

The evolution of echolocation in swiftlets

J. Jordan Price, Kevin P. Johnson and Dale H. Clayton

Price, J. J., Johnson, K. P. and Clayton, D. H. 2004. The evolution of echolocation in swiftlets. – J. Avian Biol. 35: 135–143.

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.

J. J. Price (correspondence), Department of Biology, St. Mary's College of Maryland, St. Mary's City, MD 20686 USA. E-mail: jjprice@smcm.edu. K. P. Johnson, Illinois Natural History Survey, Champaign, IL 61820 USA. D. H. Clayton, Department of Biology, University of Utah, Salt Lake City, UT 84112 USA.

An ability to orient by means of echolocation has evolved in only two groups of birds: the Neotropical oilbird (Steatornithidae) and members of the Palearctic 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.

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

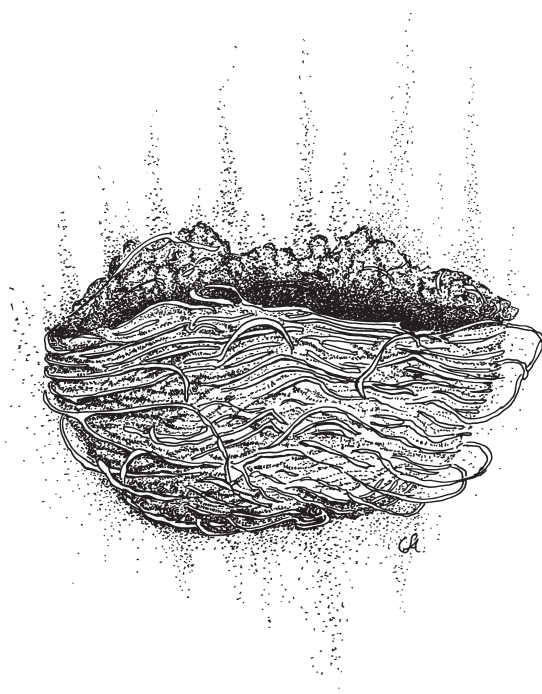


Fig. 1. Voucher nest of a pygmy swiftlet *Collocalia troglodytes* located in total darkness approximately 30 meters inside the entrance of Balete cave on Samal Island, Philippines. The nest, which was anchored to a vertical wall with copious amounts of saliva cement, was composed of fine vegetable fibers 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.

Table 1. Tissue samples included in the phylogenetic analysis.

Taxon*	Voucher Information†	Geographic Location
<i>Aerodramus salangana natunae</i>	DMT002	Gomantong caves, Sabah, Malaysia
<i>Aerodramus salangana natunae</i>	DMT047	Gomantong caves, Sabah, Malaysia
<i>Aerodramus salangana natunae</i>	DMT048	Gomantong caves, Sabah, Malaysia
<i>Aerodramus fuciphagus vestitus</i>	DMT027	Gomantong caves, Sabah, Malaysia
<i>Aerodramus fuciphagus vestitus</i>	DHC40	Gomantong caves, Sabah, Malaysia
<i>Aerodramus fuciphagus germani</i>	DHC04	Balambangan Is., Sabah, Malaysia
<i>Aerodramus elaphrus</i>	DHC59	Seychelles
<i>Aerodramus elaphrus</i>	DHC60	Seychelles
<i>Aerodramus elaphrus</i>	DHC61	Seychelles
<i>Aerodramus francicus</i>	DHC52	Mauritius
<i>Aerodramus francicus</i>	DHC53	Mauritius
<i>Aerodramus spodiopygius assimilis</i>	DHC36	Suva, Fiji
<i>Aerodramus spodiopygius assimilis</i>	DHC37	Suva, Fiji
<i>Aerodramus spodiopygius spodiopygius</i>	DHC31	Western Samoa
<i>Aerodramus spodiopygius assimilis</i>	DHC39	Suva, Fiji
<i>Aerodramus vanikorensis palawanensis</i>	DHC01	Balambangan Is., Sabah, Malaysia
<i>Aerodramus vanikorensis palawanensis</i>	DHC06	Balambangan Is., Sabah, Malaysia
<i>Aerodramus mearnsi</i>	SEA116	Mindanao, Philippines
<i>Aerodramus bartschi</i>	DHC77	Oahu, Hawaii
<i>Aerodramus sawtelli</i>	W3	Atiu, Cook Islands
<i>Aerodramus vanikorensis lugubris</i>	UWBM58708	Rennell Is., Solomons
<i>Aerodramus maximus lowi</i>	DHC117	Madai Caves, Sabah, Malaysia
<i>Aerodramus maximus lowi</i>	DHC120	Madai Caves, Sabah, Malaysia
<i>Aerodramus maximus lowi</i>	DMT040	Gomantong caves, Sabah, Malaysia
<i>Aerodramus maximus lowi</i>	DMT042	Gomantong caves, Sabah, Malaysia
<i>Aerodramus maximus lowi</i>	DHC03	Balambangan Is., Sabah, Malaysia
<i>Aerodramus brevirostris vulcanorum</i>	DHC66	Tangkuban Prahur, Java, Indonesia
<i>Aerodramus terraereginae terraereginae</i>	DHC200	Tully Gorge, Queensland, Australia
<i>Aerodramus terraereginae terraereginae</i>	DHC30	Tully Gorge, Queensland, Australia
<i>Aerodramus terraereginae terraereginae</i>	DHC28	Tully Gorge, Queensland, Australia
<i>Aerodramus whiteheadi</i>	CMNH37000	Mindanao, Philippines
<i>Collocalia esculenta cyanoptila</i>	DHC88	Lahad Datu, Sabah, Malaysia
<i>Collocalia esculenta cyanoptila</i>	DHC97	Lahad Datu, Sabah, Malaysia
<i>Collocalia esculenta cyanoptila</i>	DMT051	Sandakan, Sabah, Malaysia
<i>Collocalia esculenta cyanoptila</i>	DMT050	Sandakan, Sabah, Malaysia
<i>Collocalia esculenta cyanoptila</i>	DMT057	Selangor, Malaysia
<i>Collocalia esculenta cyanoptila</i>	DMT059	Selangor, Malaysia
<i>Collocalia esculenta bagobo</i>	ATP92.131	Mindanao, Philippines
<i>Collocalia esculenta bagobo</i>	ATP92.280	Mindanao, Philippines
<i>Collocalia esculenta marginata</i>	FMNH358301	Sibuyan, Philippines
<i>Collocalia esculenta marginata</i>	FMNH358303	Sibuyan, Philippines
<i>Collocalia esculenta becki</i>	UWBM60227	Isabel Is., Solomons
<i>Collocalia esculenta nitens</i>	MSP068	New Guinea
<i>Collocalia linchi</i>	DHC72	Bogor, Java, Indonesia
<i>Collocalia troglodytes</i>	FMNH358312	Sibuyan, Philippines
<i>Apus affinis</i>	SMG3775	Pakistan
<i>Apus apus</i>	PL05	United Kingdom
<i>Apus melba</i>	CTC	South Africa
<i>Cypsiurus balasiensis</i>	DHC45	Kuala Lumpur, Malaysia
<i>Cypsiurus parvus</i>	LSU B34256	South Africa
<i>Hirundapus caudacutus</i>	UWBM46916	Russia
<i>Chaetura pelagica</i>	MCP96.1343	USA
<i>Chaetura vauxi</i>	ELB05	Oregon, USA
<i>Chaetura chapmani</i>	JWF86.065	Brazil
<i>Chaetura cinereiventris</i>	SML1187	Peru
<i>Neafrapus cassini</i>	PRS2081	Central African Republic
<i>Cypseloides niger</i>	CTC02	USA
<i>Streptoprocne zonaris</i>	PRS791	Venezuela
<i>Cypseloides phelpsi</i>	GFB3015	Venezuela
<i>Hemiprocne comata</i>	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 linci* 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*
<i>Collocalia troglodytes</i>	Philippines	D. H. Clayton
<i>Aerodramus elaphrus</i>	Seychelles	D. H. Clayton
<i>Aerodramus elaphrus</i>	Seychelles	A. Gretton (NSA-28945)
<i>Aerodramus francicus</i>	Mauritius	D. H. Clayton
<i>Aerodramus spodiopygius spodiopygius</i>	Samoa	R. I. Orenstein (LNS-20510)
<i>Aerodramus terraereginae</i>	Queensland, Australia	From literature [†]
<i>Aerodramus brevirostris vulcanorum</i>	Java, Indonesia	D. H. Clayton
<i>Aerodramus salangana natunae</i>	Sabah, Malaysia	BBC (NSA-CDR16/17)
<i>Aerodramus vanikorensis palawanensis</i>	Palawan, Philippines	C. Collins
<i>Aerodramus bartschi</i>	Hawaii, USA	D. H. Clayton
<i>Aerodramus sawtelli</i>	Atiu, Cook Islands	J. H. Fullard (9 recordings)
<i>Aerodramus maximus lowi</i>	Sabah, Malaysia	BBC (NSA-MB6/17)
<i>Aerodramus maximus lowi</i>	Sabah, Malaysia	BBC (NSA-CDR16/24)
<i>Aerodramus maximus lowi</i>	Sarawak, Malaysia	Lord Medway (NSA-22458)
<i>Aerodramus fuciphagus germani</i>	Pahang, Malaysia	A. B. Vanden Berg (LNS-36421)
<i>Aerodramus fuciphagus vestitus</i>	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 *Hemiprogne* 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. palawa-*

nensis. 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).

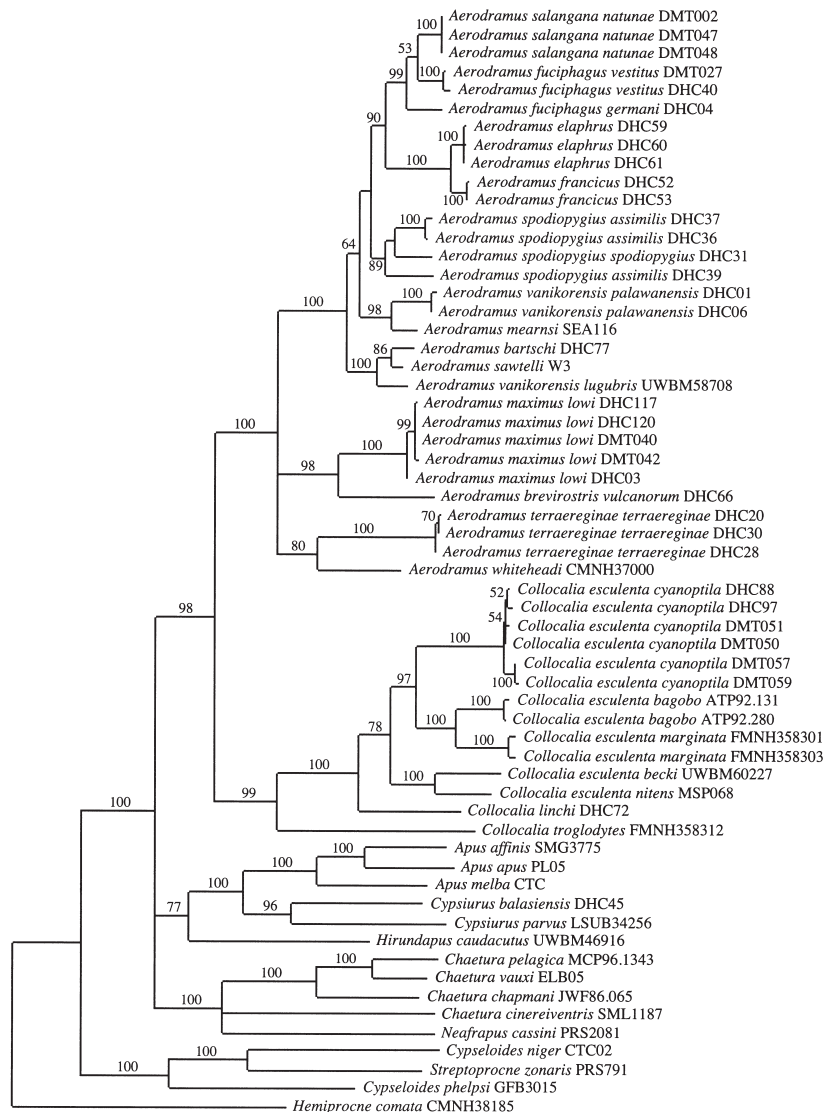


Fig. 2. Consensus of 12 most parsimonious trees (length = 3014, CI = 0.428) produced from combined analysis of *cyt-b* and ND2 sequences for swifts and swiftlets. Numbers associated with branches indicate percent support from 1000 bootstrap replicates. Branch lengths are proportional to the number of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

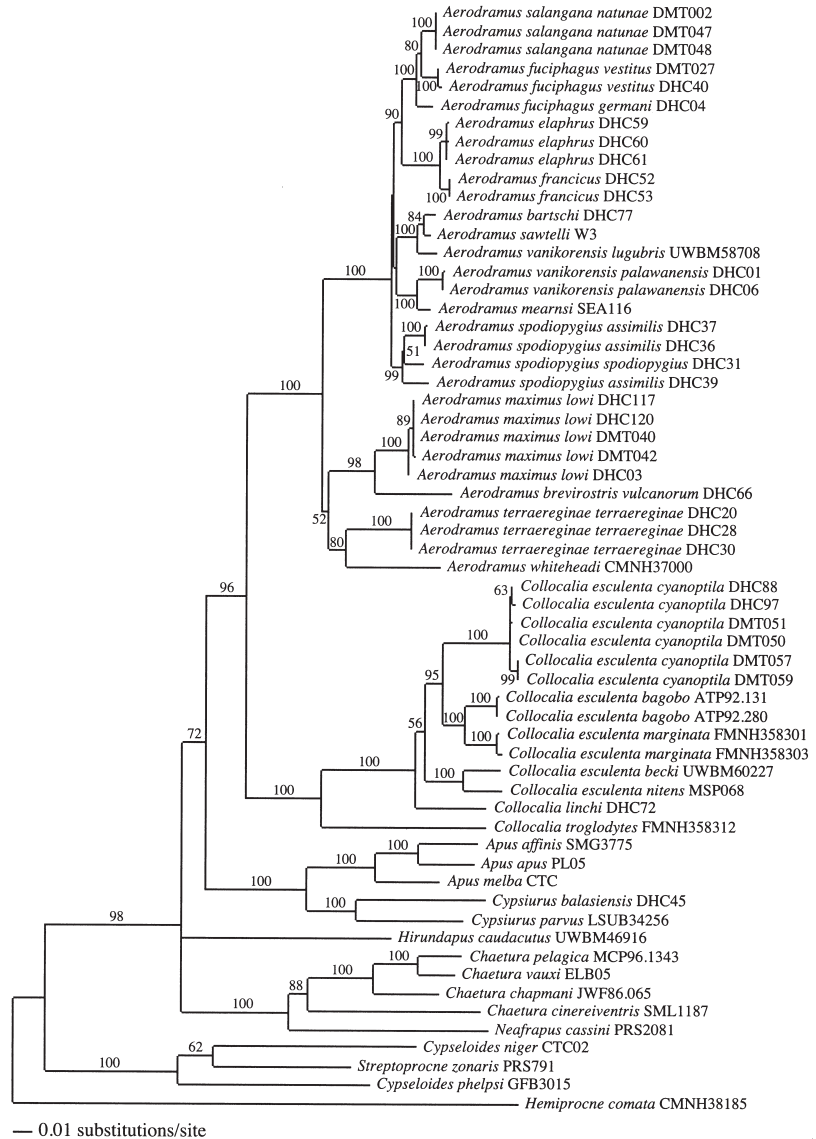
Discussion

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

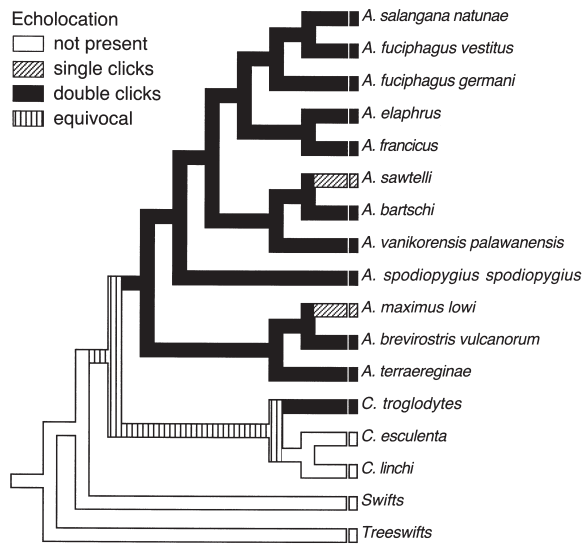


Fig. 4. The presence of echolocation and two types of echolocation sounds, single clicks and double clicks, reconstructed onto the maximum likelihood phylogeny of swiftlet taxa.

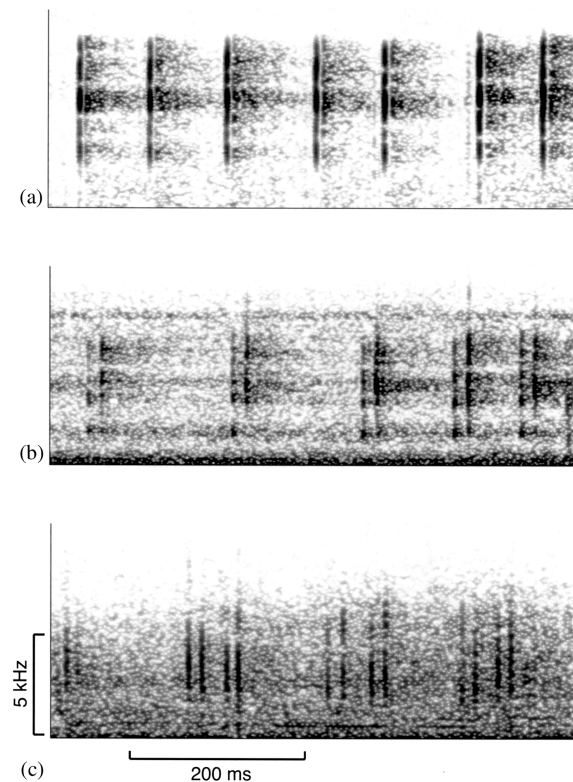


Fig. 5. Spectrograms of echolocation sounds produced by (a) *Aerodramus sawtelli*, (b) *A. bartschi* and (c) *Collocalia troglodytes*. The double clicks of *A. bartschi* (b) and *C. troglodytes* (c) are similar in acoustic structure, yet both are comparatively different from the single clicks of *A. 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.

Acknowledgements – We thank the following individuals and institutions for providing tissue material for this study: Charlie Collins, Mike Putnam, American Museum of Natural History,

Cincinnati Museum of Natural History, Field Museum of Natural History, Louisiana State University Museum of Natural Science, and the University of Washington Burke Museum of Natural History. We are also grateful to the British Museum Natural Sound Archive, the Macaulay Library of Natural Sounds, and especially to Charlie Collins and James Fullard for providing recordings of swiftlets. Charlie Collins and several anonymous reviewers provided useful comments on the manuscript. We thank L. Heaney and the Philippine government for making fieldwork in the Philippines possible. We also thank S. Bush for field assistance and for drawing Fig. 1. Financial support was provided by NSF CAREER award DEB 9703003 to DHC.

References

- Brooke, R. K. 1972. Generic limits in Old World Apodidae and Hirundinidae. – *Bull. Br. Ornith. Club* 92: 53–57.
- Chantler, P. and Driessens, G. 1995. *Swifts, a Guide to the Swifts and Treeswifts of the World*. – Pica Press, Sussex, UK.
- Chantler, P., Wells, D. R. and Schuchmann, K. L. 2000. Family Apodidae (Swifts). – In: del Hoyo, J., Elliot, A. and Sargatal, J. (eds). *Handbook of the Birds of the World*. Vol. 5. Lynx Edicions, Barcelona, Spain, pp. 338–457.
- Collins, C. T. and Murphy, R. 1994. Echolocation acuity of the Palawan swiftlet (*Aerodramus palawanensis*). – *Avocetta* 17: 157–162.
- Cranbrook, Earl of and Lord Medway. 1965. Lack of ultrasonic frequencies in the calls of swiftlets (*Collocalia* spp.). – *Ibis* 107: 258.
- Farris, J. S., Källersjö, M., Kluge, A. G. and Bult, C. 1994. Testing significance of incongruence. – *Cladistics* 10: 315–319.
- Farris, J. S., Källersjö, M., Kluge, A. G. and Bult, C. 1995. Constructing a significance test for incongruence. – *Syst. Biol.* 44: 570–572.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. – *Evolution* 39: 783–791.
- Fenton, M. B. 1975. Acuity of echolocation in *Collocalia hirundinacea* (Aves: Apodidae), with comments on the distributions of echolocating swiftlets and molossid bats. – *Biotropica* 7: 1–7.
- Fullard, J. H., Barclay, R. M. R. and Thomas, D. W. 1993. Echolocation in free-flying Atiu swiftlets (*Aerodramus sawtelli*). – *Biotropica* 25: 334–339.
- Griffin, D. R. 1954. Acoustic orientation in the oil bird, *Steatornis*. – *Proc. Natl. Acad. Sci. USA* 39: 884–893.
- Griffin, D. R. 1958. Listening in the Dark. – Yale University Press, New Haven, Conn.
- Griffin, D. R. and Suthers, R. A. 1970. Sensitivity of echolocation in cave swiftlets. – *Biol. Bull.* 139: 495–501.
- Griffin, D. R. and Thompson, D. 1982. Echolocation by cave swiftlets. – *Behav. Ecol. Sociobiol.* 10: 119–123.
- Hackett, S. J. 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). – *Mol. Phyl. Evol.* 5: 368–382.
- Harshman, J. 1996. Phylogeny, evolutionary rates, and ducks. – Unpubl. thesis, University of Chicago, Ill.
- Huelsenbeck, J. P. and Crandall, K. A. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. – *Annu. Rev. Ecol. Syst.* 28: 437–466.
- Johnson, K. P. 2001. Taxon sampling and the phylogenetic position of Passeriformes: evidence from 916 avian cytochrome *b* sequences. – *Syst. Biol.* 50: 128–136.
- Johnson, K. P. and Clayton, D. H. 2000. Nuclear and mitochondrial genes contain similar phylogenetic signal for pigeons and doves. – *Mol. Phyl. Evol.* 14: 141–151.
- Johnson, K. P. and Sorenson, M. D. 1998. Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome *b* and ND2) in the dabbling ducks (Tribe: Anatini). – *Mol. Phyl. Evol.* 10: 82–94.
- Klicka, J., Johnson, K. P. and Lanyon, S. M. 2000. New World nine-primaried oscine relationships: constructing a mitochondrial DNA framework. – *Auk* 117: 321–336.
- Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Paabo, S., Villablanca, F. X. and Wilson, A. C. 1989. Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. – *Proc. Natl. Acad. Sci. USA* 86: 6196–6200.
- Konishi, M. and Knudsen, E. I. 1979. The oilbird: hearing and echolocation. – *Science* 204: 425–427.
- Lee, P. L. M., Clayton, D. H., Griffiths, R. and Page, R. D. M. 1996. Does behavior reflect phylogeny in swiftlets (Aves: Apodidae)? A test using cytochrome *b* mitochondrial DNA sequences. – *Proc. Natl. Acad. Sci. USA* 93: 7091–7096.
- Maddison, D. R. and Maddison, W. P. 2000. *MacClade: Analysis of Phylogeny and Character Evolution*. Version 4.0. – Sinauer and Associates, Sunderland, Mass.
- Marchant, S., Higgins, P. J. and Davies, S. J. J. F. 1999. *Handbook of Australian, New Zealand and Antarctic Birds*. Vol. 4. – Oxford University Press, Oxford.
- Medway, Lord 1959. Echo-location among *Collocalia*. – *Nature* 184: 1352–1353.
- Medway, Lord 1962. The swiftlets (*Collocalia*) of Niah cave, Sarawak. Ecology and the regulation of breeding. – *Ibis* 104: 228–245.
- Medway, Lord 1967. The function of echolocation among swiftlets. – *Anim. Behav.* 15: 416–420.
- Medway, Lord and Pye, J. D. 1977. Echolocation and the systematics of swiftlets, Chap. 19. – In: Stonehouse, B. and Perrins, C. (eds). *Evolutionary Ecology*. University Park Press, Baltimore, pp. 225–238.
- Posada, D. and Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. – *Bioinformatics* 14: 817–818.
- Price, J. J. and Lanyon, S. M. 2002a. Reconstructing the evolution of complex bird song in the oropendolas. – *Evolution* 56: 1514–1529.
- Price, J. J. and Lanyon, S. M. 2002b. A robust phylogeny of the oropendolas: polyphyly revealed by mitochondrial sequence data. – *Auk* 119: 335–348.
- Smyth, D. M. and Roberts, J. R. 1983. The sensitivity of echolocation by the grey swiftlet *Aerodramus spodiopygius*. – *Ibis* 125: 339–345.
- Sorenson, M. D. and Quinn, T. W. 1998. Numts: a challenge for avian systematics and population biology. – *Auk* 115: 214–221.
- Suthers, R. A. and Hector, D. H. 1982. Mechanism for the production of echolocation clicks by the grey swiftlet, *Collocalia spodiopygia*. – *J. Comp. Physiol.* 148: 457–470.
- Swofford, D. L. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods)*, Version 4.0. – Sinauer Associates, Sunderland, Mass.
- Thomassen, H. A., Wiersma, A. T., de Bakker, M. A. G., de Knijff, P., Hetebrij, E. and Povel, G. D. E. 2003. A new phylogeny of swiftlets (Aves: Apodidae) based on cytochrome-*b* DNA. – *Mol. Phyl. Evol.* 29: 86–93.

(Received 14 January 2003, revised 23 April 2003, accepted 28 April 2003.)