 38 species and subspecies of swifts and swiftlets (Table 1). Taxonomy follows Chantler et al. (2000). A treeswift, *Hemiprocne comata*, was used as an outgroup to root the tree. We used PCR to amplify a portion of the cyt-*b* gene and all of the ND2 gene using the primers L14841 (Kocher et al. 1989) and H4a (Harshman 1996) for cyt *b* and L5215 (Hackett 1996) and H6313 (Johnson and Sorenson 1998) for ND2. Reaction conditions followed Johnson and Clayton (2000). Tissue from *Hydrochous gigas*, the waterfall swiftlet, was obtained from the study of Lee et al. (1996), but we were unable to obtain any PCR amplification from this sample.

Direct sequencing of PCR products was performed for each gene using the PCR primers and the internal primers H15299 (Kocher et al. 1989) and L15517 (Johnson and Sorenson 1998) for cyt *b* and L5758s (5′-GGY TGA ATR GGA CTW AAC CAR AC-3′) and H5766s (5′-GAT GAG AAG GCY AGG ATT TTT CG-3′) for ND2. Sequencing was performed as described by Johnson and Clayton (2000). We reconciled complementary strands and aligned sequences across species using Sequencher (GeneCodes, Madison, Wisconsin). Portions of the flanking tRNA genes were included in the alignment, but indels were infrequent making alignment straightforward. We found no evidence for nuclear copies, based on the lack of indels, stop codons, or multiple peaks in our sequencing analyses (Sorenson and

Table 1. Tissue samples included in the phylogenetic analysis.

Taxon*	Voucher Information†	Geographic Location
Aerodramus salangana natunae	DMT002	Gomantong caves, Sabah, Malaysia
Aerodramus salangana natunae	DMT047	Gomantong caves, Sabah, Malaysia
Aerodramus salangana natunae	DMT048	Gomantong caves, Sabah, Malaysia
Aerodramus fuciphagus vestitus	DMT027	Gomantong caves, Sabah, Malaysia
Aerodramus fuciphagus vestitus	DHC40	Gomantong caves, Sabah, Malaysia
Aerodramus fuciphagus germani	DHC04	Balambangan Is., Sabah, Malaysia
Aerodramus elaphrus	DHC59	Seychelles
Aerodramus elaphrus	DHC60	Seychelles
Aerodramus elaphrus	DHC61	Seychelles
Aerodramus francicus	DHC52	Mauritius
Aerodramus francicus	DHC53	Mauritius
Aerodramus spodiopygius assimilis	DHC36	Suva, Fiji
Aerodramus spodiopygius assimilis	DHC37	Suva, Fiji
Aerodramus spodiopygius spodiopygius	DHC31	Western Samoa
Aerodramus spodiopygius assimilis	DHC39	Suva, Fiji
Aerodramus vanikorensis palawanensis	DHC01	Balambangan Is., Sabah, Malaysia
Aerodramus vanikorensis palawanensis	DHC06	Balambangan Is., Sabah, Malaysia
Aerodramus mearnsi	SEA116	Mindanao, Philippines
Aerodramus bartschi	DHC77	Oahu, Hawaii
Aerodramus sawtelli	W3	Atiu, Cook Islands
Aerodramus vanikorensis lugubris	UWBM58708	Rennell Is., Solomons
Aerodramus maximus lowi	DHC117	Madai Caves, Sabah, Malaysia
Aerodramus maximus lowi	DHC120	Madai Caves, Sabah, Malaysia
Aerodramus maximus lowi	DMT040	Gomantong caves, Sabah, Malaysia
Aerodramus maximus lowi	DMT042	Gomantong caves, Sabah, Malaysia
Aerodramus maximus lowi	DHC03	Balambangan Is., Sabah, Malaysia
Aerodramus brevirostris vulcanorum	DHC66	Tangkuban Prahu, Java, Indonesia
Aerodramus terraereginae terraereginae	DHC20	Tully Gorge, Queensland, Australia
Aerodramus terraereginae terraereginae	DHC30	Tully Gorge, Queensland, Australia
Aerodramus terraereginae terraereginae	DHC28	Tully Gorge, Queensland, Australia
Aerodramus whiteheadi	CMNH37000	Mindanao, Philippines
Collocalia esculenta cyanoptila	DHC88	Lahad Datu, Sabah, Malaysia
Collocalia esculenta cyanoptila	DHC97	Lahad Datu, Sabah, Malaysia
Collocalia esculenta cyanoptila	DMT051	Sandakan, Sabah, Malaysia
Collocalia esculenta cyanoptila	DMT050	Sandakan, Sabah, Malaysia
Collocalia esculenta cyanoptila	DMT057	Selangor, Malaysia
Collocalia esculenta cyanoptila	DMT059	Selangor, Malaysia
Collocalia esculenta bagobo	ATP92.131	Mindanao, Philippines
Collocalia esculenta bagobo	ATP92.280	Mindanao, Philippines
Collocalia esculenta marginata	FMNH358301	Sibuyan, Philippines
Collocalia esculenta marginata	FMNH358303	Sibuyan, Philippines
Collocalia esculenta becki	UWBM60227	Isabel Is., Solomons
Collocalia esculenta nitens	MSP068	New Guinea
Collocalia linchi	DHC72	Bogor, Java, Indonesia
Collocalia troglodytes	FMNH358312	Sibuyan, Philippines
Apus affinis	SMG3775	Pakistan
Apus apus	PL05	United Kingdom
Apus melba	CTC	South Africa
Cypsiurus balasiensis	DHC45	Kuala Lumpur, Malaysia
Cypsiurus parvus	LSU B34256	South Africa
Hirundapus caudacutus	UWBM46916	Russia
Chaetura pelagica	MCP96.1343	USA
Chaetura vauxi	ELB05	Oregon, USA
Chaetura chapmani	JWF86.065	Brazil
Chaetura cinereiventris	SML1187	Peru
Neafrapus cassini	PRS2081	Central African Republic
Cypseloides niger	CTC02	USA
Streptoprocne zonaris	PRS791	Venezuela
Cypseloides phelpsi	GFB3015	Venezuela
Hemiprocne comata	CMNH38185	Philippines

*Taxonomy follows Chantler et al. (2000). †ATP = A. Townsend Peterson; CMNH = Cincinnati Museum of Natural History; CTC = Charlie Collins; DHC = Dale Clayton; DMT = Dan Tompkins; ELB = Evelyn Bill; FMNH = Field Museum of Natural History; GFB = George Barrowclough; JWF = John Fitzpatrick; LSU = Louisiana State University; MSP = Michael Putnam; PL = Pat Lee; PRS = Paul Sweet; SEA = Sarah Al-Tamimi; SMG = Steve Goodman; SML = Scott Lanyon; UWBM = University of Washington Burke Museum; W = Graham Wragg.

Quinn 1998). We treated gaps as missing data in all analyses. The aligned region for cyt *b* totaled 1058 bp and that for ND2 totaled 1078 bp. These sequences have been deposited in GenBank under the accession numbers AY294424 to AY294483 and AY204486 to AY294545.

Phylogenetic analyses

PAUP* (Swofford 2002) was used for all analyses of the molecular data. We conducted a partition homogeneity test (Farris et al. 1994, 1995, Swofford 2002) to evaluate possible evidence for conflicting signal between the cyt-*b* and ND2 data sets. Because there was no evidence for significant conflict ($P = 0.48$), we conducted all further analyses on the combined data. We first analyzed the combined data set using unordered parsimony with 100 random addition TBR replicates. To assess the sensitivity of the tree to character resampling, we bootstrapped the data set (Felsenstein 1985) using 1000 bootstrap replicates.

To assess the sensitivity of the tree topology to method of analysis, we further analyzed the data using maximum likelihood. We estimated the best fit model that could not be rejected in favor of a simpler model (Huelsenbeck and Crandall 1997) using Modeltest (Posada and Crandall 1998). This analysis revealed that a model incorporating unequal base frequencies, a general time reversible substitution matrix, invariant sites, and rate heterogeneity according to a gamma distribution (GTR+I+G) could not be rejected in favor of simpler models. We used the parameters estimated by this analysis in maximum likelihood searches of the combined data (10 random addition replicates with TBR branch swapping). We also used 100 bootstrap replicates to assess the sensitivity of the tree topology to character resampling.

Analysis of echolocation sounds

Tape-recordings of swiftlet echolocation clicks were obtained in the field by D. H. Clayton and from a variety of other sources (Table 2). Orientation sounds of *Collocalia troglodytes* and *Aerodramus brevirostris vulcanorum* were recorded after releasing single individuals into dark rooms. Individuals of *Collocalia linchi* and *C. esculenta* were also released into dark rooms to ensure that they could not echolocate. As in previous studies of these latter species (Medway 1967, Fenton 1975), the birds produced no audible clicks during these trials and repeatedly flew into obstacles when deprived of visual cues. Most recordings of other echolocating species were of free-flying birds entering or leaving their roost caves. Sounds of several to many individuals were typically present on a recording. For each taxon, we selected a few of the least distorted examples of echolocation clicks for analysis. We did not obtain recordings of the Australian swiftlet, *Aerodramus terraereginae* (formerly *Collocalia spodiopygia*). However, echolocation in this species has been described in detail in previous investigations (e.g., Griffin and Thompson 1982, Suthers and Hector 1982, Smyth and Roberts 1983, Marchant et al. 1999), so we included this information in our study. In all, we examined the echolocation clicks of 12 of the 16 *Aerodramus* taxa included in the molecular analysis, as well as the clicks of *C. troglodytes*.

Spectrograms of swiftlet echolocation sounds were generated using Canary sound analysis software (Version 1.2.4, Cornell Laboratory of Ornithology; sampling frequency 22.05 kHz, frequency resolution 349.7 Hz, temporal resolution 11.6 ms, 93.75% overlap of frames in successive transforms). We classed swiftlets into those with single clicks and those with double clicks by visual inspection of on-screen spectrograms. For taxa with double clicks, we measured the pause between the two pulses, or intraclick interval, for several examples from each individual. Previous analyses have suggested that intraclick intervals can vary within an individual (Suthers and Hector 1982) and do not differ consistently

Table 2. Recordings of echolocation sounds included in the study.

Taxon	Geographic Location	Recordist/Source*
Collocalia troglodytes	Philippines	D. H. Clayton
Aerodramus elaphrus	Seychelles	D. H. Clayton
Aerodramus elaphrus	Seychelles	A. Gretton (NSA-28945)
Aerodramus francicus	Mauritius	D. H. Clayton
Aerodramus spodiopygius spodiopygius	Samoa	R. I. Orenstein (LNS-20510)
Aerodramus terraereginae	Queensland, Australia	From literature†
Aerodramus brevirostris vulcanorum	Java, Indonesia	D. H. Clayton
Aerodramus salangana natunae	Sabah, Malaysia	BBC (NSA-CDR16/17)
Aerodramus vanikorensis palawanensis	Palawan, Philippines	C. Collins
Aerodramus bartschi	Hawaii, USA	D. H. Clayton
Aerodramus sawtelli	Atiu, Cook Islands	J. H. Fullard (9 recordings)
Aerodramus maximus lowi	Sabah, Malaysia	BBC (NSA-MB6/17)
Aerodramus maximus lowi	Sabah, Malaysia	BBC (NSA-CDR16/24)
Aerodramus maximus lowi	Sarawak, Malaysia	Lord Medway (NSA-22458)
Aerodramus fuciphagus germani	Pahang, Malaysia	A. B. Vanden Berg (LNS-36421)
Aerodramus fuciphagus vestitis	Sabah, Malaysia	BBC (NSA-CDR16/19)

*NSA = British Museum National Sound Archive; LNS = Macaulay Library of Natural Sounds, Cornell Laboratory of Ornithology; BBC = British Broadcasting Corporation. †Click characteristics obtained primarily from Suthers and Hector (1982).

among some species (Medway and Pye 1977), however, no previous study has compared this trait across a large number of swiftlet taxa. We did not include measures of frequency characteristics in our analysis because this sound component is more likely to be influenced by such factors as acoustic characteristics of the recording environment, the distance between the bird and microphone, and characteristics of the various recording equipment used. Free-flying birds were recorded under conditions ranging from small caves with entrances less than 1 m in diameter to enormous caverns more than 100 m tall. Variation in these conditions between recordings would have obscured any actual differences in frequency measures between taxa, like those documented in the vocalizations of other taxa (Price and Lanyon 2002a). We mapped the presence or absence of echolocation and aspects of click structure onto the molecular phylogeny using simple parsimony in MacClade (Version 4, Maddison and Maddison 2000).

Results

Of the 1058 sites for cyt *b*, 401 (37.9%) were variable and 315 (29.8%) were potentially phylogenetically informative. Genetic divergences ranged from 0.0% (between members of the same subspecies) to 15.1% (between *Hemiprocne* and some members of the ingroup). Of the 1078 sites for ND2, 528 (49.0%) were variable and 417 (38.7%) were potentially phylogenetically informative. Genetic divergences for ND2 ranged between 0.0% and 19.0%.

Unweighted parsimony analysis of the combined gene regions produced 12 trees, the consensus of which is shown in Fig. 2. Although there are several most parsimonious trees, the consensus is generally well resolved and bootstrap support for most nodes is generally high. Importantly, both the genera *Aerodramus* and *Collocalia* are monophyletic (bootstrap 100% and 99%, respectively). In addition, unlike the results of Lee et al. (1996), swiftlets (*Aerodramus*+*Collocalia*) are recovered as a monophyletic group with strong support (98%).

Within *Aerodramus*, there are three strongly supported clades: *whiteheadi*+*terraereginae*, *maximus*+*brevirostris*, and the remainder of *Aerodramus*. Some species do not appear to be monophyletic. *Aerodramus vanikorensis palawanensis* is sister to *A. mearnsi*, while *A. v. lugubris* is sister to *A. sawtelli*+*A. bartschi*. *A. fuciphagus vestitus* is sister to *A. salangana natunae* to the exclusion of *A. f. germani*, although this is only weakly supported (53%). Between subspecies, genetic divergence is often considerable, ranging from 1.8% between *A. fuciphagus vestitus* and *A. f. germani* to 3.7% between *A. vanikorensis lugubris* and *A. v. palawanensis*. Within a subspecies, in contrast, genetic variation is generally less than 0.5%, with the exception of two $>2\%$ divergent haplotypes of *A. spodiopygius assimilis*.

In *Collocalia*, *C. troglodytes* is sister to the remainder of the genus. Monophyly of *Collocalia esculenta* is strongly supported (78%). However, genetic divergence between subspecies of *C. esculenta* is considerable, ranging from 3.0% between *C. e. bagobo* and *C. e. marginata* to 5.8% between *C. e. becki* and *C. e. cyanoptila*. Like in *Aerodramus*, divergence within subspecies generally runs less than 0.5%.

For the most part, monophyly of other swift genera is supported. However, there is strong support for paraphyly of *Cypseloides*, with *Streptoprocne zonaris* the sister taxon of *Cypseloides niger* with 100% bootstrap support. *Cypseloides*+*Streptoprocne* form the sister taxon to all other swifts with strong support (100%), which agrees with previous suggestions that these taxa constitute a separate subfamily (Brooke 1972).

Maximum likelihood analysis produced a single tree that was well resolved and well supported by bootstrap analyses (Fig. 3). In most respects this tree is similar to the parsimony tree, differing mostly in the arrangement of some weakly supported nodes. Importantly, swiftlets are monophyletic (96%) as are *Aerodramus* (100%) and *Collocalia* (100%). Other relationships discussed above also appear in the maximum likelihood tree.

Mapping echolocation onto the molecular tree shows that this ability either evolved at the base of the swiftlets and was then lost on the branch leading to *Collocalia esculenta*+*C. linchi*, or arose independently in *C. troglodytes* and the genus *Aerodramus* (Fig. 4). As found in previous studies (Medway 1959, Medway and Pye 1977, Fullard et al. 1993), *Aerodramus maximus* and *A. sawtelli* differ from other swiftlets in producing echolocation pulses with a single click design (Fig. 5). All other echolocating taxa produce double clicks, including *C. troglodytes*. Based on our reconstructions of this feature on the molecular tree (Fig. 4), single clicks appear to be a specialization that evolved independently in *A. maximus* and *A. sawtelli*, whereas the double clicks of other *Aerodramus* taxa and of *C. troglodytes* represent the more ancestral character state.

Intraclick intervals measured in this study varied among species no more than they did within individuals. All *Aerodramus* taxa with double clicks had intervals of approximately 15 to 20 ms (mean = 16.8 ms, SE = 0.5 ms), which agrees with the results of a smaller survey by Medway and Pye (1977) but is slightly lower than values measured for *Aerodramus terraereginae* by Suthers and Hector (1982; 18 to 25 ms). Interestingly, the double clicks of *C. troglodytes* have a mean intraclick interval of 16.9 ms, well within the range measured in *Aerodramus* species (compare Fig. 5b and c).

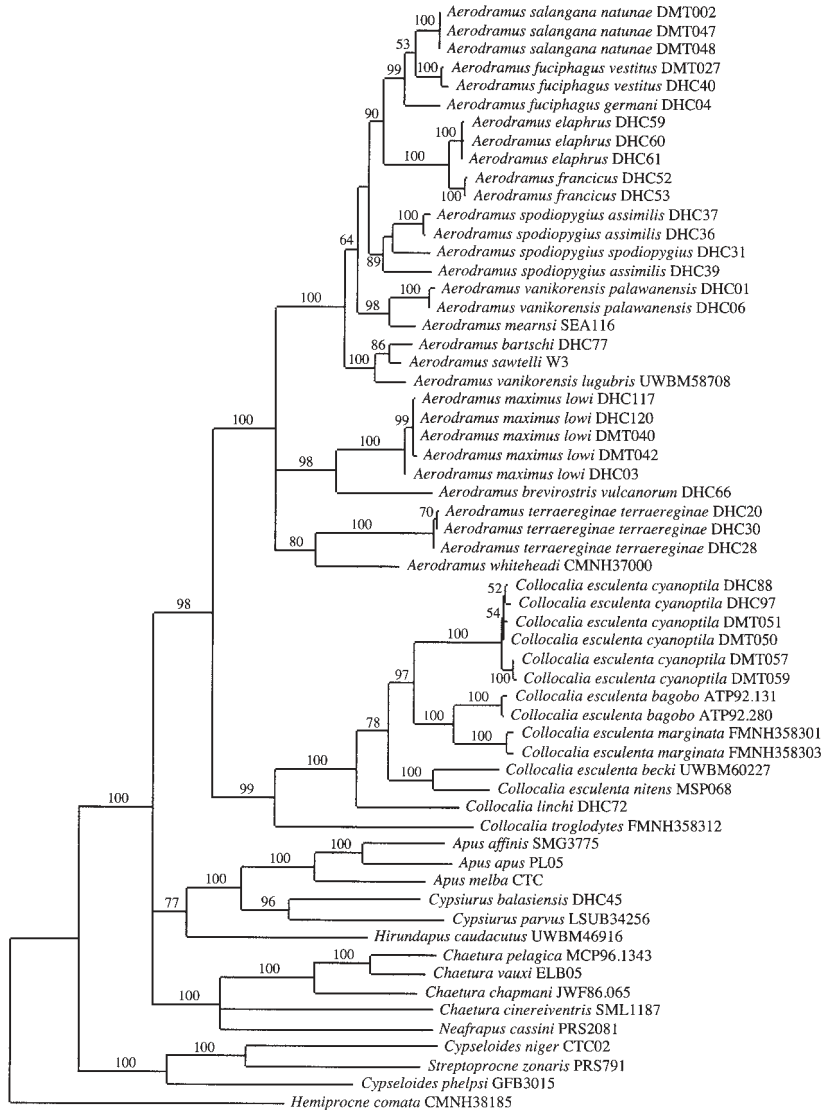

Fig. 2. Consensus of 12 most parsimonious trees (length = 3014, CI = 0.428) produced from combined analysis of cyt-*b* and ND2 sequences for swifts and swiftlets. Numbers associated with branches indicate percent support from 1000 bootstrap replicates. Branch lengths are proportional to the number of reconstructed changes.

Discussion

Phylogenetic analysis of combined cyt-*b* and ND2 sequences for swifts and swiftlets produced trees that were generally well supported and resolved (Figs. 2 and 3). Monophyly of swiftlets was strongly supported, as was the reciprocal monophyly of *Aerodramus* and *Collocalia*. With our discovery of echolocation in *C. troglodytes*, our phylogeny reveals that the evolution of this ability in the swiftlets has traced an historical pattern much different than previously thought. As a result, echolocation can no longer be considered a useful character for separating these two genera.

In a previous study, Lee et al. (1996) concluded that an ability to echolocate evolved once in the immediate ancestor of the *Aerodramus* genus. Echolocation in *Collocalia* was at that time unknown and their molecular analysis indicated paraphyly of swiftlets. In contrast to those results, our data indicate that echolocation is unlikely to have evolved in this way. Rather, echolocation arose either (1) once at the base of the swiftlets and was secondarily lost on the branch leading to *Collocalia esculenta* and *C. linchi*, or (2) twice independently among swiftlets. Our phylogeny does not resolve which of these possibilities is more likely (Fig. 4). However, the striking similarities between the orientation clicks of *Collocalia troglodytes* and those of most *Aerodramus* taxa (Fig. 5) suggest that these sounds are homologous rather than independently derived. More detailed comparisons of click structure in which recordings are made

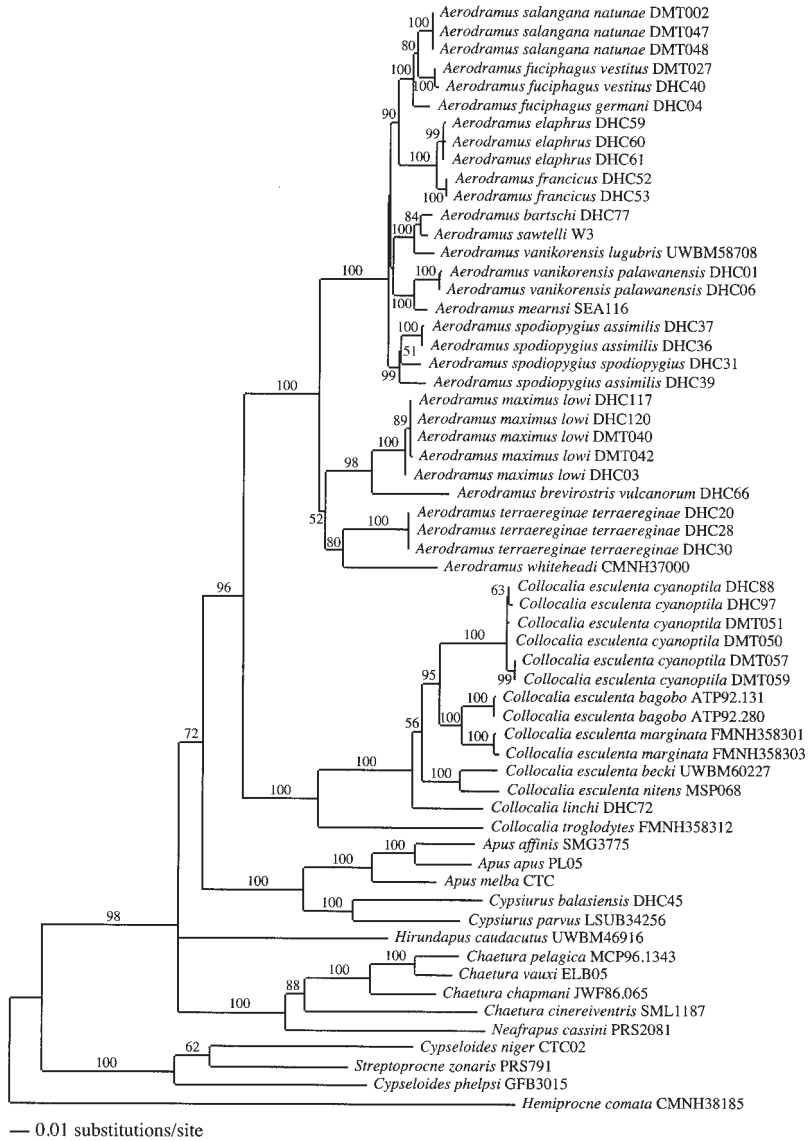

Fig. 3. Most likely tree (L = 16,830.3715) resulting from combined likelihood analysis of cyt-*b* and ND2 gene regions under a model with base frequencies (A = 0.3294, C = 0.4026, G = 0.0657, T = 0.2023), general time reversible substitutions (A-C = 0.3922, A-G = 13.3884, A-T = 0.5225, C-G = 0.2016, C-T = 6.2253, G-T = 1.0), gamma shape parameter = 1.1988, and fraction of invariant sites = 0.5008. Numbers associated with branches indicate support from 100 bootstrap replicates. Branch lengths are proportional to the length optimized under the likelihood model (scale indicated).

under much more standardized conditions, as well as comparative analyses of syringeal morphology, are needed to further investigate this possibility.

Single clicks have evolved from double clicks on at least two separate occasions during swiftlet evolution. In theory, a single click design might be more effective for acoustic orientation than a double click by allowing birds to avoid potential pulse-echo overlap (Fullard et al. 1993). However, producing single clicks might also require special physiological mechanisms. Detailed studies by Suthers and Hector (1982) of the physiology of click production suggest that the double nature of these sounds is a byproduct of the manner in which they are produced. Each double click is the result of a bird momentarily closing its sound producing organ, the syrinx, in the midst of producing a longer squeak-like sound, thus separating this vocalization into two brief clicks with high bandwidth and abrupt onset and offset times. Producing a single click probably involves the additional step of suppressing one of these sounds (Suthers and Hector 1982). Evolutionary changes in click design, as well as the possible loss of this ability in some taxa, therefore might reflect trade-offs in different swiftlet lineages between the physiological costs of click production and the need for an effective navigation system.

The strongly supported monophyly of swiftlets (>95%) in this analysis is perhaps surprising given

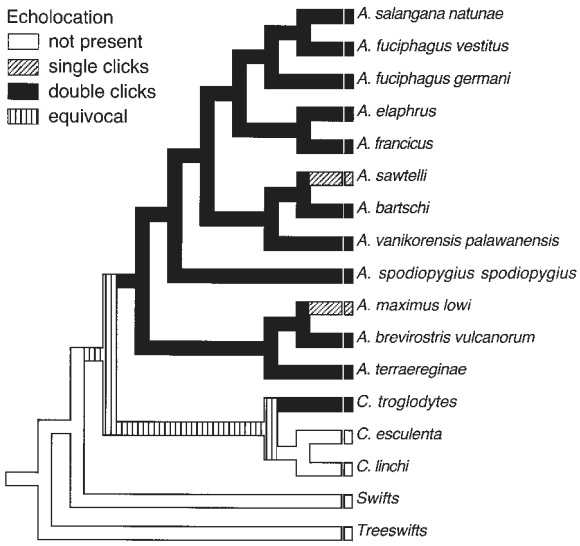

Fig. 4. The presence of echolocation and two types of echolocation sounds, single clicks and double clicks, reconstructed onto the maximum likelihood phylogeny of swiftlet taxa.

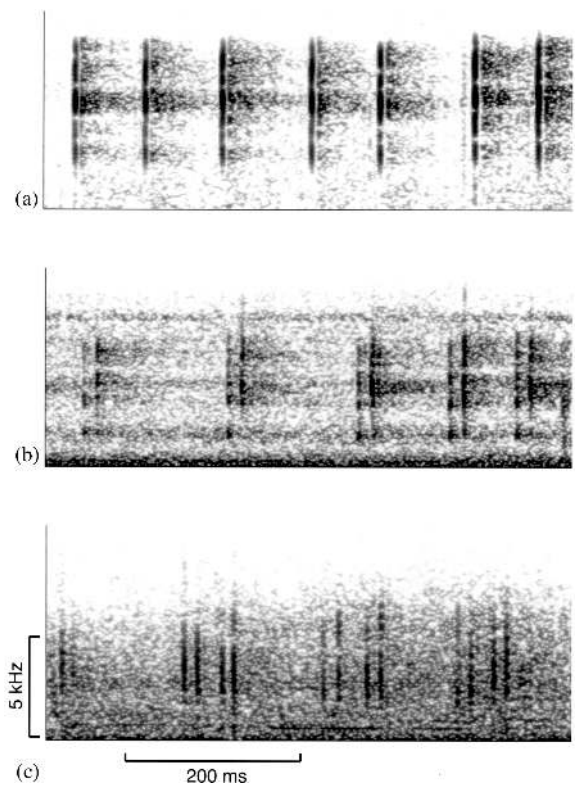

Fig. 5. Spectrograms of echolocation sounds produced by (a) *Aerodramus sawtelli*, (b) *A. bartschi* and (c) *Collocalia troglodytes*. The double clicks of *A. bartschi* (b) and *C. troglodytes* (c) are similar in acoustic structure, yet both are comparatively different from the single clicks of *A. sawtelli* (a), which is sister taxon to *A. bartschi* (recordings by J. H. Fullard (a) and D. H. Clayton (b and c)).

that a previous phylogenetic analysis of partial cyt-*b* sequences (Lee et al. 1996) resolved swiftlets as paraphyletic, with *Collocalia* and *Aerodramus* belonging to separate clades. However, Lee et al. (1996) analyzed only 406 bp of the cyt-*b* gene, less than 20% of the number of base pairs included in the present study. Since paraphyly of the swiftlets was not strongly supported in the Lee et al. (1996) analysis, it seems likely that the difference in results between their study and ours is explained largely by the difference in the number of base pairs analyzed. Caution should thus be exercised when drawing conclusions from weakly supported trees based on a limited number of base pairs.

A more recent analysis of swiftlet phylogeny by Thomassen et al. (2003) using longer cyt-*b* sequences (1143 bp) from a limited number of taxa has provided further support for swiftlet monophyly, in agreement with our findings. Unlike our study, however, they suggest paraphyly of *Aerodramus* by placing the non-echolocating waterfall swiftlet *Hydrochous gigas* within that genus, a result that could indicate either the loss of echolocation in *H. gigas* or the appearance of echolocation multiple times in different *Aerodramus* taxa (Thomassen et al. 2003). Support for this placement of *H. gigas* was weak, however, and the analysis by Thomassen et al. (2003) sampled only four *Aerodramus* species and did not include data from *Collocalia troglodytes*. Inclusion of the 406 bp sequence of *H. gigas* from the study of Lee et al. (1996) in the current analysis resulted in a weakly supported sister relationship between *Aerodramus* and *Hydrochous*, still recovering swiftlet monophyly and the monophyly of *Aerodramus*. However, since we were unable to reamplify the *Hydrochous* tissue for a proper investigation, we treat these results with caution and refrain from drawing conclusions about echolocation based on this species. Perhaps sequencing additional genes in this enigmatic swiftlet will help resolve its relationships and provide a clearer picture of how echolocation has evolved in the group.

Swiftlets have been perhaps the most taxonomically difficult group of birds, and this is evidenced by our results. Although many species relationships are well resolved, several species are shown to be paraphyletic. Additional sampling of species and subspecies in *Aerodramus* and *Collocalia* is needed to resolve many of these outstanding taxonomic issues. On the other hand, deep phylogenetic relationships among many of the swifts are strongly supported by our data, unlike many previous studies of mitochondrial genes in birds (e.g., Johnson and Clayton 2000, Klicka et al. 2000, Johnson 2001, Price and Lanyon 2002b). This result underscores the strong potential for mitochondrial gene sequences to answer other unresolved questions in swift systematics.

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