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Phylogenetic relationships amongst swifts and swiftlets: A multi locus approach

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Abstract

We recently reconstructed the troublesome swiftlet phylogeny using cytochrome-*b* mitochondrial DNA sequences. The relationship of the giant swiftlet (*Hydrochous gigas*) with swiftlets of the genus *Aerodramus* was, however, unresolved. In an attempt to clarify this issue, we now incorporated mitochondrial 12S rRNA and nuclear β-fibrinogen intron 7 nuclear DNA sequences with the cyt-*b* sequences of six swiftlet, two swift, and one hummingbird outgroup species. A partition homogeneity (PH) test, used to determine the congruence of phylogenetic signal between two sets of sequences, suggested that cyt-*b* and Fib7 sequences were incongruent and therefore should not be combined. However, further analyses revealed that the apparent incongruence was probably due to the high amount of variation in cyt-*b* sequences. Separate and combined analyses of the three sequences unambiguously placed *H. gigas* as the sister-group of *Aerodramus* and supported monophyly of the swiftlets. These results were supported by analyses of combined NADH dehydrogenase subunit-2 (ND2) and cyt-*b* sequences of *H. gigas* in combination with sequences previously published by other workers. Recently, it was shown that the pygmy swiftlet (*C. troglodytes*)—in our phylogenetic analyses consistently placed with other, non-echolocating, *Collocalia* species—is in fact able to echolocate. Echolocation thereby lost its value to distinguish between different swiftlet genera. Furthermore, the phylogenetic distribution of echolocation can be explained either by its single evolution at the base of the swiftlets, with subsequent loss, or by independent evolution in *Aerodramus* and *C. troglodytes*. Because yet unpublished data suggest that only the auditory nuclei in swiftlet brains show adaptations to echolocation, the latter explanation seems the more likely one.

Keywords: Swiftlet, phylogeny; 12S; β-Fibrinogen intron 7; NADH dehydrogenase subunit-2; Cytochrome-*b*; Echolocation; Partition homogeneity test

1. Introduction

Swifts (Apodidae) and swiftlets (Apodidae: Collocaliini) have since long been controversial in terms of their taxonomy and phylogeny. Swiftlets are highly unusual among birds for their echolocation ability, which is only shared with the South American oilbird (*Steatornis caripensis*). Among the swifts, the taxonomic and phylogenetic classification of particularly the swiftlets (a tribe within the swifts) has proven to be difficult. Mayr (1937) stated that "their classification presents the most difficult problem in the taxonomy of birds." As a reason for this he mentioned that "most of the species are of practically the same dull sooty gray coloration with almost the same development of the structural characters." Originally, all swiftlets were placed into a single genus, *Collocalia* (Gray, 1840), and this classification has been used for over a hundred years. However, in 1959 echolocation was discovered in swiftlets almost simultaneously by Medway (1959) and Novick (1959).

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This ability of several species to echolocate was for Brooke (1970, 1972) the main reason to revise swiftlet taxonomy. Brooke (1970, 1972) split the genus *Collocalia* s.l. into three different genera, i.e., non-echolocating *Collocalia* s.s., non-echolocating *Hydrochous* (comprising only a single species, the giant swiftlet *H. gigas*), and echolocating *Aerodramus*. However, no consensus was reached on Brooke's classification. In subsequent publications, these three genera were pooled into a single genus *Collocalia* s.l. again (e.g., Chantler and Driessens, 1995; Salomonsen, 1983) or split up into two or three different genera after all (e.g., Chantler et al., 1999; Sibley and Monroe, 1993). The first attempt to create a phylogenetic rather than a taxonomic classification of swiftlets was by Lee et al. (1996), who used mtDNA sequence data. However, Lee et al. (1996) sequenced only a limited portion (406 bp) of cytochrome-*b* DNA, resulting in a phylogeny with many peculiarities and unanswered questions. Thomassen et al. (2003) sequenced the complete cytochrome-*b* gene and the resulting tree supported swiftlet monophyly. Furthermore, echolocation was assigned to a single genus, i.e., Brooke's (1970, 1972) *Aerodramus*. The placement of *Hydrochous* was nonetheless still uncertain. The high amount of variation in cytochrome-*b* may have been the reason that the position of *Hydrochous* was not resolved with certainty.

The uncertain classification of *H. gigas* was not solved either in a subsequent phylogeny by Price et al. (2004). Price et al. (2004) incorporated more species of swifts and swiftlets in their analyses than previously had been done. Moreover, besides most of cytochrome-*b* (1058 bp), they used sequences (1078 bp) from an additional mitochondrial gene, NADH dehydrogenase subunit-2 (ND2). The resulting tree once more supported monophyly of the swiftlets and the subdivision of swiftlets into two clades, i.e., the echolocating genus *Aerodramus* and the genus *Collocalia* whose members, until recently, were thought unable to echolocate. However, in contrast with previous results, echolocation ability could no longer be attributed to a single genus. Price et al. (2004) discovered that the pygmy swiftlet (*C. troglodytes*) is able to echolocate, but that it was grouped with other members of non-echolocating *Collocalia*. Echolocation in *C. troglodytes* was suspected before, but had never been proven to exist until the study of Price et al. (2004). Thus, echolocation ability is present in two different clades of swiftlets. Unfortunately, Price et al. (2004) did not incorporate the non-echolocating *H. gigas* in their study. The position of *H. gigas*, however, is crucial to understand the evolution of echolocation in more detail and whether echolocation in swiftlets evolved once or several times.

We therefore incorporated two additional sequences into our original dataset of cytochrome-*b* sequences (Thomassen et al., 2003) in order to investigate the phylogenetic relationship of *H. gigas* with other members of the swiftlets. The added sequences, mitochondrial 12S rRNA (12S) and nuclear non-coding β-fibrinogen intron 7 (Fib7), evolve more conservatively than cytochrome-*b*. It was expected that inclusion of these sequences in the phylogenetic analyses would resolve the uncertain phylogenetic relationship of *H. gigas* with other swiftlets.

To gain additional evidence for the placement of *H. gigas*, we also sequenced cytochrome-*b* and ND2 mtDNA of two specimens of *H. gigas*. These sequences were added to the data of Price et al. (2004). Because 12S and Fib7 sequences were not available for the taxa and specimens of Price et al. (2004), and ND2 sequences were not available from our own dataset, analyses were done on the two different datasets: our own and that of Price et al. (2004) with two specimens of *H. gigas* added. In this paper, we will present the resulting phylogenies. Furthermore, we will discuss the combined use of different genes evolving at a range of rates and with different levels of saturation in a single phylogenetic reconstruction.

2. Materials and methods

2.1. Taxa, DNA regions, and sequencing methods

Blood samples of seven swift and swiftlet species from the Indo-Australian region and two swift species of Eurasian origin were used as DNA source (Table 1). The blood samples were collected on the spot of the capture of the birds. Samples were stored on FTA papers (Whatman Bioscience, Whatman Group, USA; courtesy of Professor P. de Knijff, Leiden University, The Netherlands) and kept dry using silica gel. The samples of the alpine swift (*Apus melba*) (courtesy of Dr. P. Bize, University of Bern, Switzerland) were stored in EDTA buffer. Because hummingbirds are generally supposed to be the sister-group of the swifts (e.g., Sibley and Alquist, 1990), one hummingbird species (courtesy of W. van Gestel (M.Sc.), Wageningen University, The Netherlands) was included in the analyses, using muscle tissue as the source of DNA.

DNA isolation from dried blood samples and muscle tissue was performed using the DNeasy Tissue Kit (Qiagen, Hilden, Germany). The manufacturer's protocol for animal tissue was followed and lysis was done overnight. The blood samples stored in EDTA buffer were treated according to the protocol for isolation of genomic DNA from whole nucleated blood.

Using PCR, the complete nuclear DNA β-fibrinogen intron 7 (Fib7) and the complete mitochondrial ribosomal subunit 12S rRNA (12S) were directly amplified from the isolated DNA. The primers L1263 tRNAPhe, H1858 12S rRNA, L1753 12S rRNA, and H2294 tRNAVal (Sorenson et al., 1999) were used to amplify