

# Integrative taxonomy of Flatbill Flycatchers (Tyrannidae) reveals a new species from the Amazonian lowlands

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## Abstract

Integrative taxonomic studies continue to reveal that many current polytypic species of birds are in fact constituted by two or more species and therefore have been central in uncovering ‘hidden’ or ‘cryptic’ biodiversity. The Olivaceous Flatbill (Aves: Tyrannidae: *Rhynchocyclus olivaceus*) currently has nine recognized subspecies distributed throughout the Neotropics, but so far, no complete phylogenetic hypothesis exists to test the validity and evolutionary relationships among them. To remedy this, we conducted a multi-character integrative taxonomic revision of the genus *Rhynchocyclus*, focusing on the polytypic *R. olivaceus*. The combination of a taxonomically dense sampled multilocus phylogeny (including three mitochondrial and two nuclear genes) with phenotypic analyses including morphological and vocal characters pointed to several taxonomic inconsistencies within *R. olivaceus*. The analyses strongly support that *R. olivaceus* is paraphyletic, with an exclusively cis-Andean clade (where the topotypic *R. olivaceus* is found) clustering as sister to *Rhynchocyclus fulvipectus*, to the exclusion of a clade grouping trans-Andean and western Amazonian populations currently placed in *R. olivaceus*—one of which is unnamed and fully diagnosable based on vocal and genetic characters. Consistent with the phylogenetic results, our vocal analyses identified at least four morphologically cryptic lineages within *R. olivaceus* that can be mutually diagnosed from each other by different loudsongs and call parameters. Therefore, we provide evidence for splitting these four groups into separate species, two of which are sympatric but not syntopic in western Amazonia, including an unnamed species described herein—*Rhynchocyclus cryptus*, sp. nov. urn:lsid:zoobank.org:act:2DC17190-2BDD-49EC-88E6-4CF2FC2562A3.

## KEYWORDS

bioacoustics, cryptic speciation, molecular systematics, neotropics, phylogeography

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## 1 | INTRODUCTION

Birds represent one of the vertebrate groups for which the largest amount of taxonomic, evolutionary and ecological data are available (Tobias et al., 2020). However, recent studies have shown the existence of large gaps in this knowledge, especially among tropical lineages (Barrowclough et al., 2016; Whitney & Cohn-Haft, 2013). For example, many new species were discovered and named from Amazonia in recent years, revealing a far from complete knowledge about Amazonian bird biodiversity (e.g. Carneiro et al., 2012; Moncrieff et al., 2017; Portes et al., 2013; Whitney & Cohn-Haft, 2013; Whittaker et al., 2013). Moreover, recent studies have proposed that several taxa (including many polytypic species) with wide distributions across the Amazon basin are in fact complexes of multiple species (Aleixo et al., 2013; Batista et al., 2013; Rodrigues et al., 2013; Thom & Aleixo, 2015; Cerqueira et al., 2016; Ferreira et al., 2017; Reis et al., 2019; Schultz et al., 2017; Smith et al., 2017). These cryptic taxa usually represent allopatric populations with strong genetic differentiation, distinct vocal parameters, but which are very similar in external morphology. Certainly, new studies will continue to reveal a greater diversity than that currently known for most Amazonian groups, especially if an integrative approach to taxonomy is included in systematic reviews (Carneiro et al., 2012; Padial et al., 2010; Pante et al., 2014; Venkatraman et al., 2019). Herein, we present an integrative study on the systematics of Flatbills (*Rhynchocyclus* Cabanis & Heine, 1859), with a focus on the polytypic and widely distributed Olivaceous Flatbill—*Rhynchocyclus olivaceus* (Temminck, 1820).

Flatbill flycatchers belong to the Tyrannidae, an exclusively New World family (del Hoyo et al., 2021). *Rhynchocyclus* currently includes four species (del Hoyo et al., 2021; Gill et al., 2021; Remsen et al., 2021; Figure S1): *R. olivaceus* (Temminck, 1820), *Rhynchocyclus brevirostris* (Cabanis, 1847), *Rhynchocyclus pacificus* (Chapman, 1914) and *Rhynchocyclus fulvipectus* (Sclater, 1860). Two of these are monotypic (*R. pacificus* and *R. fulvipectus*), and the other two are polytypic (*R. olivaceus* and *R. brevirostris*). While *R. pacificus* and *R. brevirostris* share trans-Andean distributions, *R. fulvipectus* is exclusively Andean, whereas *R. olivaceus* is widely distributed in both Cis and Trans-Andean lowland evergreen forests (Del Hoyo et al., 2021).

As the most widespread species in the complex, all nine subspecies of *R. olivaceus* are very similar to each other, with a remarkable constancy in morphometric characters and plumage colour patterns, which evidences their cryptic nature (del Hoyo et al., 2021). All are large-headed, and flat-billed; with whitish eye rings; upper parts olive-green; wings and tail dark brown with olive-green (edged feathers); lower parts greyish-green, striated

with yellowish; centre of the abdomen yellowish; iris dark-brown; maxilla blackish; mandible white; and tarsi bluish-grey (del Hoyo et al., 2021; Novaes & Lima, 2009). However, unlike morphology, vocal patterns among subspecies of *R. olivaceus* appear to have conspicuous differences. Boesman (2016) suggested the existence of at least two groups diagnosed by vocalizations—a conclusion reached from a brief analysis of recordings available from online databases. The two acoustic groups were considered related to different subspecies groups, which were named the *olivaceus* and *aequinoctialis* subspecies groups (del Hoyo et al., 2021). The former group included subspecies *Rhynchocyclus olivaceus olivaceus*, *Rhynchocyclus olivaceus guianensis* and *Rhynchocyclus olivaceus sordidus* (distributed in eastern Amazonia and the Atlantic Forest), while the latter grouped subspecies *Rhynchocyclus olivaceus aequinoctialis*, *Rhynchocyclus olivaceus bardus*, *Rhynchocyclus olivaceus mirus*, *Rhynchocyclus olivaceus flavus* and *Rhynchocyclus olivaceus jelambianus* (found in western Amazonia, Trans-andean South America and Central America; Boesman, 2016). The taxonomic proposal of Boesman (2016) was never formally presented in a peer-reviewed journal, and it is not widely adopted (e.g. Gill et al., 2021; Remsen et al., 2021; but see del Hoyo et al., 2021).

It is therefore clear that a broad and integrative systematic review of flatbills is needed. Moreover, there is no proposed phylogenetic hypothesis with an inclusive taxon sampling across *Rhynchocyclus*. Five studies used sequences of up to three species of *Rhynchocyclus* (Ohlson et al., 2008, 2012, 2013; Rheindt et al., 2007; Tello et al., 2009), with only one previous study (Harvey et al. (2020), sampling all currently recognized species in the genus. It is important to note that none of these studies was focused on *Rhynchocyclus*, but instead on higher-level relationships within either Suboscine passerines or the Tyrannidae/Rhynchocyclidae families.

Considering the absence of a modern study focused on the phylogenetic systematics of *Rhynchocyclus*, we conducted an integrative, multi-character, taxonomic review of *Rhynchocyclus*, focusing on the subspecies of *R. olivaceus*.

## 2 | METHODS

### 2.1 | Genetic sampling

To infer the phylogenetic relationships of *Rhynchocyclus*, we used DNA sequence data from 99 specimens belonging to all recognized species in the genus (Gill et al., 2021). Of these, 83 samples come from five of the subspecies of *R. olivaceus* (*R. o. aequinoctialis*, *R. o. bardus*,

*R. o. guianensis*, *R. o. olivaceus* and *R. o. sordidus*; Gill et al., 2021). Unfortunately, we could not sample the remaining recognized subspecies (*R. o. jelambianus*, *R. o. tamborensis*, *R. o. flavus* and *R. o. mirus*) because there were no tissue samples available to us. The remaining 10 samples sequenced belong to other *Rhynchocyclus* species (*R. brevirostris* [ $n = 1$ ]; *R. fulvipectus* [ $n = 4$ ]; and *R. pacificus* [ $n = 4$ ] — Figure S1). In addition, three samples of two species of the sister genus *Tolmomyias* (*T. poliocephalus* and *T. flaviventris*) were used as outgroups and to root the tree (see Harvey et al., 2020). For a complete list of all specimens sequenced and their institutions of origin, see Table S1.

DNA was extracted from tissue samples (muscle or liver) using a phenol–chloroform method as described in Sambrook and Russel (2001) for samples obtained from MPEG, and using a DNeasy tissue extraction kit (Qiagen), to extract samples from other collections (Table S1). We used PCR to amplify three mitochondrial genes (*Cytochrome B*—Cytb, 634 pb; *NADH subunit 2*—ND2, 815 pb; and *Cytochrome oxidase I*—COI, 728 pb) and two nuclear loci (*Glyceraldehyde-3-phosphodehydrogenase intron 11*—G3PDH, 431 pb; and *Muscle-specific receptor tyrosine kinase intron 3*—MUSK, 599 pb—see Table S4 for primer and PCR conditions). PCR products were purified with polyethylene glycol (PEG8000, 20% filtered) and sequenced with Big Dye Terminator 3.1 (Applied Biosystems) following the manufacturer's protocols. All procedures were performed at the molecular biology laboratory of the Museu Paraense Emílio Goeldi (MPEG).

## 2.2 | Genetic data processing and phylogenetic analyses

Sequences of each gene were edited manually in Bioedit v7.2.5 (Hall, 1999) and aligned using the default settings of MAFFT (Katoh et al., 2002). Heterozygous and ambiguous positions were coded according to the IUPAC nomenclature. Alignments were concatenated in SequenceMatrix (Vaidya et al., 2011). We used PartitionFinder (Lanfear et al., 2012) to determine the best evolutionary model for each gene, and the best partitioning scheme using the criterion of Bayesian information. The evolutionary models selected for the genes used were HKY+I+G for COI; GTR+G/K80+G/HKY+I+G for the three different codons of CytB; K80+I+G for G3PDH; HKY for MUSK; and HKY+G/HKY+I/GTR for the three different codons of ND2. To infer the phylogenetic tree, we used MrBayes V3.2.6 (Ronquist & Huelsenbeck, 2003). Tree search was ran for 100,000,000 generations, with two heated chains, and trees and parameters sampled every 10,000 generations. We discarded 10% of the total sampled trees, as burn-in. We used TRACER software v1.6 (Rambaut

et al., 2013) to analyse whether stationarity was reached by Markov chains, and posterior values of effective sample sizes of parameters met the threshold of  $>200$ .

To investigate the genetic structure patterns in *R. olivaceus*, we built haplotype networks for each gene with the HaplotypeViewer software (Salzburger et al., 2011). To this end, we excluded all missing data, retaining a matrix with the following dataset: COI (728 bp), ND2 (815 bp), CYTB (634 bp), G3PDH (382 bp) and MUSK (543 bp). Because this software requires a phylogeny as an input, we used MrBayes to perform a Bayesian inference of the phylogenies for each gene. Tree search was run for 1,000,000 of generations, with two heated chains, and trees and parameters sampled every 10,000 generations.

We used MEGA 6.06 (Tamura et al., 2013) to calculate uncorrected 'p' distances within and between groups, for each gene separately, with the bootstrap method chosen and 500 replicates made. The groups considered were those recovered from the phylogenetic tree.

## 2.3 | Morphological analyses

We examined 113 skins, belonging to clades recovered in the molecular phylogeny and from the subspecies *R. o. olivaceus*, *R. o. guianensis*, *R. o. sordidus* and *R. o. aequinoctialis*; Gill et al., 2021—Fig. S1). All specimens examined are housed at MPEG, Belém, Brazil. We used Smithe (1975) as a standard colour reference when describing plumage of the throat, chest, belly, wing (upper, middle, lower and border portions) and tail. Measurements were taken with a digital calliper to the nearest 0.1 mm by CCS for each specimen. We measured six characters: (a) BL—bill length from distal point of the nostrils to bill tip; (b) BW—bill width at the distal point of the nostrils; (c) BH—bill height at the point above the nostrils; (d) WL—wing length; (e) TL—tarsus length; and (f) TailL—tail length. Because no statistically significant differences were detected between sexes for any of the morphometric characters measured, both males and females were combined in the analyses described below. We compared specimens based on groups (clades) recovered by the molecular phylogeny and used only skins from specimens that were sequenced in this study and whose sex could be determined. To test differences in individual measurements among groups, we performed ANOVA with a Tukey test for a posteriori comparison. We performed a principal component analysis (PCA) to assess variation in the morphometric space and test multivariate differences among samples. Descriptive statistics, ANOVA and PCA were performed on R 3.6.1 (R Development Core Team, 2020). A complete list of specimens used in the morphological analyses can be found in Table S2.

## 2.4 | Vocal analyses

We analysed 84 song recordings of *R. olivaceus*, belonging to all subspecies currently recognized, except *R. o. mirus*. We also analysed recordings of *R. brevirostris* ( $n = 16$ ), *R. fulvipectus* ( $n = 9$ ) and *R. pacificus* ( $n = 10$ ; Figure S1 and Table S3). Only one song emission per recording was measured and used in the analyses. As *Rhynchocyclus* species have a wide variety of vocal emissions in different situations, vocalizations were tentatively categorized into two main vocal types: loudsongs and calls. Loudsongs consist on series of notes ascending or descending in pitch, normally uttered under natural conditions while birds participate in mixed flocks. Some variation in song length exists even within some populations, with shorter versions called herein ‘short songs’ usually consisting of an introductory longer note, followed by a small series of shorter notes. In contrast, calls are shorter series of more uniformly shaped notes descending in frequency and with an overall rough or hoarse quality, normally uttered in alarm situations.

Vocalizations are quite different between groups analysed, and due to uncertainties about homology of calls, we used just loudsongs in statistical analyses. For loudsongs, we measured six characters: (a) number of notes—NN; (b) duration of total phrase—D; (c) peak frequency of total phrase, a measure of frequency at which maximum power occurs within a song bout—PF; (d) peak frequency of first note—PF1; (e) peak frequency of last note—PF2; and (f) delta peak frequency, calculated as the difference in peak frequencies between the first and last notes. Delta peak frequency was used to characterize the overall ascending (negative values) or descending (positive values) structure of loudsongs. Before taking measurements, all recordings were converted to ‘.wav’ file format with 44.1 kHz sampling rate and 16 bits in mono channel. As in morphometric analyses, we performed ANOVA with Tukey tests and a PCA to compare clades recovered by the molecular analyses. Vocal characters were measured from spectrograms and oscillograms using Raven Pro 1.5 (Bioacoustics Research Program, 2013). Figures derived from spectrograms and oscillograms were made using following parameters: window type—Blackman; window size—512 samples; time grid—95% overlap; and DFT size—1,024 samples. Detailed information about sound recordings used is available in Table S3.

## 3 | RESULTS

### 3.1 | Phylogenetic analysis

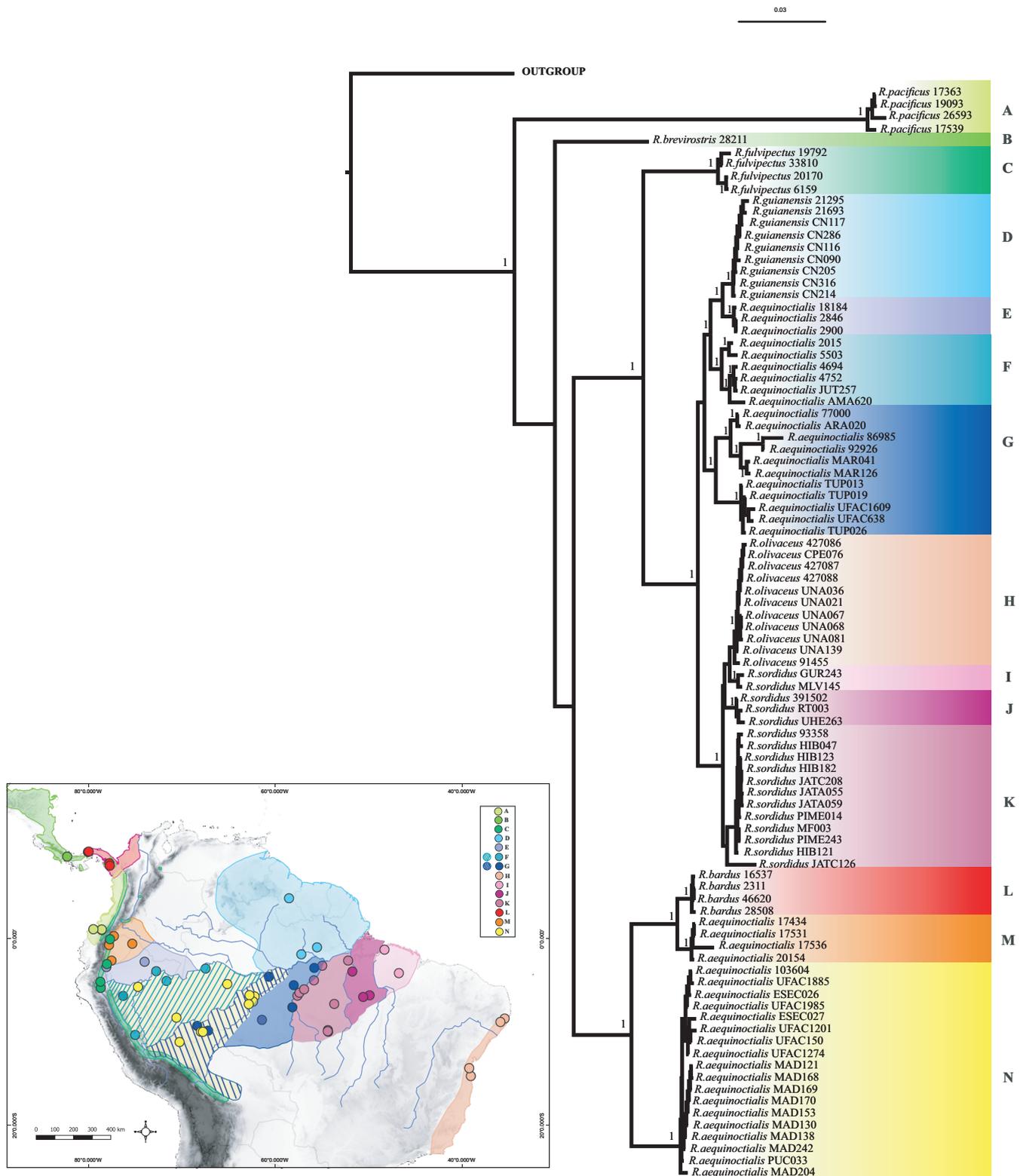
The inferred topology recovered several *Rhynchocyclus* taxa (including one currently recognized species) as

para- or polyphyletic (Figure 1). Because of the evident disagreement with current taxonomy, we refer to the recovered *Rhynchocyclus* clades (phylogroups) by individual labels (Clades A through N), and associate taxon names to these labels when possible—names and labels are shown in the recovered phylogeny (Figure 1). *Rhynchocyclus pacificus* (Clade A) was recovered as monophyletic and as the sister group of all remaining *Rhynchocyclus* (Clades B–N). Only a single specimen of *R. brevirostris* (Clade B) was included in the analysis, and therefore, the monophyly of this species could not be tested (Figure 1). The specimen was recovered as the sister to all remaining *Rhynchocyclus* (Clades C–N), except *R. pacificus*. *Rhynchocyclus fulvipectus* (Clade C) was recovered as a monophyletic species, but nested with high statistical support within a widely paraphyletic *R. olivaceus* (Clades D–N).

Our genetic analysis included five of the nine subspecies of *R. olivaceus*. Based on the geographic overlap between phylogroup distribution and type localities of the different taxa grouped under *R. olivaceus* (contained in Amadon et al., 1979), specimens assigned to *R. o. bardus* (Clade L), *R. o. guianensis* (Clade D) and *R. o. olivaceus* (Clade H) were all recovered as monophyletic (Figure 1). However, all of them were nested within other non-monophyletic taxa. For example, *R. o. bardus* (L) is nested in a clade with two of the groups recovered for *R. o. aequinoctialis* (Clades M and N), whereas *R. o. guianensis* (D) is nested within the remaining three groups currently grouped under *R. o. aequinoctialis* (Clades E, F and G). Moreover, *R. o. olivaceus* (clade H) is nested with *R. o. sordidus* (clades I, J and K). The recently proposed polytypic species *Rhynchocyclus aequinoctialis* (Boesman, 2016) was recovered as polyphyletic and divided into five phylogroups (Clades E, F, G, M and N).

The genetic structure recovered by the phylogeny was also recovered by the haplotype networks, especially those of the mitochondrial genes (Figure 2). Clades L, M and N are separated from the other clades for all genes (except for the M clade, which does not have sequences for the ND2 and nuclear genes). In addition, sharing of haplotypes in the nuclear genes network occurs mainly amongst groups D, E, F and G (Figure 2).

Levels of mitochondrial divergence within *R. olivaceus* lineages were smallest in clades L and N but greatest in closely related clades D, E, F and G (Table S5). Considering distances between lineages, higher values of mitochondrial divergence were observed mainly between the non-sister clades comprising lineages L, M and N and that including lineages D, E, F, G, H, I, J and K. In contrast, much lower divergence values were observed in the nuclear genes, as demonstrated by the widespread clade joining lineages D, E, F, G, H, I, J and K, where almost no variation was seen (Table S5).



**FIGURE 1** Bayesian phylogenetic tree estimated with the multilocus dataset obtained for the genus *Rhynchocyclus*. Inset map shows the approximate geographic distribution of tissue samples used in the analysis. Colours and capital letters refer to the distinct reciprocally monophyletic clades recovered in the genus, as follows: Clade A: *Rhynchocyclus pacificus*; Clade B: *Rhynchocyclus brevisrostris*; Clade C: *Rhynchocyclus fulvipectus*; and clades D–N: *Rhynchocyclus olivaceus*. Taxonomy follows Gill et al. (2021). The hatched area in south-western Amazonia indicates the sympatry among clades F, G and N

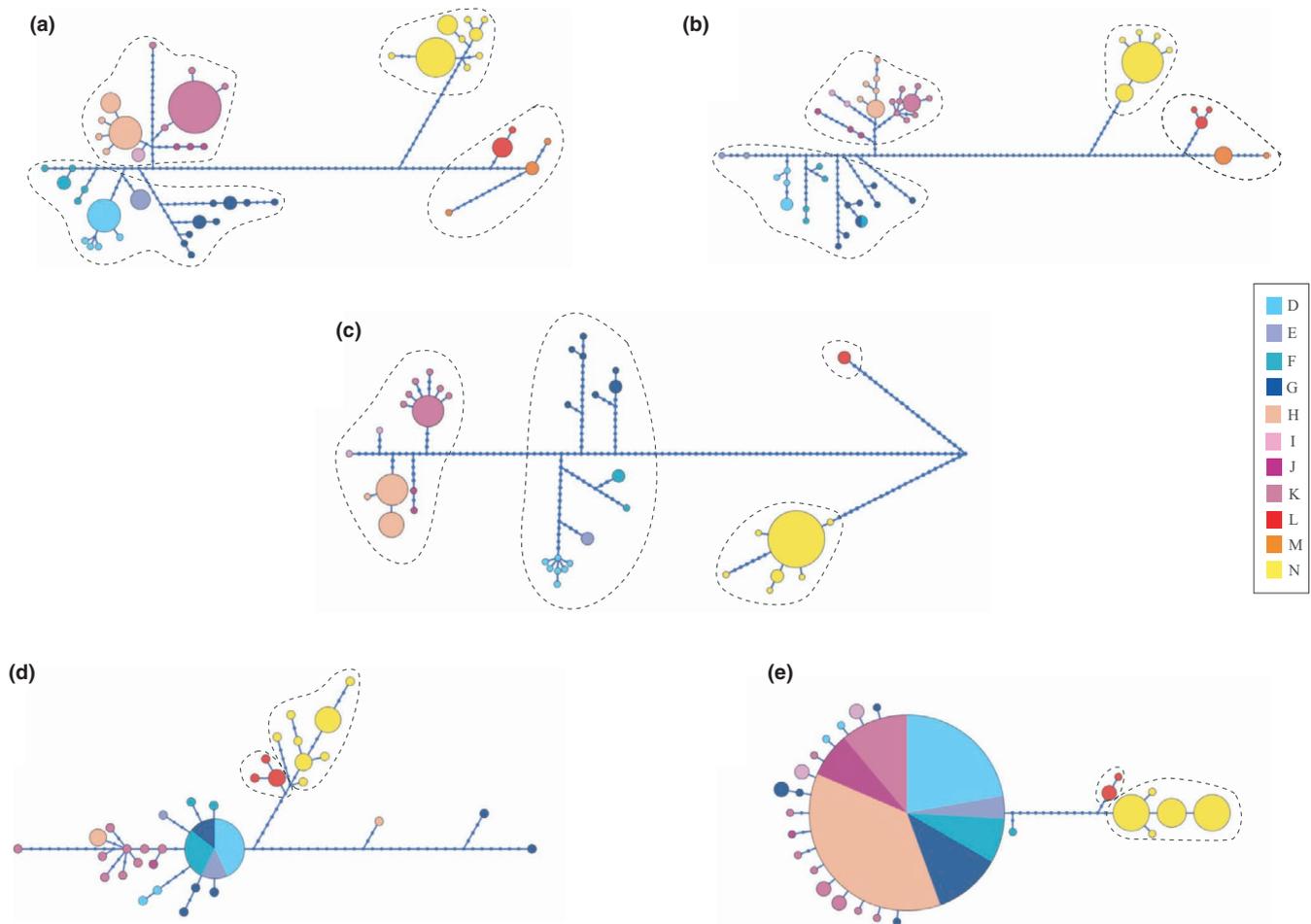


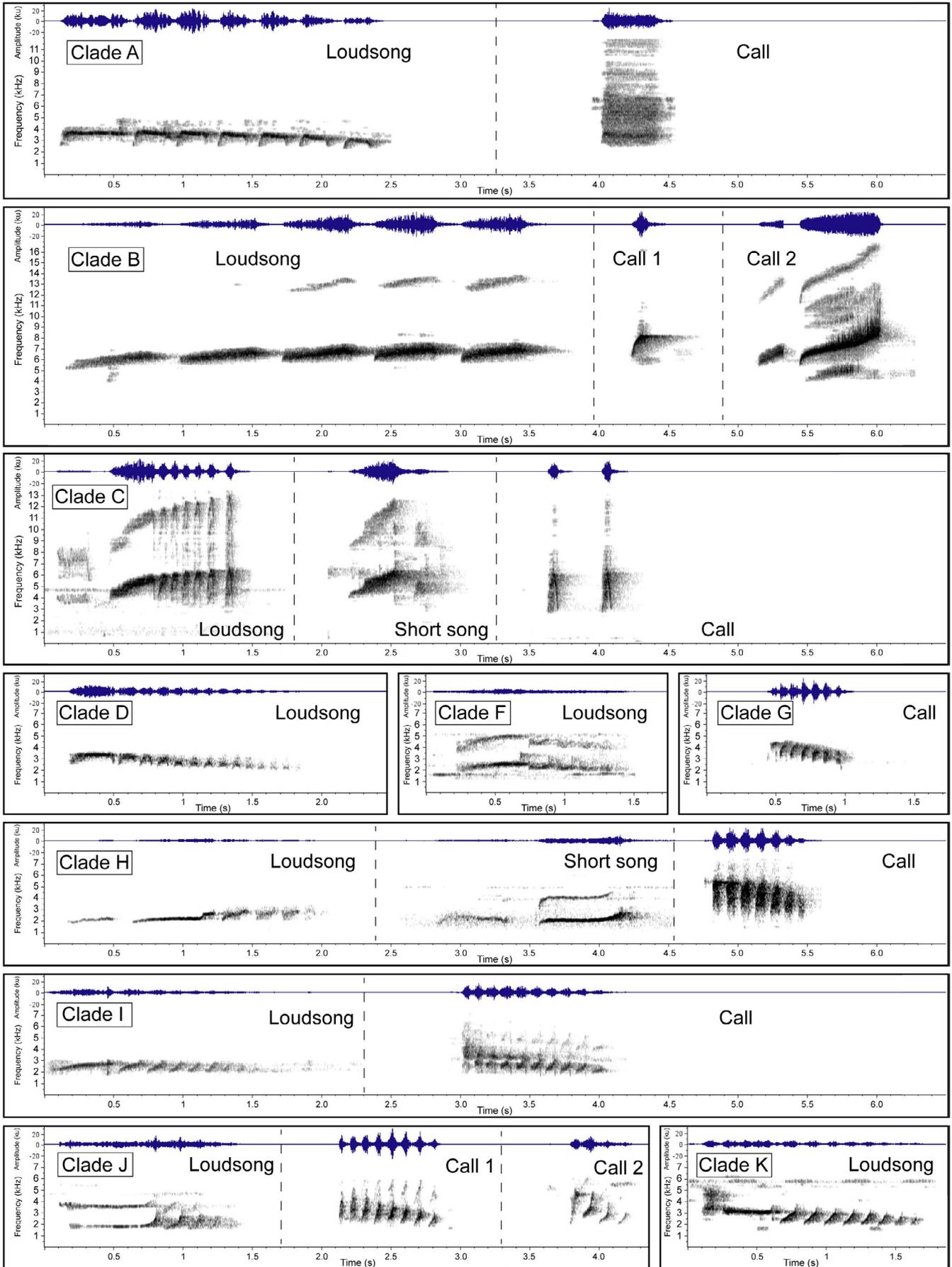
FIGURE 2 Haplotype networks estimated for the polytypic *Rhynchocyclus olivaceus* based on the mitochondrial genes: (a) Cytochrome oxidase I—COI, (b) Cytochrome B—Cytb and (c) NADH subunit 2—ND2; and nuclear genes: (d) Glyceraldehyde-3-phosphodehydrogenase intron 11—G3PDH and (e) Muscle-specific receptor tyrosine kinase intron 3—MUSK. Colours and clade letters are the same as in Figure 1

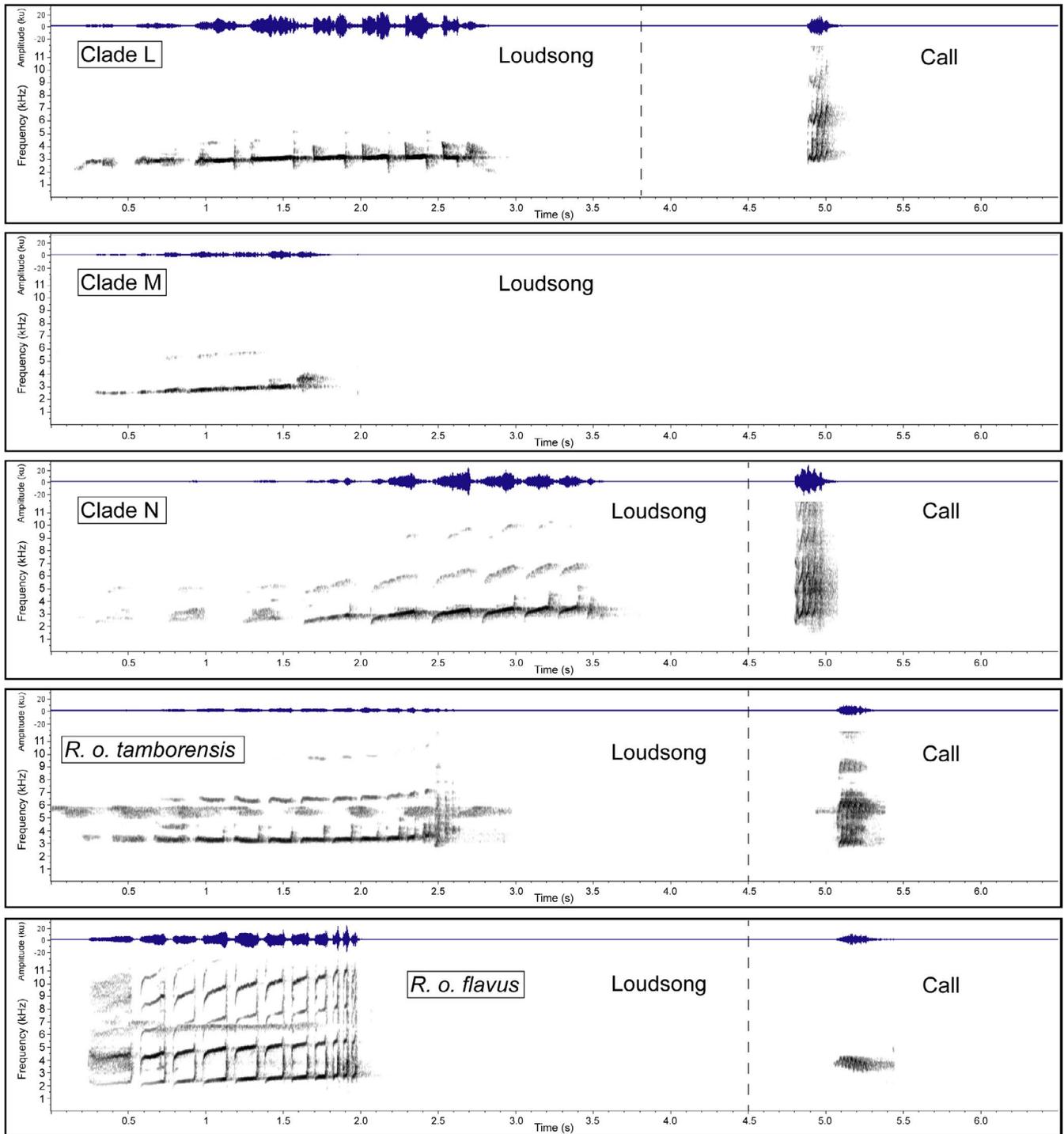
### 3.2 | Morphology

Although the phylogeny indicates a marked genetic structure and suggests the existence of several independent lineages within *R. olivaceus*, most populations currently referred to *R. olivaceus* are morphologically undistinguishable. Regarding plumage colour variation, all analysed groups are very similar to each other with no diagnostic characters, although individuals from the Guiana Shield (clade D; *R. o. guianensis*) show generally a brighter yellow belly than other clades (Figure S2; Table S7).

We analysed morphological measurements of 52 specimens included in the phylogenetic analyses, covering the following lineages: D ( $n = 7$ ), F ( $n = 1$ ), G ( $n = 8$ ), H ( $n = 6$ ), I ( $n = 2$ ), J ( $n = 2$ ), K ( $n = 10$ ) and N ( $n = 16$ ). The morphological characters of clades E, L and M were not measured directly through specimens (which were not available in the collections studied), but digital pictures available from public repositories concur with the same general pattern of very little plumage variation across these lineages as well (<https://ebird.org/species/olifla1>). All morphometric characters measured presented statistical differences across the genetic

FIGURE 3 Spectrograms and oscillograms of vocalization types (loudsong and call) for *Rhynchocyclus* clades analysed corresponding to *Rhynchocyclus pacificus* (Clade A; loudsong: XC66966, call: XC21789), *Rhynchocyclus brevirostris* (Clade B; loudsong: XC65695, call 1: XC229086 and call 2: XC274068), *Rhynchocyclus fulvipectus* (Clade C; loudsong: XC534048, short song: XC264697 and call: XC59422) and 'vocal group' 1 (VG1) of *Rhynchocyclus olivaceus* (Clade D—loudsong: XC395114; Clade F—loudsong: XC90673; Clade G—call: XC66668; Clade H—loudsong: WA287318, short song: XC550474, and call: WA3011277; Clade I—loudsong: XC84087 and call: XC423771; Clade J—loudsong: WA3855889, call 1: WA2002197 and call 2: XC5153; and Clade K—loudsong: XC147429). No recordings were available for Clade E. Repository abbreviations: XC—Xeno-canto ([www.xeno-canto.org](http://www.xeno-canto.org)) and WA—Wikiaves ([www.wikiaves.com.br](http://www.wikiaves.com.br))





**FIGURE 4** Spectrograms and oscillograms of vocalization types (loudsong and call) for *Rhynchoyclus* clades and taxa analysed corresponding to 'vocal group' 2 (VG1) of *Rhynchoyclus olivaceus* (Clade L—loudsong: XC363933, call: XC17201; Clade M—loudsong: XC249877; Clade N—loudsong: ML75277, call: XC452946; and subspecies *Rhynchoyclus olivaceus flavus*—loudsong: ML69829, call: XC229089; and *Rhynchoyclus olivaceus tamborensis*—loudsong: XC18231, call: XC18230). Repository abbreviations: XC—Xeno-canto ([www.xeno-canto.org](http://www.xeno-canto.org)), WA—Wikiaves ([www.wikiaves.com.br](http://www.wikiaves.com.br)), ML—Macaulay Library ([www.macaulaylibrary.org](http://www.macaulaylibrary.org))

lineages recovered (Table S8), but none could uniquely diagnose any of them, although clade H specimens (from the Atlantic Forest) had greater measurements overall when

compared to all remaining lineages. Accordingly, a PCA analysis based on all specimens and morphometric characters measured retained 66% of the total variance in the first

two principal components (PC1: 41% and PC2: 25%), with higher contributions by bill, tarsus and tail loadings, and which showed a distinct cluster grouping all individuals of clade H (Figure S3).

### 3.3 | Bioacoustics

Unlike many other suboscine passerines, *Rhynchocyclus* are usually quiet most of the time and representativeness of sound recordings in databases is an important bottleneck for robust quantitative vocal analyses. Nonetheless, we evaluated at least one type of song of all species of *Rhynchocyclus* and most of described subspecies and clades recovered in *R. olivaceus*, except *R. o. mirus* and clade E birds, which were unavailable. Descriptions of vocalizations for each clade and subspecies sampled are provided below (see also Figures 3 and 4), with sound frequency being relative to peak frequency measurements. The acoustic analyses revealed conspicuous differences in vocalizations among some of species, taxa and genetic lineages recovered.

The loudsong of Clade A (*R. pacificus*) consists of a series of 4–7 notes, with subsequent notes slightly decreasing in frequency, conferring a descending overall quality to the song; the call is a rapid hissing note (Figure 3). The loudsong of Clade B (*R. brevirostris*) is a series of 3–5 squeaky notes with each note rising in pitch, giving an ascending quality to the overall song; the most common call is a single short squeaky note (call 1), sometimes presenting longer duration or two notes (call 2; Figure 3). The loudsong of Clade C (*R. fulvipectus*) is a series of 4–7 notes, with the first note being longer in duration and possessing frequency modulation, followed by shorter notes with increasing frequencies, conferring an overall ascending nature to the song. Some variation exists in this general theme, with some recordings consisting of ‘short songs’ including an initial longer note, followed by only 1–3 shorter notes (XC264697, Figure 3); the call is a short raspy note similar in shape to an inverted ‘U’ letter.

Within the remaining clades (D–N) and taxa making part of the polytypic and paraphyletic *R. olivaceus*, two highly variable loudsong and call types could be identified, each corresponding to reciprocally monophyletic groups. One of these vocal groups includes clades D, F, G, H, I, J and K, whereas the other clusters clades L–N, plus subspecies *R. o. tamborensis* and *R. o. flavus*, which were not sampled genetically (Figures 3 and 4).

In the first group, the loudsong of Clade D (*R. o. guianensis*), associated with the Guiana shield in north-eastern Amazonia, is a series of 11–18 notes decreasing in pitch, resulting in an overall descending quality (Figure 3). This main theme varies commonly with the presence of a

longer first note, whereas its complete absence is usually linked to excitement or agonistic behaviour. The variation without a longer first note is similar in structure to the calls of Clades H, I and J (Figure 3). We could not identify or relate any recordings to Clade E (from north-western Amazonia), but the loudsong of closely related Clade F (from south-western Amazonia; recovered as the sister group to clades D + E) is a series of 5 notes with a similar overall pattern consisting of a longer first note, followed by notes with progressively lower frequencies (Figure 3). Interestingly, Clade F is sympatric (but apparently not syntopic; see Discussion) with phylogenetically distant Clade N birds, whose vocalizations are entirely different (Figure 4). Just one recording is available for Clade G (found in southern Amazonia between the Purus and Tapajós rivers), and it consisted of a short series of 7–10 notes decreasing slightly in frequency, resembling closely the calls of clades H, I and J from south-eastern Amazonia and the Atlantic Forest (Figure 3). The loudsong of Clade H (*R. o. olivaceus*; endemic to the Atlantic Forest) consists of a series of 4–10 notes, with the first one being longer in duration and with frequency modulation, followed by shorter notes with slightly rising frequencies, resulting in an ascending overall quality (Figure 3). Clade H calls consist of a series of 6–13 raspy notes decreasing progressively in frequency (Figure 3). Another vocal type in Clade H is a ‘short song’ presenting only the first longer notes of the original loudsong (Figure 3). In contrast to other clades clustering in the same ‘DEFGHIJK’ major monophyletic group, the loudsong of Clade H is the only one presenting ascending rather than descending notes. The loudsongs of clades I, J and K (*R. o. sordidus*; from south-eastern Amazonia) are similar in general structure, with a longer first note followed by 4–8 notes with progressively slightly lower frequencies, conferring a descending quality to the song (Figure 3). Similarly, the calls of clades I and J are a series of 4–9 raspy notes with similar duration but progressively lower frequencies (no call recordings were available for Clade K; Figure 3).

The second vocal group identified in *R. olivaceus* includes clades L, M and N, in addition to recording samples putatively belonging to subspecies *R. o. tamborensis* and *R. o. flavus* based on currently accepted range limits (Gill et al., 2021; Figure 4). The loudsong of Clade L (*R. o. bardus*; from north-western Colombia and Panama) is a series of 7–8 sharp flat notes, with no frequency modulation, but with each subsequent note being slightly higher in frequency than the previous, hence having an overall ascending quality; sometimes, first loudsong notes are weak and raspy, whereas the call consists of a very short hoarse trill emission (Figure 4). We identified and assigned a few poor quality loudsong recordings to Clade M (*R. o. aequinoctialis* from

north-western Amazonia based on reference to range and type locality; Amadon et al., 1979, Gill et al., 2021; see also below), which consisted of a series of 6–7 sharp notes with each subsequent note slightly increasing in frequency, conferring an overall quality very similar to its sister group Clade L; we could not identify any recordings that could be assigned as calls for Clade M (Figure 4). The loudsong of Clade N (from south-western Amazonia) is a series of 5–11 notes, with individual sequential notes rising in frequency (modulation), conferring an overall ascending quality to the song. Even though the loudsong structure in Clade N is similar to that in closely related clades L and M, the individual notes have a readily diagnostic distinct upslurred shape (Figure 4). The call of Clade N birds is a short hoarse trill similar to that uttered by those in Clade L, but with an overall lower peak frequency. Loudsong and calls similar to those documented for clades L–M were also observed in Colombian and Venezuelan populations tentatively assigned to *R. o. tamborensis* and *R. o. flavus* (which were not sampled genetically) based on reference to range and type locality; Amadon et al., 1979, Gill et al., 2021). The single loudsong recording assigned to *R. o. tamborensis* analysed consisted on a series of 13 notes slightly rising in pitch, with individual note shape and overall structure like those in Clade L (*R. o. bardus*); the call is a short hoarse trill emission, also very similar in structure to that in Clade L (Figure 4). The loudsong attributed to *R. o. flavus* is a series of 8–11 notes, with individual notes rising in frequency (modulation), but with the fundamental frequency lower than *R. o. tamborensis*, but approaching more that in Clade L (*R. o. bardus*; Figure 4). The call is a short trill emission but with higher peak frequency than that observed for other groups presenting trill calls. Finally, the lone recording (call) obtained attributed to *R. o. jelambianus* from coastal north-eastern Venezuela (XC229090) was disregarded due to uncertainty concerning the identification of the bird uttering the vocalization, as reported by the recordist.

For statistical analyses of quantitative characters measured for loudsongs, we considered only good quality recordings attributed to the polytypic *R. olivaceus* (Gill et al., 2021), and therefore excluded recordings of *R. pacificus*, *R. brevisrostris* and *R. fulvipectus* due to high disparity and variance in measurements. Four vocal measurements presented significant statistical differences across clades and taxa (PF, PF1, PF2 and NN), but with no single group being unequivocally diagnosed by any of the characters measured, given overlap in measurements between two or more groups (Table S10). Nevertheless, a PCA analysis retaining 81% of the total variance in two first principal components (PC1: 53%

and PC2: 28%), recovered along PC2 the two main vocal groups reported above, one including clades D, H, I, J and K (hereafter called vocal group 1—VG1) and another containing clades L, M and N, plus the samples attributed to *R. o. tamborensis* and *R. o. flavus* (hereafter called vocal group 2—VG2; Figure S4). The interpretation of PC1 is more challenging, and it seems to primarily divide VG1 into two main sub-groups, roughly corresponding to phylogenetic relationships, although samples of Clade I birds are found in both sub-groups; with respect to VG2, no clear trend could be discerned (Figure S4).

## 4 | DISCUSSION

### 4.1 | Phylogenetic relationships and vocal differentiation

An integrative analysis of genetic and acoustic data for *Rhynchocyclus* revealed that current taxonomy (Gill et al., 2021; Remsen et al., 2021) is at odds with evolutionary history in the genus, particularly with respect to its most widespread species (*R. olivaceus*), which was recovered as paraphyletic, with *R. fulvipectus* nested in it (Figure 1). Furthermore, each of the two main paraphyletic groups recovered for *R. olivaceus* were also strongly differentiated with respect to both loudsong and calls, despite both having extremely similar plumages and morphometry. In spite of the sampling limitations of our study, we were able to re-evaluate species limits within *R. olivaceus* by adding a phylogenetic perspective to the interpretation of patterns of vocal variation in the group, which were previously explored by Boesman (2016). Even though our results agreed with those in Boesman (2016) in recovering two main vocal groups in *R. olivaceus*, the incorporation of phylogenetic data based on the densest sampling ever available for the whole genus, resulted in very different taxonomic implications from that study, as discussed below. Other similar instances of remarkable vocal differentiation between otherwise morphologically undistinguishable non-sister species have been documented for other avian taxa (e.g. Carneiro et al., 2012; Dantas et al., 2021).

The most complete phylogeny for the genus *Rhynchocyclus* available before this study was that of Harvey et al. (2020), which sampled only one specimen of each currently recognized species (Gill et al., 2021). As such, Harvey et al. (2020) did not test the monophyly of any *Rhynchocyclus* species, including *R. olivaceus*, which was recovered herein as paraphyletic (Figure 1). Nonetheless, their phylogeny agreed with the one presented herein in that *R. fulvipectus* and *R. o. olivaceus* were

recovered with high statistical support as monophyletic (Harvey et al., 2020). However, an important difference was the strongly supported monophyly of *R. brevirostris* and *R. pacificus* (Harvey et al., 2020), which were recovered as paraphyletic herein, albeit with low statistical support. While the genomic-level sampling employed by Harvey et al. (2020) may have better resolved the relationships between *R. brevirostris* and *R. pacificus*, the absence of any additional samples of *R. olivaceus* in that study precludes any further comparison with our results.

When the polytypic *R. olivaceus* is considered, the most recent taxonomic appraisal (based solely on vocal characters) indicated the existence of two very distinct subspecies groups roughly corresponding to the VG1 and VG2 diagnosed herein (Boesman, 2016), providing the basis for their subsequent treatment as distinct subspecies groups (del Hoyo et al., 2021). According to this subdivision, the Eastern Olivaceous Flatbill subspecies group included the nominate form plus populations sharing the same VG1 loudsong type and associated calls (i.e. *R. o. guianensis*, *R. o. sordidus*, distributed in eastern Amazonian and the Atlantic Forest; del Hoyo et al., 2021). The second group was called the western Olivaceous Flatbill and included populations sharing the VG2 loudsong type and the same distinct call type (*R. o. bardus*, *R. o. mirus*, *R. o. flavus* and *R. o. aequinoctialis*).

Even though our molecular results indicated with strong support that VG1 and VG2 correspond to statistically well-supported clades, which are paraphyletic with respect to each other given the strongly supported sister relationship between the Eastern Olivaceous Flatbill subspecies group and *R. fulvipectus* (Figure 1), samples attributed to *R. o. aequinoctialis* were recovered in both unrelated *R. olivaceus* clades and were also found to utter vocalizations typical of both VG1 and VG2 (Figures 3 and 4). In fact, samples occurring within the range of *R. o. aequinoctialis* and attributed to this taxon grouped in non-sister clades E, F and G (belonging to VG1) and in non-sister clades M and N (belonging to VG2), therefore rendering this taxon polyphyletic (Figures 1, 3 and 4). Also, our results demonstrated that clade N (belonging to VG2) overlaps broadly in distribution with distantly related clades F and G (both belonging to VG1) in different parts of south-western Amazonia (Figure 1), clearly indicating that these lineages may have already acquired a significant level of reproductive isolation consistent with their paraphyletic status, and current sympatry. This sympatry in south-western Amazonia between different clades of *R. o. aequinoctialis* is apparently mediated by occupancy of distinct habitat types. Out of the 25 specimens sequenced attributed to *R. o. aequinoctialis* and belonging to clades F, G and N,

a clear pattern emerged: specimens from closely related clades F + G ( $N = 9$ ) were collected mostly in upland *terra-firme* forest ( $N = 5$ ), transitional forest ( $N = 2$ ) and bamboo patches ( $n = 2$ ), whereas those in distantly related clade N ( $N = 16$ ) were obtained from *várzea* seasonally flooded forest ( $N = 12$ ), riparian forest ( $N = 2$ ) and secondary forest ( $N = 1$ ) (Table S2). Our sampling revealed that sequenced specimens of distantly related clades G and N are found within a few kilometres from each other in suitable habitats in the Rio Branco capital area in the Brazilian state of Acre, with birds in clade G associated with bamboo patches, and those in clade N with seasonally flooded forests with varying levels of disturbance along the banks of the Rio Acre (Figure 1; Table S2). We anticipate that a similar pattern of local ecological replacement across upland and seasonally flooded habitats may also occur between birds in clades F and N in the westernmost part of the Amazon (Figure 1; Tables S2 and S3). These genetically, vocally and ecologically divergent lineages of the Olivaceous Flatbill lineages overlapping in south-western Amazonia are apparently undistinguishable from a morphological perspective, when both plumage and morphometric variation are considered (Table S8, Figures S2 and S3), highlighting another remarkable case of cryptic diversification in the Neotropics. Based on these findings, we discuss and propose below a new taxonomic treatment for the polytypic *R. olivaceus* based on the results of the integrative analyses presented herein.

## 4.2 | Taxonomic review of the Olivaceous Flatbill

Based on the integrative analyses of all combined data presented and discussed in this study, we propose a new taxonomic treatment for the current polytypic *R. olivaceus* that aims to minimize any changes based on yet preliminary or incomplete datasets, while it incorporates the major patterns of ecological, genetic and vocal divergences reported herein. Under a conservative taxonomic perspective, intended to minimize the number of changes with respect to current taxonomy, our study provides strong evidence that *R. olivaceus* should be split in at least two species, which have already diverged enough to become sympatric in a significant part of their mutual ranges. In this case, each major paraphyletic group recovered for *R. olivaceus* herein (Figure 1), and which were also found to diverge vocally and ecologically, would consist independent species, to which the taxon names applicable with priority would be *R. olivaceus* (grouping lineages and taxa corresponding to clades D–K) and *R. aequinoctialis* (grouping lineages and taxa

corresponding to clades L–N). However, our data and analyses also clearly show that within these two major *R. olivaceus* groups, there is still significant molecular and diagnostic vocal variation allowing for the delimitation of additional species occupying distinct biomes and river drainages across the Neotropics (Figures 1–3; Figure S4; Tables S5 and S11). Namely, vocal and genetic patterns of variation allow for the split of the major *R. olivaceus* and *R. aequinoctialis* groups into at least two additional species level taxa each, as justified below. Therefore, we propose the recognition of four species in the former paraphyletic and polytypic Olivaceous Flatbill (Figure S5): *R. olivaceus* (corresponding to clades H, I, J and K), *Rhynchocyclus guianensis* (corresponding to clades D, E, F and G), *R. aequinoctialis* (referring to clades L and M) and a fourth species corresponding to clade N—described herein as:

#### 4.2.1 | *Rhynchocyclus cryptus*, sp. nov

u r n : l s i d : z o o b a n k .  
org:act:2DC17190-2BDD-49EC-88E6-4CF2FC2562A3  
English Name: Cryptic Flatbill  
Portuguese Name: Bico-chato-críptico

##### *Holotype*

Museu Paraense Emílio Goeldi 58,915. Skin, adult female, netted in riparian forest on 14 August 2005 by A. Aleixo and M. S. Brígida at Estacao Ecológica (ESEC) Rio Acre, state of Acre, Brazil (11°03'05.2'S;70°12'59'W) on the Brazil/Peru border. Tissue samples deposited at the Laboratório de Biologia Molecular, MPEG under accession number ESEC 027. Sequences deposited in GenBank (Table S1).

##### *Paratypes*

Museu Paraense Emílio Goeldi 57,166. Skin, male, netted in floodplain riparian forest by F. Poletto, D. C. P. Neto and A. Aleixo on 21 June 2003 at Igarapé Lontra, Base Petrobras—Urucú, Tefé (04°52'S, 65°07'W), state of Amazonas, Brazil. Tissue samples deposited at the Laboratório de Biologia Molecular, MPEG under accession number PUC 033. Sequences deposited in GenBank (Table S1). MPEG 60,026. Skin, female, netted by E. Guilherme in disturbed 'capoeira' forest on 25 February 2006 at the Acre Federal University campus (09°57'S; 67°57'W) in Rio Branco, state of Acre, Brazil. Tissue samples deposited at the Laboratório de Biologia Molecular, MPEG under accession number UFAC 0150. Sequences deposited in GenBank (Table S1). MPEG 63,792. Skin, male, netted by E. Guilherme and J. N. S. Brígida in várzea floodplain forest on 18 November 2007 at Novo Porto, mouth of Paraná do Ouro (8°27'35.5'S;7 0°33'22.9'W) on

the Envira River, Feijó, state of Acre, Brazil. Tissue samples deposited at the Laboratório de Biologia Molecular, MPEG under accession number UFAC 1885. Sequences deposited in GenBank (Table S1). MPEG 73357. Skin, male, netted in floodplain várzea forest by B. D. Silva, J. N. S. Brígida and A. Aleixo on 19 August 2011 at Muanense, Humaitá (07°00'42,9'S;62°49'16,8'W), state of Amazonas, on the right (eastern) bank of the Madeira River, Brazil. Tissue samples deposited at the Laboratório de Biologia Molecular, MPEG under accession number MAD 121. Sequences deposited in GenBank (Table S1). MPEG 73440. Skin, male, netted by B. D. Silva, J. N. S. Brígida and A. Aleixo in floodplain várzea forest on 25 August 2011 at Maloca do Baeta, state of Amazonas, on the left (western) bank of the Madeira River (06°11'20,8'S;62°11'24,5'W), Brazil. Tissue samples deposited at the Laboratório de Biologia Molecular, MPEG under accession number MAD 204. Sequences deposited in GenBank (Table S1).

##### *Etymology*

The new species is named after its remarkable morphological cryptic nature, which strongly contrasts with its high levels of vocal and genetic differentiation, two characteristics which probably allow for the sympatry with *R. guianensis* throughout its range.

##### *Diagnosis*

No apparent consistent morphological diagnosis exists with respect to either sex of *R. olivaceus*, *R. guianensis* and *R. aequinoctialis*, as delimited herein (see below; Figure S2, Tables S7–S9). In contrast, *R. cryptus* includes specimens grouped in the statistically well-supported clade N (Figure 1), which diverges by average uncorrected mitochondrial *p*-distances of 7.4% (cytb), 10% (ND2) and 8.2% (COI) from *R. olivaceus* and *R. guianensis* (Table S6). Average mitochondrial divergence with respect to its sister species *R. aequinoctialis* is 4.3% (cytb), 5.8% (ND2) and 4.9% (COI), hence approaching values obtained between sister species *R. olivaceus/guianensis* (clades D–M in Figure 1) and *R. fulvipectus* (5.3%—cytb; 7.5%—ND2; and 5.3%—COI). Vocally, the loudsongs and calls of *R. cryptus* are readily separable from those of *R. pacificus*, *R. brevirostris*, *R. fulvipectus*, *R. olivaceus* and *R. guianensis* by their distinct structures consisting, respectively, on 5–11 rising notes in frequency, typical of VG2, and a hoarse short trill (Figure 4, Table S11). With respect to closely related *R. aequinoctialis*, the loudsong of *R. cryptus* can also be easily diagnosed by the shape of individual notes, which rise steadily in frequency (modulation), giving it a more powerful quality that contrasts with the flat shape (no modulation) of the loudsong of the former species (Figure 4). The calls between *R. cryptus* and *R. aequinoctialis* are similar overall, but with those of the former species possessing a lower peak frequency (Figure 4).

*Description of holotype*

Throat greyish olive (43); chest greyish olive (43); belly straw yellow (56); Wing (upper part) greenish olive (49); wing (middle part) vandyke brown (121); wing (lower part) vandyke brown (121); wing (border) cinnamon (39); and tail olive brown (28).

*Measurements of holotype*

Bill length from distal point of the nostrils to bill tip—8.5 mm; bill width at the distal point of the nostrils—8.8 mm; bill height at the point above the nostrils—4.72 mm; wing length—65.71 mm; tarsus length—17.29 mm; tail length—67.55 mm.

*Range*

South of the Amazon River and on islands along its main channel (e.g. island Yanamono near Iquitos), from both banks of the Madeira River (on the eastern bank, apparently limited to the belt of floodplain *várzea* forests) in the states of Amazonas and Acre in Brazil, westwards to the Marañón River in Peru (Loreto and Madre de Dios), and southwards to north/central Bolivia in the Mamoré and likely Beni drainages in the departments of Cochabamba and possibly Beni, La Paz and Pando (Table S3).

*Habitat, ecology and conservation*

The new species is associated with seasonally flooded floodplain forests (*várzea*), including degraded patches close to human settlements such as in the Rio Branco metropolitan area in the Brazilian state of Acre. As discussed above, *R. cryptus* is sympatric with clades F and G of *R. guianensis* (as delimited below), but they replace each other across the floodplain/upland forest gradient locally, as documented for the Rio Branco capital area (see above).

*Rhynchocyclus cryptus* is widespread throughout the Inambari area of endemism. The main threats for this species are deforestation caused by rapid progress of livestock farms, monocultures and implementation of large hydroelectric dams on the Madeira River, which affect the flow of sediments supporting seasonally flooded forests along its banks. However, most parts of the species' distribution are in areas of limited access and within conservation units. The extent of occurrence (EOO) of *R. cryptus* based on the area covered by our genetic sampling is estimated as 704,000 km<sup>2</sup>, hence not reaching the threshold to be treated as Vulnerable (VU) under distribution range criterion B1 (<20,000 km<sup>2</sup>). Therefore, *R. cryptus* should be treated as least concern (LC) according to IUCN (2021) guidelines.

The ranges, taxon names, and diagnostic genetic and vocal characters associated with each of the remaining three species recognized here but previously treated under the polytypic Olivaceous Flatbill (sensu Gill et al., 2021) are described below.

4.2.2 | *Rhynchocyclus olivaceus* (Temminck, 1820)

English Name: Olivaceous Flatbill

Portuguese Name: Bico-chato-grande

*Platyrhynchus olivaceus* Temminck, 1820; Nouv. Rec. Pl. Color., pl12, Figure 1: 'Brésil'; restricted to Rio de Janeiro by Pinto, 1944, Cat. Aves Brasil, pt 2, p. 214.

*C[otinga] virescens* Thunberg, 1823; Mém. Soc. Imper. Nat. Moscou, 6, p.178: Brazil; restricted to Rio de Janeiro by Zimmer, 1939, Amer. Mus. Novit., n. 1045, p. 23.

*Todus olivaceus* Lichtenstein, 1823; Dubl. Berliner Mus., p.51: Bahia.

*Platyrhynchus sulphurescens* Spix, 1825; Av. Bras., 2, p.10, pl.12, Figure 2 (specimen in Munich Museum examined by Hellmayr, 1927).

*Platyrhynchus nuchalis* Wied, 1831; Beitr. Naturg. Bras., 3 (2), p. 971: southeastern Brazil (albinistic variety, in Allen, 1889; Bull. Amer. Mus. Nat. Hist., 2, p.233).

*Cyclorhynchus olivaceus* Burmeister, 1856; Syst. Übers. Th. Bras., 2, p. 503.

*Cyclorhynchus nuchalis* Burmeister, 1856; l.c., p. 503 (ex Wied).

*Craspedoprion olivaceus* Ihering, 1907; Cat. Faun. Braz., I, p. 265.

*Rhynchocyclus olivaceus sordidus* Todd, 1952; Ann. Carnegie Mus., 32, p. 296.

*Type locality*

Rio de Janeiro, restricted by Pinto (1944).

*Range*

Two currently allopatric populations distributed in the Atlantic Forest of eastern Brazil between Pernambuco to Rio de Janeiro (*R. o. olivaceus*) and south-eastern Amazonia east of the Tapajós River (*R. o. sordidus*, type locality Santarém, Pará state, Brazil; Todd, 1952; Figure 1). The Marajó Island population of the former Olivaceous Flatbill (two specimens from Anajás; Table S2) is tentatively assigned here to *R. olivaceus*, given that no specimens from it were sequenced and no vocal samples are available. As shown for several other avian lineages, Marajó Island populations of widespread Amazonian taxa are more closely related to other lineages found south of the Amazon River than those on the Guiana shield (Silva et al., 2019).

*Remarks*

Despite their allopatric distributions, our molecular data failed to recover the reciprocal monophyly between Atlantic Forest (*R. o. olivaceus*; Clade H) and Amazonian (*R. o. sordidus*; clades I, J and K), consistent with their overall low levels of genetic differentiation (maximum

average pairwise uncorrected genetic distances of 0.6%–1.3%, depending on the mitochondrial marker considered; Table S6), and a likely very recent separation (Figures 1 and 2). Morphometric analyses indicated overall higher measurements for *R. o. olivaceus*, especially in tarsus length, for which no overlap was found in measurements obtained for any other clades grouped under the same species (H, I, J and K) and those in *R. guianensis*, *R. aequinoctialis* and *R. cryptus* (Table S8). From a vocal perspective, loudsongs of both *R. o. olivaceus* and *R. o. sordidus* clades share a typical structure consisting on a longer introductory note followed by 3–9 (*R. o. olivaceus*) or 4–8 (*R. o. sordidus*) subsequent notes. However, while subsequent notes slightly rise in frequency in *R. o. olivaceus*, those in *R. o. sordidus* follow a consistent opposite decreasing trend (Figure 3 and results section). Similarly, call structure is equivalent between *R. o. olivaceus* and *R. o. sordidus*, but with distinct ‘upward’ and ‘downward’ trends, respectively (Figure 3 and results section). The interpretation of this conspicuous geographic variation in vocal characters is complicated by the extensive paraphyly of the Amazonian (*R. o. sordidus*) populations with respect to the Atlantic Forest one (*R. o. olivaceus*; Figure 1), and due to a low geographic coverage of vocal sampling for *R. o. sordidus* (Figure S1). In terms of habitat preferences, all populations of *R. olivaceus* are found in forest with varying degrees of disturbance, with Amazonian populations being mostly confined to upland *terra-firme* forest, except for a few specimens documented in seasonally flooded *várzea* and *igapó* forests along the Xingu River and on Marajó Island (Table S2).

#### 4.2.3 | *Rhynchocyclus guianensis* McConnell, 1911

English Name: Guianan Flatbill

Portuguese Name: Bico-chato-da-guiana

*Rhynchocyclus olivaceus guianensis* McConnell, 1911; Bull. B. O. C., 27, p. 106.

*Craspedoprion intermedius* Todd, 1912; Ann. Carnegie Mus., 8, p. 207.

*Craspedoprion olivaceus guianensis* Cherrie, 1916; Mus. Brookl. Inst., Sci. Bull., 2, p. 235.

*Craspedoprion guianensis* Chubb, 1921; Birds Brit. Guiana, 2, p. 148.

##### Type locality

British Guiana (Abary River; Zimmer, 1939).

##### Range

Four major monophyletic allopatric and parapatric populations were recovered herein (Figure 1), distributed as follows: (a) north of Amazon River on the Guiana shield

(eastern Venezuela, Guyana, Suriname, French Guiana and north-eastern Amazonian Brazil in the states of Amapá, Amazonas and Pará—Clade D); (b) north of the Amazon, apparently west of the Napo River in northern Peru (Loreto) and southern Ecuador (Morona-Santiago) to the base of the Andes (Clade E; Figure 1); (c) south of Amazon River from at least the Jutáí River in Brazil (Amazonas) westward to the Marañón River in Peru (Loreto, Pasco, and San Martín; Clade F); and (d) south of the Amazon River between the Tapajós and the Purus rivers in Amazonian Brazil (states of Acre, Amazonas and Pará; Clade G).

##### Remarks

Our molecular data indicated that these four disjunct populations are reciprocally monophyletic with high statistical support and strongly structured geographically, diverging from each other by maximum average pairwise uncorrected genetic distances of 1.8%–3.8%, depending on the mitochondrial marker considered (Table S5). In contrast, no plumage or morphometric characters could diagnose any of them separately (Figure S3; Table S8). From a vocal perspective, sample sizes were quite small for this species, but a distinct loudsong pattern emerged for the Guiana shield population (Clade D), consisting on a series of 11–18 notes decreasing progressively in pitch, and with distinct shapes and shorter durations than those in the loudsong of *R. olivaceus* (Figure 3, Table S11; see also above). While no vocal samples were obtained for the north-western Amazonian population (Clade E), the only loudsong of the south-western Amazonian population (Clade F) analysed resembled that of the Guianan shield population, but it consisted on a shorter series of five notes, with a longer introductory note (Figure 3). No loudsong recordings were available for the southern Amazonian population between the Purus and Tapajós rivers (Clade G), but the only call recording obtained for this group resembled closely the calls of *R. olivaceus* (see above; Figure 3). No other taxon names seem available for any of the lineages grouped under *R. guianensis* as delimited herein, and it is possible that more than one species is present within this group consisting on clades D, E, F and G (Figure 1), pending future analyses based on an improved geographical sampling. From an ecological standpoint, all populations of *R. guianensis* are found mainly in upland *terra-firme* forest, although at least one specimen of the Guianan shield population (Clade D) was collected in *várzea* forest (Table S2).

#### 4.2.4 | *Rhynchocyclus aequinoctialis* (Sclater, 1858)

English Name: Equinoctial Flatbill

Portuguese Name: Bico-chato-do-equador

*Cyclorhynchus aequinoctialis* Sclater, 1858; Proc. Zool. Soc. London, 26, p. 70.

*Craspedoprion olivaceus bardus* Bangs and Barbour, 1922; Bull. Mus. Comp. Zool., 65, p. 216.

*Rhynchocyclus olivaceus mirus* de Schauensee, 1950; Notulae Naturae 221, p. 13.

*Rhynchocyclus olivaceus tamborensis* Todd, 1952; Ann. Carnegie Mus., 32, p. 297.

*Craspedoprion aequinoctialis flavus* Chapman, 1914; Bull. Amer. Mus. Nat. Hist., 33, p. 175.

*Rhynchocyclus olivaceus jelambianus* Avelado and Perez, 1994; Boletín de la Sociedad Venezolana de Ciencias Naturales 148, p. 243.

#### Type locality

Upper Rio Napo (Quijos; Napo province), Ecuador (Sclater, 1858).

#### Range

At least two major apparent allopatric populations were recovered herein for this species (Figure 1), corresponding to clades L (*Rhynchocyclus aequinoctialis bardus*) from north-western Colombia (Antioquia northwards) and Panamá (Darién to Colón), and M (*Rhynchocyclus aequinoctialis aequinoctialis*) from north-western Amazonia in Ecuador (Morona-Santiago, Napo, Sucumbios) and possibly Peru and Colombia (Figure 1; Table S1). The status of the remaining Cis and Trans-Andean Colombian and Venezuelan populations assigned to taxa *mirus*, *tamborensis*, *flavus* and *jelambianus* could not be directly assessed due to the unavailability of tissues to our study.

#### Remarks

Our molecular data indicated that these two reciprocally monophyletic allopatric populations diverge from each other by maximum average pairwise uncorrected genetic distances of 1.2%–1.9%, depending on the mitochondrial marker considered (Table S5). Our sampling lacked tissues of taxa *mirus*, *tamborensis*, *flavus* and *jelambianus*, but call and loudsong patterns suggest that at least *tamborensis* and *flavus* are vocally close to *R. a. bardus*, and therefore are also treated herein under *R. aequinoctialis*. In fact, based on our limited sampling, little vocal differentiation seems to exist between *R. a. aequinoctialis*, *R. a. bardus*, *Rhynchocyclus aequinoctialis flavus* and *Rhynchocyclus aequinoctialis tamborensis* (Figure 4; Table S10), but additional analyses with greater sample sizes are needed. Finally, subspecies *mirus* has been reported from a very small area in north-western Colombia close to the range of *R. a. bardus* and it has been postulated that it could represent a hybrid individual between the latter taxon and *R. pacificus* (Amadon et al., 1979), whereas *jelambianus* proposed range overlaps broadly with that reported for *flavus* (Amadon et al., 1979),

from which it can be diagnosed based only on paler underparts (Avelado Hostos & Pérez Chinchilla, 1994). Based on these subtle differences and close geographic proximity to previously described taxa, we tentatively include *mirus* and *jelambianus* in *R. aequinoctialis* before the validity of those former taxa can be properly assessed. From an ecological perspective, all taxa grouped herein under *R. aequinoctialis* inhabit humid forest with varying degrees of disturbance, including upland *terra-firme* in north-western Amazonia, where it remains to be determined to what extent the ranges of the latter species and *R. guianensis* (Clade E) overlap with each other (Figure 1).

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