Cryptic speciation in the Lesser Elaenia *Elaenia chiriquensis* (Aves: Passeriformes: Tyrannidae)

FRANK E. RHEINDT¹⁴, NIELS KRABBE², ALISON K.S. WEE¹ & LES CHRISTIDIS¹

¹Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543
²Zoological Museum, University of Copenhagen, Universitetsparken 15, DK-2100, Copenhagen, Denmark
³National Marine Science Centre, Southern Cross University, Coffs Harbour, NSW Australia 2450
⁴Corresponding author. E-mail: dbsrfe@nus.edu.sg

Abstract

Tyrant-flycatchers (Tyrannidae) are a taxonomically confusing bird group containing a large degree of cryptic diversity that has only recently begun to be unraveled through the application of acoustic and molecular methods. We investigated all three subspecies of the Lesser Elaenia, *Elaenia chiriquensis* Lawrence, across their range using sound recordings as well as nuclear and mitochondrial markers. We show that two of the three subspecies, the nominate race from southern Central America and the widespread South American subspecies *E. c. albivertex* Pelzeln, have undergone very low levels of vocal and molecular differentiation across their fragmented range. In contrast, the isolated taxon *E. c. brachyptera* Berlepsch, endemic to the western and also, as recently shown, eastern slopes of the northern Andes, is phylogenetically and vocally distinct from other Lesser Elaenias, indicating that it constitutes a separate biological species.

Key words: Neotropics, Elaeniinae, diversification, cryptic differentiation, bioacoustics, Andes, Panamá, vocalization, mitochondrial DNA, nuclear DNA, Coopman’s Elaenia

Introduction

With 300–400 species in approximately 70 genera, the tyrant-flycatchers (Tyrannidae) are one of the richest bird families worldwide, but equally one of the least understood in terms of taxonomy (Fitzpatrick 2004). With generally inconspicuous plumage that often looks near-identical even across generic boundaries, many species have evaded formal recognition until recently (Fitzpatrick 2004), and additional cryptic species continue to be discovered (e.g. Coopmans & Krabbe 2000; Alonso & Whitney 2001; Johnson & Jones 2001; Zimmer et al. 2001; Herzog et al. 2008). Moreover, new insights into the importance of vocalizations for species recognition (Alström & Ranft 2003), especially in tyrannid flycatchers (Rheindt et al. 2008a), have revealed further tyrannid species diversity (e.g. Lanyon 1978; Reynard et al. 1993; Zimmer & Whittaker 2000). The rapid advancement of molecular methods in the field of phylogenetics over the past decade has also added to our knowledge of alpha taxonomy in tyrant-flycatchers (García-Moreno et al. 1998; Chesser 2000; Johnson & Cicero 2002; Joseph et al. 2003a, b; Joseph & Wilke 2004; Rheindt et al. 2008a, b, c; 2009a, b; 2013).

The genus *Elaenia* is a fairly homogeneous group of tyrant-flycatchers comprising 18 currently recognized species distributed mostly throughout drier and scrubber parts of the Neotropics (Hosner 2004). The identification of *Elaenia* flycatchers requires extreme care, as some species are challenging to distinguish even in the hand; this has created widespread confusion over distributional boundaries, vocalizations and even species limits within the genus (Zimmer 1941; Traylor 1982; Ridgely & Tudor 1994; Fitzpatrick 2004; Hosner 2004).

The Lesser Elaenia *Elaenia chiriquensis* is one of the most widespread members of the genus, occurring in open country throughout much of the Neotropics (Figure 1). The three subspecies are distributed as follows: nominate *chiriquensis* in Panama and Costa Rica; *E. c. brachyptera* in the border region between Ecuador and Colombia on the west slope of the Andes in the Chocó Region; *E. c. albivertex* throughout much of tropical and subtropical lowland South America, except for Amazonia (Figure 1). Recently a cis-Andean population tentatively attributed to *brachyptera* has been documented (Moore et al. 2013).
The three subspecies of *E. chiriquensis* differ in the hues of their body colouration, with *brachyptera* additionally being slightly smaller (Hosner 2004). Otherwise, they appear to differ little from one another. Within widespread *E. c. albivertex*, Zimmer (1941) found no clear plumage distinctions in a series of over 240 specimens from different parts of South America, and Bates *et al.* (2003) demonstrated comparatively low levels of mitochondrial divergence (c. 0.25% cytochrome-*b* and 0.28% ND2) between populations from the savannas of Amapá, Brazil, in the Guianan Shield and the cerrado regions of Dpto. Santa Cruz, Bolivia. They concluded that cerrado birds such as *E. chiriquensis* must have maintained higher levels of gene flow than Neotropical forest understorey birds with similarly wide distributions, or that cerrado may have expanded to parts of its present-day distribution fairly rapidly. Ridgely & Greenfield (2001) reported that the vocalizations of *E. c. brachyptera* differ from those of widespread *E. c. albivertex*, suggesting that the former taxon may deserve to be elevated to species rank.

As part of an on-going wider study on the evolutionary dynamics of the flycatcher genus *Elaienia*, we generated nuclear and mitochondrial DNA sequences for all three subspecies from across their range. Additionally, we analysed sound recordings of all three subspecies. We here present our molecular and vocal data on taxon differentiation within *E. chiriquensis* and discuss their taxonomic implications.

**FIGURE 1.** Distribution map of the *E. chiriquensis* complex roughly following Hosner (2004) with minor modifications; red: nominate subspecies (*chiriquensis*); blue: *brachyptera*; purple: *albivertex*; note that the eastern Andean population from the Río Maranon drainage of southern Ecuador into Peru is here shown to belong to *albivertex*.

**Material and methods**

**Molecular sampling regime.** We generated full-length sequences of the mitochondrial gene NADH dehydrogenase subunit 2 (ND2) for five samples of *E. c. albivertex* from large parts of its distribution, two samples of *E. chiriquensis* from Panama, and two samples of a population from the eastern Andean slope of Ecuador tentatively assigned to *brachyptera* by Moore *et al.* (2013; Table 1). Sequence data were also obtained for the nuclear β-fibrinogen intron 5 (Fib5) for the latter two Ecuadorian samples (Table 1). Our dataset was complemented by ND2 and Fib5 sequences of an additional *E. c. albivertex* reported by Tello & Bates (2007), and two *E. c. albivertex* individuals and one *E. c. brachyptera* from the west slope of the Andes as reported by Rheindt...
et al. (2008b). ND2 and Fib5 sequences of two *E. mesoleuca* (Deppe) individuals were used as outgroups to root the trees. The latter species was identified as the sister taxon of the *E. chiriquensis* complex in phylogenetic analyses including all but one *Elaenia* species (Rheindt et al. 2008b).

**Extraction, sequence generation and alignment.** For most samples, we extracted genomic DNA from frozen and ethanol-preserved tissue following standard extraction procedures as outlined by Gemmel & Akiyama (1996). For the two Ecuadorian east-slope samples, we extracted genomic DNA from ethanol-preserved tissue using the Exgene Clinic SV Genomic DNA Purification kit (GeneAll Biotechnology Co., Ltd, Seoul) following the manufacturer’s protocol. The complete ND2 gene (and partial sequence of the adjacent tRNA-Met) was amplified and sequenced in two overlapping fragments of approximately 370 and 750 base pairs (bp), using the primer pairs L5215 with H5578 (Hackett 1996) and FRND2.1 (Rheindt et al. 2008d) with H6315 (Kirchman et al. 2001), respectively. Additional sequences were amplified as a single fragment using L5215 and H6315. The Fib5 intron was amplified and sequenced using primer pairs Fib5 and Fib6 (Driskell & Christidis 2004). PCR conditions for both gene regions were similar to those described by Driskell & Christidis (2004). Amplified fragments were purified using the GFX Gel Band and PCR purification Kit (Amersham Bioscience Corp., Piscataway, New Jersey), the AMPure reagent (Agencourt Bioscience Corp., Beverly, Massachusetts), or ExoSAP-IT PCR cleanup reagent (Affymetrix, Inc., California). Purified PCR products were sequenced by cycle sequencing and dye terminator methods with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, California) on an automated sequencer (ABI 3130xl Genetic Analyzer, Applied Biosystems, California) or on a MegaBACE 1000 capillary DNA sequencer utilising the methods described by Norman et al. (2007).

We aligned and edited sequences using the program CodonCode Aligner 4.2.5 (CodonCode Corp., Massachusetts). All sequences were double-checked by eye. We used ORF Finder (www.ncbi.nlm.nih.gov/orffinder/orfig.cgi) to check for internal stop codons, reading frame shifts, anomalous substitution patterns, and deviant base composition.

**Phylogenetic analysis.** Phylogenetic inference was conducted using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference methods. MP and ML analyses were carried out in MEGA 5.2.2 (Tamura et al. 2011). We analyzed each data partition (ND2 and Fib5) separately. MEGA 5.2.2 was also used to perform raw ‘p’ sequence divergence calculations using the pairwise deletion option.

MP analyses were performed using a heuristic search of tree space with tree-bisection-reconnection branch swapping. Parsimony bootstrap tests were conducted with 10,000 replicates of full heuristic searches. For ML analyses, we used the software jModelTest (Posada 2008) to evaluate the likelihoods of 88 models of nucleotide sequence evolution using default settings. The best-fitting model was chosen according to the Bayesian Information Criterion. The optimal model selected for ND2 was the Hasegawa-Kishino-Yano model with invariant sites, and we also used this model for the concatenated dataset given that the ND2 partition is about twice as large and 5–6 times as variable as the Fib5 partition. ML bootstrap tests were performed with 10,000 replicates and heuristic searches of tree space, with initial trees generated either by neighbor-joining (Saitou & Nei 1987) or BIONJ, a modified neighbor-joining method (Gascuel 1997). Subtree-pruning-regrafting branch swapping was conducted on the dataset.

Bayesian inference was performed with BEAST v1.8 (Drummond & Rambaut 2007). Markov Chain Monte Carlo (MCMC) chains were run with a Hasegawa-Kishino-Yano model of nucleotide substitutions with invariant site and Yule tree prior. Four runs were completed with a randomly generated starting-tree topology. For each run, posterior estimates were obtained by sampling every 1000th MCMC step from a total of 10,000,000 steps. Using Tracer, we determined a reasonable burn-in for our runs of 200,000, with the corresponding ESS value >200, confirming that our runs had reached convergence. The remaining sampled trees were used to build a maximum clade credibility tree with TreeAnnotator v.1.8 (Drummond & Rambaut 2007) with a 2% burn-in.

**Acoustic Sampling Regime and Analyses.** Our vocal dataset consisted of 10 recordings of vocalizations of nominate *E. c. chiriquensis*, 109 recordings of *E. c. albivertex*, 19 recordings of *E. c. brachyptera* (7 of which are from its western Andean range, and 12 are from the eastern Andean slope population), 49 recordings of the outgroup species *Elaenia mesoleuca*, and 93 recordings of various other *Elaenia* species, for a total of 280 recordings. For the exact identity of recordings and their accession numbers in public sound libraries, see Appendix 1.

For spectrographic analysis, we used Cool Edit Pro (Syntrillium Software). Because vocalizations of *brachyptera* differ so strikingly from other *E. chiriquensis* groups, we struggled to assign call types to their
TABLE 1. Voucher or sample numbers, names of institutions, GenBank accession numbers, taxon identity and collection localities of all *E. chiriquensis* specimens and two outgroup samples; samples for which both ND2 and Fib5 were generated are printed in **bold**; abbreviations: FMNH—Field Museum of Natural History (Chicago); USNM—United States National Museum (Washington, D.C.); AMNH—American Museum of Natural History (New York); MECN—Museo Ecuatoriano de Ciencias Naturales (Quito); MG—Museu Goeldi (Belem); UFP—Universidade Federal de Pernambuco; LSUMZ—Louisiana State University Museum of Natural Science; ZMUC—Zoological Museum at the University of Copenhagen; LGEMA—Laboratório de Genética e Evolução Molecular de Aves (São Paulo); USP—Universidade de São Paulo.

<table>
<thead>
<tr>
<th>Institution and voucher or sample number</th>
<th>Genbank accession number</th>
<th>Taxon name</th>
<th>Collection locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voucher skin MG 53532 (field number MG CH-245); tissue FMNH 391476 **</td>
<td>ND2: EU310947, Fib5: EU310940</td>
<td><em>E. c. albivertex</em></td>
<td>Brazil: Amapá, Faz. Casimiro</td>
</tr>
<tr>
<td>Voucher skin FMNH 391476 **</td>
<td>EU310949</td>
<td><em>E. c. albivertex</em></td>
<td>Brazil: Pará, Monte Alegre</td>
</tr>
<tr>
<td>Voucher skin UFP uncatalogued (field number UFP MTE-084); tissue FMNH 392574</td>
<td>EU310942</td>
<td><em>E. c. albivertex</em></td>
<td>Guyana</td>
</tr>
<tr>
<td>Voucher skin USNM 625968; tissue USNM B12343</td>
<td>EU310946</td>
<td><em>E. c. albivertex</em></td>
<td>Guyana</td>
</tr>
<tr>
<td>Voucher skin USNM 649004; tissue USNM B4398</td>
<td>EU310943</td>
<td><em>E. c. albivertex</em></td>
<td>Venezuela: Bolivar, Auyan Tepui</td>
</tr>
<tr>
<td>Voucher skin AMNH 831265 (field number GFB 2854); tissue DOT 4789</td>
<td>EU310941</td>
<td><em>E. c. albivertex</em></td>
<td>Bolivia: Sta. Cruz, Velasco</td>
</tr>
<tr>
<td>Voucher skin LSUMZ 150966 **</td>
<td>ND2: EU310948, Fib5: EU310941</td>
<td><em>E. c. albivertex</em></td>
<td>Ecuador: Esmeraldas, 10 km W Lita</td>
</tr>
<tr>
<td>Voucher skin MECN 6363 (field number NK2-20.8.92); tissue ZMUC 125668 **</td>
<td>ND2: EU310951, Fib5: EU310939</td>
<td><em>E. c. brachyptera</em></td>
<td>Ecuador: E Andean slope at Rio Quijos, Napo</td>
</tr>
<tr>
<td>Voucher skin MECN 8740 (field number NK1-16.8.12); tissue ZMUC 148519</td>
<td>ND2: KP696505, Fib5: KP938993</td>
<td><em>E. c. brachyptera</em></td>
<td>Ecuador: E Andean slope at Rio Quijos, Napo</td>
</tr>
<tr>
<td>Voucher skin ZMUC 138277 (field number NK2-16.8.12); tissue ZMUC 148520</td>
<td>ND2: KP696506, Fib5: KP938994</td>
<td><em>E. c. brachyptera</em></td>
<td>Ecuador: E Andean slope at Rio Quijos, Napo</td>
</tr>
<tr>
<td>Voucher skin UFP uncatalogued (field number UFP MTE-017); tissue FMNH 392559</td>
<td>EU310950</td>
<td><em>E. c. albivertex</em> (museum skin erroneously labeled as <em>E. flavogaster</em>)</td>
<td>Brazil: Pará, Monte Alegre</td>
</tr>
<tr>
<td>Voucher skin UFP 53525 (field number MG CH-217); tissue FMNH 391472 **</td>
<td>ND2: DQ294543, Fib5: DQ294455</td>
<td><em>E. c. albivertex</em></td>
<td>Brazil: Amapá, Faz. Casimiro</td>
</tr>
<tr>
<td>Voucher skin USP uncatalogued; tissue LGEMA P2177 **</td>
<td>ND2: EU310945, Fib5: EU310937</td>
<td><em>E. mesoleuca</em> (outgroup)</td>
<td>Brazil</td>
</tr>
<tr>
<td>Voucher skin USP uncatalogued; tissue LGEMA P2176 **</td>
<td>ND2: EU310944, Fib5: EU310938</td>
<td><em>E. mesoleuca</em> (outgroup)</td>
<td>Brazil</td>
</tr>
</tbody>
</table>

* sequence from Tello & Bates (2007)
** sequence from Rheindt *et al.* (2008b)
homologous counterparts in sister taxa. Therefore, our bioacoustic analysis is largely qualitative and aims at the documentation of different call types within each taxon.

Results

Molecular characterization. We did not detect any stop codons or indels (insertions or deletions) in the ND2 gene or indels in Fib5. Consequently, alignments were straightforward. The ND2 fragment was 1066 bp long and contained 118 variable sites (=11.1%), whereas the Fib5 fragment was 544 bp long and contained 23 variable sites (=4.2%). Fib5 had a noticeably smaller proportion of variable sites as compared to ND2, and is therefore presumably of less value in phylogenetic inference at this taxonomic level.

Uncorrected ND2 sequence divergence (shown across taxa in Table 2) ranged from 7.9–8.3% between members of the *E. chiriquensis* complex and the outgroup *E. mesoleuca*. Within *E. chiriquensis*, the two individuals from the eastern Andean slope in Ecuador were characterized by very low (0.6%) ND2 divergence from the *brachyptera* sample from the western Andean Chocó region, suggesting a close affinity of east-slope birds not with the widespread taxon *albivertex*, but with *brachyptera* from west of the Andes. In contrast, the group comprising *brachyptera* (from Chocó) and Ecuadorian Andean east-slope individuals exhibited ND2 divergences of 3.7–4.3% from the other two taxa (*albivertex* and nominate *chiriquensis*). ND2 differentiation between the latter two subspecies was low (0.8–1.2%). Within widespread *albivertex*, divergences ranged from 0.1–0.6% despite the pronounced geographic separation of some of these samples.

Sequence divergence in the more slowly-evolving nuclear Fib5 intron (Table 2) ranged from 0.2–2.3% between the *E. chiriquensis* complex and the outgroup *E. mesoleuca*. Comparable levels of divergence were also recorded within the *E. chiriquensis* complex, with 0.2–3.1% between *E. c. brachyptera* and the other two taxa, and 0–1.9% between *albivertex* and nominate *chiriquensis*.

| TABLE 2. Uncorrected ND2 and Fib5 p-divergences between and within taxa in the *E. chiriquensis* complex and the outgroup *E. mesoleuca*; bold print indicates intra-taxon divergences. |
|----------------------------------|-----------------|----------------|-----------------|---------------|
|                                | albivertex      | chiriquensis   | brachyptera     | mesoleuca     |
| ND2                             |                 |                |                 |               |
| albivertex                      | 0.1–0.6         |                |                 |               |
| chiriquensis                    | 0.8–1.2         | 0.2            |                 |               |
| brachyptera                     | 3.9–4.3         | 3.7–3.9        | 0.0–0.6         |               |
| mesoleuca                       | 7.9–8.3         | 8.1–8.2        | 8.3             | 0.4           |
| Fib5                            |                 |                |                 |               |
| albivertex                      | 0.4–1.7         |                |                 |               |
| chiriquensis                    | 0.0–1.9         | 0.4            |                 |               |
| brachyptera                     | 0.9–3.1         | 0.2–1.3        | 0.0–0.2         |               |
| mesoleuca                       | 0.4–1.3         | 0.2–1.1        | 1.7–2.3         | 0.8           |

Molecular phylogenetics. MP, ML and Bayesian analyses of the ND2 dataset all recovered a deep division of the complex into a clade containing the three *brachyptera* samples versus a clade consisting of all *albivertex* and nominate *chiriquensis* samples with high branch support for each clade (Figure 2). Within each of these two clades, there was only shallow differentiation among samples. Apart from variations in the exact branch support values, there were limited differences among trees resulting from different analytical modes. The same topology and general branch support were recovered in MP, ML and BI analyses of the concatenated dataset (Figure 3). This may reflect a lack of phylogenetic resolution in the Fib5 partition, which produced completely unresolved and polytomous trees with all analytical modes (not shown). Given the limited phylogenetic utility of Fib5 in *Elaenia* flycatchers at this taxonomic level, subsequent discussions are based on analysis of the ND2 sequences only.
FIGURE 2. Bayesian tree of the ND2 partition; numbers at nodes indicate MP bootstrap support / ML bootstrap support / Bayesian posterior probability. Support values below 90 not shown.

FIGURE 3. Bayesian tree of the concatenated dataset (using both partitions); for explanation of nodal support values, see Figure 2.
Bioacoustic analysis. The vocalizations of *E. c. chiriquensis* and *E. c. albivertex* were found to be strikingly similar (Figure 4a–d). Vocalizations of *E. c. brachyptera*, on the other hand, differed from the former two taxa as much as from any other species of *Elaenia* (Figures 4–5). Dawn song of brachyptera differed distinctly in the number and quality of its elements (easily recognizable units); one call type, a simple up-down-stroke (rise and fall in pitch) (Figure 6), differed in pitch and relative volume, and another call (Figure 7f), perhaps best described as a rattle, differed in so many respects that no homologous call of chiriquensis or albivertex was apparent. A simple up-down-stroke call is generally widespread in the genus, in some species including *E. mesoleuca* and *E. flavogaster* only given as a burred note (i.e. a rapid series of alternating up- and down-strokes), in others, such as *E. albiceps* and *E. pallatangae*, both in a burred and a pure whistled form. Therefore, its absence or presence in a taxon may not provide much phylogenetic information. The rattle call of brachyptera, however, was not detected in any other taxon in the genus and must be considered tentatively a vocal autapomorphy.

Dawn song: The most complex tyrannid vocalization is the dawn song, which is normally only given by the male for a brief period at predawn and early dawn and occasionally at dusk, but only very rarely during the day (Ridgely & Tudor 1994). We suspect that in some species the dawn song may be a pair duet. The dawn song is rarely heard outside the breeding season. Most tyrannid dawn songs, including those of *Elaenia* mesoleuca and all three forms of *E. chiriquensis*, consist of two phrases, one fairly simple and the other more complex. Typically, the simple phrase is repeated several times before the song is terminated with a complex phrase. At the peak of song activity, pauses between songs may be so brief that it is difficult to determine when one song ends and the next begins. The number of times the simple phrase is repeated varies from song to song, and sometimes the complex phrase may be repeated a few times. Occasionally an individual may repeat only one of the two phrases for extended periods of time.

An examination of available dawn songs in xeno-canto (www.xeno-canto.org) and Macaulay Library of Natural Sounds (www.macaulaylibrary.org) showed that for the genus *Elaenia* as a whole, the introductory phrase of the dawn song is usually rather stereotyped throughout the range of a species except for slight differences in pitch among individuals, whereas the more complex termination may be subject to some variation, not only among individuals and regions, but also by the same individual during a bout of songs. Unless occasional call notes are included, variations of complex phrases usually only involve a change in the order of the elements in the phrase; a new element is almost never included.

In the dawn songs examined of nominate *Elaenia chiriquensis* (n=2) and *E. c. albivertex* (n=31), structure varied little, and in 25 dawn song recordings of *E. mesoleuca* the only structural variation was omission (n=8) of the first element of the complex phrase, and replacement (n=1) of the second element with a call note.

In dawn songs of *E. c. chiriquensis* and *E. c. albivertex* (Figures 4a–d and 5b) the complex phrase consisted of three elements. The first element was identical to the simple phrase and was a double-noted tirí, the higher first note a pure up-downstroke and the second note a rising burred note. The second element of the complex phrase was usually given twice but sometimes thrice, and was occasionally just given once. There was minor variation in the length and quality of mid-notes between individuals.

FIGURE 4. Complex (terminal) phrase in dawn songs of three subspecies of *Elaenia chiriquensis*. a: *E. c. chiriquensis*; b–d: *E. c. albivertex* (b from Venezuela, c from southeastern Brazil, d from the Río Marañón drainage, southernmost Ecuador); e: *E. c. brachyptera* (from eastern Ecuador). Note the similarity of *E. c. chiriquensis* and *E. c. albivertex* and the difference of *E. c. brachyptera*. In *chiriquensis* and *albivertex* the number of times the mid-element was repeated varied even in the same individual; it was usually repeated once, but sometimes twice, and was occasionally just given once. There was minor variation in the length and quality of mid-notes between individuals.

FIGURE 5. Complex (terminal) phrase in dawn songs of three closely related forms of the genus *Elaenia*. A: *E. mesoleuca*; B: *E. chiriquensis albivertex*; C: *E. chiriquensis brachyptera*. Note the peak volume at high pitch, the fewer elements in *brachyptera*, and the differences in quality of the elements between all three taxa.

FIGURE 6. Whistled calls of *Elaenia chiriquensis*. a: *E. c. chiriquensis*; b–d: *E. c. albivertex* (b from Venezuela, c from Bolívia, d from the Río Marañón drainage in southernmost Ecuador); e–f: *E. c. brachyptera* (e from eastern, f from western Ecuador). Note the higher pitch and the asymmetry in e and f. This is the most commonly heard call of these forms.

FIGURE 7. Other calls of *Elaenia c. chiriquensis* (a), *E. c. albivertex* (b–c), and *E. c. brachyptera* (d–f). The “rattle” of *brachyptera* (f) is quite unlike calls of other taxa in the genus.
In *E. mesoleuca* (Figure 5A) the complex phrase was usually also composed of three elements, but it was the second element that is identical to the simple phrase. The first element (omitted in 8 of 25 recordings) was a sharp, high-pitched, rising (1.1–5 kHz) pure note, 0.03–0.05 s long, occasionally ending with a faint down-stroke, quite unlike any call given by the species or related forms. The second element was a rising (1–4 kHz), burred note, the first half of which was composed of fast-paced strokes, the second half of slow-paced strokes. The third element was a low and evenly pitched (2–3 kHz) burred note fairly constant in length (0.12–0.15 s) but subject to considerable individual variation in the number of strokes (4–11).

Owing to the scarcity of the taxon, only a single dawn song recording was available for *brachyptera* (Figures 4e and 5c). It differed distinctly from nominate *chiriquensis* and *albivertex* in three respects: (1) by having only two elements in the complex phrase, none repeated; (2) by the quality, very high pitch, and relative volume of the first element; (3) and by the sound quality of the second element. The first element was a sharply rising and high-pitched whistle similar to the simple phrase, beginning at c. 2.5 kHz and suddenly rising to over 6 kHz with most volume at high pitch. The second element was double-noted, beginning with a burred note of 5–6 up-down-strokes and continuing into a single, equally long down-up-stroke with lowest pitch four semitones below the burred note. It differed as much from dawn song of *E. c. chiriquensis* and *E. c. albivertex* as dawn songs of other species of *Elaenia* did from each other. Several individuals were heard to give similar dawn songs when the existing recording was made, so the recording was presumed to be representative, an assumption also supported by birds at two different localities in eastern Ecuador reacting to playback of this recording during the day by approaching closely, calling, and spreading their crest.

**Calls:** The recorded calls of *E. c. chiriquensis* from Panama and Costa Rica could be divided into two different call types: “whistled” calls (7 recordings), and “burred” calls (1). The whistled calls of *E. c. chiriquensis* (Figure 6a) were almost identical to whistled calls on 39 recordings of *E. c. albivertex* (Figure 6b–d). They averaged 2–3 semitones higher-pitched but did not differ in any other aspect, and only two of them exceeded the highest-pitched call recorded in *albivertex*, and then only by one and two semitones. The single recording of a burred call of nominate *chiriquensis* (Figure 7a) was fairly similar to one of the calls given by *albivertex* (Figure 7b). A variety of burred calls of *E. c. albivertex* ranged from whistled calls with a small burred section to entirely burred calls of various lengths and pitches (some shown in Figure 7b–c), all differing from the most commonly given calls of *E. mesoleuca* in both pitch and quality.

The recorded calls of *E. c. brachyptera* (Figures 6e–f and 7d–f) were from at least 18 different individuals (12 from east and 6 from west of the Andes) and could be divided into three different types: “whistled call” (15), “burred call” (10), and “rattle” (5), besides chase calls and begging calls. All differed in at least two aspects from vocalizations of *chiriquensis* and *albivertex* as well as from the outgroup *E. mesoleuca*. The whistled call was the commonest call recorded. It is presumed to be homologous to the whistled call of *albivertex*, but averages 7–8 semitones higher and is distinctly asymmetrical, with the peak volume extending to the down-stroke rather than being evenly placed around the peak frequency (Figure 6). This call led Bret Whitney (pers. comm.) to suggest that *brachyptera* should be treated as a distinct species, which led NK to collect a specimen for molecular and morphological comparisons. Burred calls of *brachyptera* averaged 1–2 semitones lower than the whistled calls but still six semitones higher than in *chiriquensis* and *albivertex*, and most showed a tendency to asymmetrical volume around the peak frequency (Figure 7d–e) as did the whistled call (Figure 6e–f). The “rattle” call of *brachyptera* (Figure 7f) was distinctly different from calls of all other taxa in the genus examined and was the vocalization that led the late Paul Coopmans (pers. comm.) to suggest that *brachyptera* should be elevated to species rank even before its distinctive dawn song was recorded. It was a 0.3–1.0 s long series of 5–15 notes with peak frequency around 5.2 kHz (4.6–6.0 kHz), usually slightly rising at first and falling at the end, notes given at a fairly even pace of about 20 (16–23) notes per s, with each note composed of two or three connected up-down-strokes, the first and last notes often of fewest strokes.

**Discussion**

Analyses of the mitochondrial ND2 gene corroborate that the subspecies *albivertex* is monophyletic, with shallow differentiation over its large range (Figures 1–3), despite the wide distribution of its savannah habitat along the margins of the Amazonian rainforest bloc. The data provide no evidence of obvious phylogeographic structuring.
within this taxon (Figures 2, 3). This result is in agreement with Bates et al. (2003), who also found comparably low ND2 divergences (0.1–0.5%) over the whole of this taxon’s range, and lower divergences between some samples from opposite ends of the range than between samples of the same provenance.

Despite their geographic separation across the Darién Gap and northern central Colombia, *albivertex* and the nominate subspecies *chiriquensis* were relatively poorly differentiated in terms of mitochondrial DNA. Their conspecific treatment is further supported by our detection of limited acoustic differentiation at best (Figures 4, 7, 8).

South American populations of *E. chiriquensis* east of the Andes have invariably been attributed to the taxon *albivertex* (e.g. Hosner 2004). However, vocal encounters with populations along the east slope of the Ecuadorian Andes cast this attribution in doubt (Moore et al. 2013). Here, our vocal and molecular analyses unequivocally demonstrate that these populations form a tight-knit group with *brachyptera*, which had heretofore been assumed to be endemic to the western Andean Chocó region.

We propose the English name “Coopmans’s Elaenia” for *E. brachyptera*, in honour of the late Paul Coopmans’s important contributions to Neotropical ornithology, and in recognition of the fact that it was through his ears that this taxon’s presence in eastern Ecuador first came to be recognised.

**Acknowledgements**

This contribution is dedicated to the memory of Paul Coopmans, who met his untimely death in January 2007. His suggestion that *brachyptera* is vocally distinct was the primary trigger for FER to pursue DNA material of the taxon. We owe a special debt of gratitude to John Moore and Robert Ridgely for helpful suggestions and comments. We thank Bret M. Whitney for first pointing out, in 1992, the distinctive vocalizations of *brachyptera*, and Dusan Brinkhuizen and Luis Salagaje for help in field identification or locating of *brachyptera* in eastern Ecuador. We thank the following people and institutions for kindly lending us *Elaenia* tissue for DNA analysis: Donna Dittmann and Robb Brumfield (Louisiana State University Museum of Natural Sciences), David Willard and Shannon Hackett (Field Museum of Natural History), Paul Sweet (American Museum of Natural History), Christopher Huddleston (Smithsonian National Museum of Natural History), Cristina Miyaki and Gustavo
Sebastián Cabanne (Laboratório de Genética e Evolução Molecular de Aves, Universidade de São Paulo) and Jon Fjeldså (Zoological Museum, University of Copenhagen). This work was supported by the following grants to FER: a National University of Singapore Department of Biological Sciences start-up grant (WBS R-154-000-583-651) and Faculty of Science start-up grant (WBS R-154-000-570-133); the Joseph Grinnell Student Research Award awarded by the Cooper Ornithological Society; Sigma Xi Grant-in-Aid of Research; and Museum Victoria 1854 Student Scholarship. Labwork was partly funded by the Ian Potter Foundation and Amersham Biosciences (now GE Healthcare).

References


http://dx.doi.org/10.1076/snef.38.2.87.15924


http://dx.doi.org/10.2307/2410897


http://dx.doi.org/10.1006/mpev.1999.0774


http://dx.doi.org/10.1046/j.1365-294x.2000.01020.x


http://dx.doi.org/10.1016/j.ympev.2003.10.017


http://dx.doi.org/10.1186/1471-2148-7-214


http://dx.doi.org/10.1039/c39795000000


http://dx.doi.org/10.1093/oxfordjournals.molbev.a025808


http://dx.doi.org/10.1016/S0168-9525(96)80005-9


http://dx.doi.org/10.1006/mpev.1996.0032


http://dx.doi.org/10.1525/auk.2008.07038


http://dx.doi.org/10.1046/j.1365-294X.2002.01588.x


http://dx.doi.org/10.1071/MU03047


http://dx.doi.org/10.1046/j.1365-2699.2003.00841.x


http://dx.doi.org/10.1016/j.ympev.2008.04.016


http://dx.doi.org/10.1642/0004-8038(2001)118[0849:PASOGR]2.0.CO;2


http://dx.doi.org/10.1011/1/j.1463-6409.2008.00369.x


http://dx.doi.org/10.1010/j.ympev.2007.09.011


http://dx.doi.org/10.1046/j.1365-2699.2003.00841.x


http://dx.doi.org/10.1111/j.1095-8312.2012.02036.x


E. chiriquensis chiriquensis: 10 (ML 7426, 46397, 165831, 165856, 165862–165863, 184520, 184527; XC 2816, 53709).


E. chiriquensis brachyptera: 19 [7 from western Colombia and Ecuador and 12 from eastern Ecuador: XC 85110, 116575, 157107, 157133–34, 157138–40, 157189–90, 157195, 158215, 169413, 169417; also a recording published by Krabbe & Nilsson (2003; cut 3), one by Moore *et al.* (2013; cut 3), an unpublished recording by Dušan Brinkhuizen, and two unpublished recordings by Paul Coopmans].

E. mesoleuca: 49 (ML 19568–78, 20050, 22170, 56344, 56348, 85625, 88083, 103881, 103886, 103903, 103906, 143848, 178137, 178166, 178168–69, 178177; XC 10454, 13510, 16330, 23765, 30466, 33238, 41818, 42051, 59830, 60392, 81538–40, 81543–44, 93328, 93406, 98813, 103906, 118643, 144990, 153670). Examined recordings in ML and XC of other species of *Elaenia* (primarily dawn songs): *E. albiceps* 30; *E. cristata* 3; *E. dayi* 2; *E. fallax* 1; *E. flavogaster* 13; *E. frantzii* 6; *E. gigas* 4; *E. martinica* 1; *E. obscura* 5; *E. pallatangae* 3; *E. parvirostris* 7; *E. pelzelni* 1; *E. ridleyana* 1; *E. ruficeps* 3; *E. spectabilis* 10; *E. strepera* 3.