

**Vocalizations of the rare and flagship species
Pharomachrus mocinno (Aves: Trogonidae):
implications for its taxonomy, evolution and conservation**

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1 **Vocalizations of the rare and flagship species *Pharomachrus mocinno* (Aves:**
2 **Trogonidae): implications for its taxonomy, evolution and conservation**

3

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24 **Vocalizations of the rare and flagship species *Pharomachrus mocinno* (Aves:**
25 **Trogonidae): implications for its taxonomy, evolution and conservation**

26

27 **Abstract**

28 The Resplendent Quetzal *Pharomachrus mocinno* is a rare Neotropical bird included in the IUCN
29 red list as Near Threatened. Fragmentation of its habitat, the cloud forest, is considered as the
30 principal threat. Two subspecies are currently recognized but genetic and morphometric studies
31 suggested they could be considered as full species. We assessed whether male vocalizations
32 would support a species delimitation hypothesis. We recorded in the field and downloaded from
33 sound archives vocalizations of 57 individuals from 30 different localities distributed in 11
34 countries. We estimated the acoustic differences of all the *Pharomachrus* taxa with multivariate
35 analyses and machine learning techniques. Our results show vocal differences between *P. m.*
36 *mocinno* and *P. m. costaricensis* that could have a molecular basis, potentially due to genetic drift
37 developed during the more than three million years of separation of *P. m. mocinno* (from Mexico
38 to Nicaragua) and *P. m. costaricensis* (Costa Rica and Panama). We therefore suggest that *P.*
39 *mocinno* could potentially be divided into two species. A possible separation of these taxa into
40 two species could have important consequences for the conservation status of the Resplendent
41 Quetzals, and redirect conservation efforts for these taxa.

42

43 Key words: territorial vocalization – *Pharomachrus mocinno* – Resplendent Quetzal –
44 Trogonidae – conservation

45

46

47 **Introduction**

48 The biological species concept is the main evolutionary concept considered to draw the
49 lists of threatened species by the International Union for the Conservation of Nature Red List
50 (IUCN 2001) which are mainly used to rule national and international policy for nature
51 conservation (Isaac et al. 2004). The species level bears a particular importance for flagship
52 species which act as symbols and attract public interest (Simberloff 1998). Most flagship species
53 are large mammals such as the African elephant (*Loxodonta africana*) for African savannah, the
54 giant panda (*Ailuropoda melanoleuca*) for Chinese bamboo forest, the Bengal tiger (*Panthera*
55 *tigris*) for Indian forest, the koala (*Phascolarctos cinereus*) for Australian eucalypt woodlands, or
56 the humpback whale (*Megaptera novaeangliae*) for oceans (Courchamp et al. 2018; Groom et al.
57 2006). Bird species are more rarely used as a nature icon. One exception is the Resplendent
58 Quetzal, *Pharomachrus mocinno* (De la Llave 1832) (Aves: Trogonidae), a rare Neotropical bird
59 with highly coloured, bright and elongated feathers (LaBastille et al. 1972) and regarded as a
60 symbol of Central American cloud forest. The Resplendent Quetzal is the centre of the
61 Guatemalan heritage since the Mayan civilizations, being represented in all sorts of arts, drawn
62 on the national flag, and used as the currency name (Bowes and Allen 1969). The Resplendent
63 Quetzal is also considered a symbol of freedom, due to the belief that the species cannot live in
64 captivity. Meanwhile there has been some successful captive reproduction programs, the task
65 require a high level of technical knowledge (Morales-Divas, 2017).

66 In addition to its social influence, the Resplendent Quetzal plays a significant ecological
67 role by dispersing the seeds of at least 32 tree species and by participating in the dynamics and
68 resilience of the cloud forest (Solórzano et al. 2000). *Pharomachrus mocinno* is ranked in the
69 Near Threatened category of the IUCN Red List (Birdlife International 2016) and listed in the
70 Convention on International Trade in Endangered Species of Wild Fauna and Flora Appendix I of

71 the most endangered species (UNEP-WCMC (Comps.) 2014). The distribution of the
72 Resplendent Quetzal shows an insular pattern limited to well preserved cloud forests in the south
73 of Mexico, Guatemala, El Salvador, Nicaragua, Honduras, Costa Rica and Panama (Solórzano et
74 al. 2003).

75 *Pharomachrus mocinno* was originally described by the Mexican naturalist Pablo de la
76 Llave from specimens collected between 1787 and 1803 in Guatemala ('Goatemala') by the
77 Royal Botanical Expedition to New Spain (De la Llave 1832). The name of the specific epithet,
78 *mocinno*, was dedicated to the naturalist José Mariano Mociño, who participated in the
79 expedition, and the genus name referred to the main body characteristics of the bird, *pharos*
80 meaning mantle and *makros* meaning long in ancient Greek. In 1869, the German ornithologist
81 Jean-Louis Cabanis revealed that male specimens from Costa-Rica were smaller than male
82 specimens from Guatemala, motivating the creation of a new subspecies named *P. m.*
83 *costaricensis* (Cabanis 1869). This subspecies distinction still persists with the populations of
84 south Mexico, Guatemala, Honduras, El Salvador and Nicaragua classified as *P. m. mocinno* and
85 the populations of Costa Rica and Panama classified as *P. m. costaricensis* (Birdlife International
86 2016). The distribution areas of the two subspecies are separated by the Nicaraguan depression, a
87 50 km wide, 600 km long lowland that contains the two largest lakes from Central America,
88 namely the Lake Nicaragua and the Lake Managua (Marshall 2007). The age of this barrier,
89 which is also known as the biological border region of Nicaragua (Weyl 1980), is not precisely
90 established but arose between the early Pliocene (5 million years ago) and the beginning of the
91 Pleistocene (1.8 million years ago), probably when the Panamanian Isthmus was formed three
92 million years ago (Keigwin 1982; Solórzano et al. 2004). Phylogenetic analyses revealed two
93 monophyletic groups, corresponding to each subspecies for which gene flow was possibly
94 interrupted for three to six million years corresponding to the age of the Nicaraguan depression

95 (Solórzano and Oyama 2009). The lack of current contact between the populations of the two
96 subspecies has also been evidenced by telemetry studies, showing that individuals of *P. m.*
97 *costaricensis* from Costa Rica had no contact with populations of *P. m. mocinno* from north
98 Nicaragua (Powell and Bjork 1995).

99 The taking of taxonomic decisions based on the “amount of genetic difference” as an
100 absolute criterion for deciding whether two operational taxonomic units are distinct species, is
101 not recommended (McDonough et al. 2008). Ideally to test the taxonomic status of candidate
102 populations for specieshood, genetic evidence should be supported by complementary character
103 evidences (Cotterill et al. 2014, Tobias et al., 2010).

104 Supporting the original observations of Jean-Louis Cabanis (1869), additional
105 observations suggest that *P. m. mocinno* has more brilliant golden feathers than *P. m.*
106 *costaricensis* and the female of *P. m. mocinno* has a slight crest when the female of *P. m.*
107 *costaricensis* has no crest (Skutch, 1944; LaBastille et al., 1972). Recent morphometry analyses
108 revealed differences in size between the two subspecies, *P. m. mocinno* being larger than *P. m.*
109 *costaricensis*, having longer wings, a wider bill and longer and wider tail cover feathers (Schulz
110 and Eisermann 2017; Solorzano et al. 2009; Solórzano and Oyama 2009). Behavioural characters
111 play an important role in species isolation, geographic variation in songs and calls being of
112 particular importance for species delimitation (Wei et al. 2015). Apart from the oscine passerines,
113 hummingbirds, parrots and some sub-oscines such as the Three-wattled Bellbird *Procnias*
114 *Tricarunculata* (Saranathan et al., 2007) there is no evidence that other birds could learn their
115 vocalizations (Kroodsma and Konishi 1991, Kroodsma, 2005). The species *P. mocinno*, as non-
116 passerine bird, would not acquire vocalizations through learning processes and therefore would
117 not be subject to cultural evolution (Wei et al. 2015). Thus, the acoustic differences between
118 populations, if they exist, could be mainly related to genetic factors (Brown and Lemon 1979).

119 Surprisingly, no comparison between the sounds produced by the two subspecies of *P. mocinno*
120 has been documented yet.

121 To clarify the subspecies vs species taxonomy (Solórzano and Oyama 2009), we
122 conducted an acoustic comparison between the two subspecies *P. m. mocinno* and *P. m.*
123 *costaricensis* and between all *Pharomachrus* taxa based on multivariate analyses and machine
124 learning techniques. Our analyses show that the acoustic signals of *P. m. mocinno* and *P. m.*
125 *costaricensis* differ, mostly in frequency parameters. We then discuss the possible consequences
126 of a taxonomy change, in terms of evolution and conservation.

127

128 **Material and methods**

129 *Acoustic analysis*

130 To assess the acoustic specificity of *P. m. mocinno* and *P. m. costaricensis*, the territorial
131 vocalization of the two subspecies were compared with each other, and with the territorial
132 vocalization of the four other closely related species *P. antisianus*, *P. auriceps*, *P. fulgidus* and *P.*
133 *pavoninus*. The territorial song of *Pharomacrus* was the only vocalization selected for this
134 comparison due to a clear functional and structural acoustic homology between species in the
135 family Trogonidae. The territorial song mainly consists of multiple repetitions of a two-note
136 syllable at fairly regular intervals, with little change in pitch (Johnsgard 2000). The territorial
137 song has already been used to make comparisons between species in the family (Ornelas et al.
138 2009).

139 Seven males of *P. m. mocinno* were visually localized and recorded in January and
140 February 2016 and 2017 during the peak of vocal activity in two protected areas of Guatemala:
141 the “Refugio del Quetzal”, San Marcos (N 14° 56' - W 91° 52', 1531 m) and “Los Andes”,
142 Suchitupéquez private reserve (14° 32' - 91° 11', 1992 m). Recordings were achieved with a

143 Tascam digital recorder DR-100 MK II (44.1 kHz sampling frequency, dynamic range of 16 bit)
144 connected to a Sennheiser ME-67 directional microphone (frequency response: 40-20000 Hz \pm
145 2.5 dB). To increase the number of individuals and include other sites and the closely related
146 species, recordings of 50 individuals available in five sound libraries (Xeno-Canto, Macaulay
147 Library, Biblioteca de Sonidos de Aves de México, Laboratorio de Bioacústica de la Universidad
148 de Costa Rica, and Borror Laboratory of Bioacoustics) were included in the analysis, collected in
149 different locations and/or on different dates, or alternatively when the sound recordists specified
150 that the vocalizations belonged to different individuals. When the libraries provided sounds in
151 compressed mp3 format that are not ideal for sound analysis in birds (Araya-Salas et al. 2017),
152 recordings were systematically requested from the authors in wav format with a minimum
153 sampling rate of 44.1 kHz and a dynamic range of 16 bit. A total of 57 individual recordings (*P.*
154 *m. mocinno*, n=21; *P. m. costaricensis*, n= 15; *P. antisianus*, n=7; *P. auriceps*, n=6; *P. fulgidus*
155 n=4; *P. pavoninus* n=4) from 30 different localities distributed in 11 countries (Mexico,
156 Guatemala, Honduras, Costa Rica, Panama, Colombia, Venezuela, Ecuador, Peru, Bolivia and
157 Brazil) could be analysed (Figure 1, Supporting Information Table S1).

158 The vocalizations were analysed with Raven Pro 1.4 software
159 (www.birds.cornell.edu/raven) directly from on-screen measurement cursors on the oscillogram
160 for time parameters (time precision = 0.0232 s) and on the spectrogram for frequency parameters
161 (Hanning window with a FFT of 1024 points and an overlap of 90% between successive
162 windows, leading to a frequency precision of 21.5 Hz and a time precision of 0.0232 s). Taking
163 measurements on the spectrogram might not be optimal due to limited time and frequency
164 precisions when proceeding formal description of vocalizations, but is valuable when doing
165 comparison between sounds when only relative differences matter. The parameters for each of
166 the two successive notes, note 1 and note 2, composing the syllable were: note duration (s), inter-

167 note separation (s), peak frequency that is the frequency of highest energy (Hz), centre frequency
168 (Hz), highest and lowest frequencies (Hz), first and third frequency quartiles (Hz) (the
169 frequencies that divide the selection into frequency intervals containing respectively 25% and
170 75% of the energy), frequency inter-quartile-range (difference between the first and third
171 frequency quartiles). The inter-syllable separation (s) was also measured. In addition, the
172 frequency modulation (FM) of each note was assessed by measuring the dominant frequency in a
173 series of 20 frequency measurements equally distributed in time along each note using the
174 package seewave 2.0.5 (Sueur et al. 2008) from the R 3.2.5 environment (Development Core
175 Team 2008). The first mathematical derivative of these time series was computed, and the
176 resulting positive and negative values were summed to obtain the positive and negative FM
177 respectively. The FM was then characterized by two features, the positive and negative FMs. In
178 total, a 57 individuals by 22 variables matrix was obtained (one temporal parameter, seven
179 frequency parameters, two FM parameters per note, one temporal parameter between notes, and
180 one temporal parameter between syllables) (Figure 2).

181 As the number of notes found for each individual varied from 12 to 639, a random
182 subsample of 40 notes was applied for the individuals that produced more than 40 notes to ensure
183 balanced datasets. A total of 1738 notes were analysed. For each note, the average of each
184 parameter per individual was calculated. The spectrograms were generated with seewave with a
185 Fourier transform made of 2048 samples tapered with a Hanning window and with an overlap of
186 87.5%.

187 To test how the 22 acoustic features could classify correctly *P. m. mocinno*, *P. m.*
188 *costaricensis* and the closely related species, two supervised classification methods were
189 applied, used in machine learning, namely a multiclass linear discriminant analysis (LDA) for the
190 subspecies comparison (Fisher 1936) and a balanced random forest analysis (RF) (Breiman 2001)

191 including the subspecies and the other *Pharomachrus* species. For the LDA, the data were first Z-
192 transformed and reduced to two dimensions with a principal component analysis (PCA). The
193 coordinates of the recordings according to the first two PCA axes were used as input data for the
194 LDA. The taxa names were used as an explained (dependent) variable so that the LDA classified
195 the recordings according to subspecies. A LDA confusion matrix was built to estimate the
196 percentage of correct classification, and PCA scores were plotted as a function of latitude to test
197 whether the territorial vocalizations of *P. m. mocinno* and *P. m. costaricensis* intergrade along
198 their distribution. Both PCA and LDA analyses were carried out with the R package *ade4* (Dray
199 et al. 2016).

200 For the RF, a Breiman's RF algorithm was applied on the 57 by 22 matrix with the help of
201 the *randomForest* R package (Liaw and Wiener 2015). The RF analysis was designed so that the
202 six *Pharomachrus* taxa were defined as the explained (dependent) variable and the 22 acoustic
203 features as the explaining (independent) variables. A total of 4000 decision trees were built based
204 on a random sample with replacement among 63% of the observations. A confusion matrix was
205 built with an average error rate based on the observations not sampled, known as the out-of-bag
206 observations. The relative importance of the explaining variables, *i.e.* of the acoustic features,
207 was calculated using the Gini index.

208 A Chi-square test was conducted to evaluate if the number of individuals classified by the
209 LDA or the RF was significantly higher than a classification expected by chance.

210

211 ***Molecular analyses***

212 The 255 bp of the mitochondrial Control Region, of the 16 individuals of *P. m. mocinno* and 9
213 individuals of *P. m. costaricensis*, published by Solórzano *et al.* (2004), were reanalysed using
214 other statistics (Da, dxy, uncorrected sequence divergence) classically used to assess genetic

215 differentiation between two lineages. All analyses were performed in DNAsp 6.0 (Rozas et al.
216 2017).

217

218 **Results**

219 Spectrograms of the typical territorial vocalizations of each *Pharomachrus* taxa are compared in
220 Figure 3. The first two axes of the PCA, which explained 61.17% of the total variance, showed a
221 difference between *P. m. mocinno* and *P. m. costaricensis* (Figure 4). Plotting the PCA scores
222 with respect to latitude did not indicate that the territorial song intergrades and did not show any
223 trend according to latitude within each sub-species (Figure 5). The LDA obtained from the PCA
224 scores showed a clear differentiation between *P. m. mocinno* and *P. m. costaricensis*. The
225 confusion matrix returned 89.88% of correct classification (*P. m. mocinno* 19 of 21 individuals
226 assigned correctly, *P. m. costaricensis* 13 of 15 individuals assigned correctly), and exceeded
227 classification expected by chance (Chi-square test, d.f.=1, $\chi^2=18.37$, $p < 0.001$) (Table 2). The
228 acoustic features of the two subspecies of *Pharomachrus* are shown in Table 1.

229 The RF classification showed that the most important acoustic features to classify the
230 *Pharomachrus* taxa were the peak and centre frequency of the second note, followed by the
231 centre frequency of the second and first note (Figure 6). These parameters were followed by the
232 third frequency quartile of the second and first notes, then the inter-syllable and inter-note
233 separation, the duration of the first note, the frequency inter-quartile of the second note, the peak
234 frequency of the first note and the duration of the second note. The lowest and highest
235 frequencies, the negative and positive FM and the frequency inter-quartile range of the two notes
236 did not appear as major discriminating parameters.

237 The confusion matrix built on the balanced RF classification revealed a high correct
238 classification rate for all the species and subspecies with 81.9 % for *P. m. mocinno* (17 of 21

239 individuals assigned correctly, 86.67% for *P. m. costaricensis* (13 of 15 individuals assigned
240 correctly), 100% for *P. antisianus* (7 of 7 individuals assigned correctly), 100% for *P. auriceps*
241 (6 of 6 individuals assigned correctly), 75% for *P. fulgidus* (3 of 4 individuals assigned
242 correctly), and 100% for *P. pavoninus* (4 of 4 individuals assigned correctly) (Table 3), all rates
243 exceeded classification expected by chance (Chi-square test, d.f.=25, $\chi^2=221.1$, $p < 0.001$).

244

245 *Molecular analyses*

246 The divergence statistics in the 255 bp fragment of the Control Region between *P. m. mocinno*
247 and *P. m. costaricensis* were: Da: 0.02763, dxy: 0.03091, uncorrected sequence divergence:
248 3.1%.

249

250 **Discussion**

251

252 *Acoustic difference between **Pharomachrus taxa***

253 The acoustic analysis showed relatively important differences among the *Pharomachrus* species,
254 suggesting that each taxa bears a species signature in its song, a phenomenon commonly
255 observed in birds but also in other singing species (Obrist et al. 2010). In particular, we found a
256 difference in the acoustic parameters of *P. mocinno* and *P. costaricensis*, similar as it has been
257 reported for other learning and non-learning species where species status has been promoted
258 (Cadena and Cuervo 2010; Millsap et al. 2011; Sandoval et al. 2014, 2017). The correct
259 classification between the two taxa was high as revealed by the LDA classification and confirmed
260 by the RF classification among all *Pharomachrus* taxa. In the particular case of the RF, the
261 classification of the sub-species was slightly less successful than the classification of the other

262 species except for *P. fulgidus* that included only four individuals. As non-passerine birds, species
263 of *Pharomachrus* are supposed not to learn their vocalizations (Kroodsmma and Konishi 1991;
264 Kroodsmma 2005; Saranathan et al. 2007), so such differences between species of the family
265 probably arise from genetic drift, acoustic adaptation to environments or sexual selection
266 cumulated by years of separation.

267 In numerous species, body size is negatively correlated to sound frequency, a larger
268 animal producing lower frequencies (Fletcher 2004; Martin et al. 2011). Here the peak, median,
269 lowest and highest frequencies of the territorial vocalization of males of *P. mocinno* were higher
270 than in *P. costaricensis*, when the first is significantly larger and heavier than the second (Schulz
271 and Eisermann 2017; Solórzano et al. 2009, Solórzano and Oyama 2009). Such discrepancy
272 between acoustics and morphology among-taxa has been observed for other bird species (Laiolo
273 and Rolando 2003) and might suggest the occurrence of physiological or environmental
274 evolutionary constraints. The morphological difference existing between the two species could be
275 the consequence of different sexual selective pressures within the populations of *P. mocinno* and
276 of *P. costaricensis*. This morphological difference may also indicate that following a potential
277 founder group, with representation of larger males, this characteristic is maintained by a sexual
278 selection process (Solórzano 2003). Moreover, plotting the discriminant function scores with
279 respect to latitude did not reveal trends that would suggest intergradation.

280

281 ***Integrative taxonomy of the Resplendent Quetzal and implications for conservation***

282 A discrete molecular differentiation was found between the two *P. mocinno* taxa
283 (Solórzano et al. 2004; Solórzano and Oyama 2009), implying that there is no female mediated
284 gene flow between the two subspecies. The divergence we found between the two Resplendent
285 Quetzal taxa (3.1%) is similar to that described between other bird sister-species (Frankham et al.

286 2010), and in particular within the Trogonidae (1-4% in ND2 for sister-species in the Neotropical
287 genus *Trogon* (DaCosta and Klicka 2008), 10-13% in ND2 for sister-species in the Asian genus
288 *Harpactes* (Hosner et al. 2010)). The International Ornithological Committee (IOC) taxonomy
289 (Gill and Donsker 2017) for the genus *Trogon* was based on the results from DaCosta and Klicka
290 (2008) and resulted in the elevation of several subspecies to species status (e.g. *T. mesurus*, *T.*
291 *ramoniamus*) for 'traditional species' that were not monophyletic in DaCosta and Klicka (2008).
292 Monophyletic species (e.g. *T. personatus*, *T. rufus*) with strong genetic differentiation (8%)
293 across their distribution were not split (Gill and Donsker 2017). The genetic differentiation
294 between the two *P. mocinno* subspecies is 3.1% for the analysed 255 bp of the Control Region
295 fragment which usually has a comparatively higher substitution rate than protein coding genes in
296 birds (Lerner et al. 2011). Furthermore, it could be difficult to representatively estimate the
297 genetic divergence from such a short fragment. From a phylogenetic perspective, the two
298 subspecies are reciprocally monophyletic and diverge from each other by a level of genetic
299 divergence that is the low end of the range of genetic divergence between undisputed species.
300 Hence, the short sequence data available so far need to be complemented by the analyses of
301 characters linked to the evolution of reproductive isolation (biometrics, vocalizations) are
302 necessary.

303 For ethical reasons, due to the fact that *P. mocinno* and of *P. costaricensis* are rare,
304 endangered and highly protected in Guatemala, it was not possible to conduct playback
305 experiments to test whether the individuals perceive the differences revealed by the analysis as
306 usually achieved in behavioural experiments (Freeman and Montgomery 2017). Nevertheless,
307 previous playback experiments showed that males of *P. mocinno* could respond to territorial
308 vocalizations of *P. costaricensis* (Solórzano and Oyama 2009) as actually did other species of the
309 family Trogonidae responding to the same vocalizations tested (2017 personal communication

310 from S. Solorzano to PB, unreferenced). This failure to discriminate an allospecific song has been
311 reported in other bird species (Nelson 1998; Soha et al. 2016) and does not preclude that females
312 could discriminate allospecific territorial and courtship vocalizations in a mate choice context
313 (Seddon and Tobias 2007).

314 The acoustic differences between *P. mocinno* and *P. costaricensis* are in agreement with
315 the morphology differentiation (LaBastille et al. 1972; Schulz and Eisermann 2017; Solórzano
316 and Oyama 2009), the genetic differentiation that a lack of shared haplotype implying no female
317 mediated gene flow (Solórzano and Oyama 2009), and the absence of contact due to an important
318 geographical and climatic barrier (Powell and Bjork 1995).

319 Speciation is a continuous process on which it is often very difficult, if not impossible, to
320 place a boundary among populations, especially if the populations involved are allopatric.
321 Populations distributed along the speciation continuum are often characterized by a mosaic of
322 differentiation in characters involved in the evolution of reproductive isolation and this is
323 reflected in the two *P. mocinno* subspecies.

324 The taxonomic decision to erect *P. mocinno* and *P. costaricensis* at the species level
325 would have strong consequences for conservation. Traditional subspecies nomenclature can
326 provide a misleading impression of the true geographical pattern of intraspecific differentiation
327 along the speciation gradient and can arguably misdirect conservation effort (Zink 2004). At a
328 global level, the former *P. m. mocinno* is classified as a Near Threatened species (Birdlife
329 International 2016). Even though the two subspecies are at an intermediate level of
330 differentiation, where elevating them as full species or keeping them as subspecies could be a
331 matter of debate. A species level would imply a reduction of the area of occurrence and a
332 decrease of population density for each taxon. Therefore the conservation status must be
333 reconsidered for each taxon to a higher level of danger. Moreover, both *P. m. mocinno* and *P. m.*

334 *costaricensis* are vulnerable due to a widespread deforestation, but the rate of habitat degradation
335 being higher for the former than for the later (Sofia Solórzano et al. 2003), the modification of the
336 conservation status could be higher for *P. m. mocinno*.

337 Based on multivariate acoustic analysis and machine learning techniques, we could
338 highlight an acoustic difference between *P. m. mocinno* and *P. m. costaricensis*, which could
339 support a possible separation of these taxa into two species. Nevertheless, additional high-quality
340 and well documented recordings of all *Pharomachrus* taxa would help in getting a better idea of
341 variation inside the genus and further studies of differences in ecology and behaviour between the
342 two subspecies are needed to decide if the subspecies could be regarded as two valid biological
343 species..

344

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360

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363

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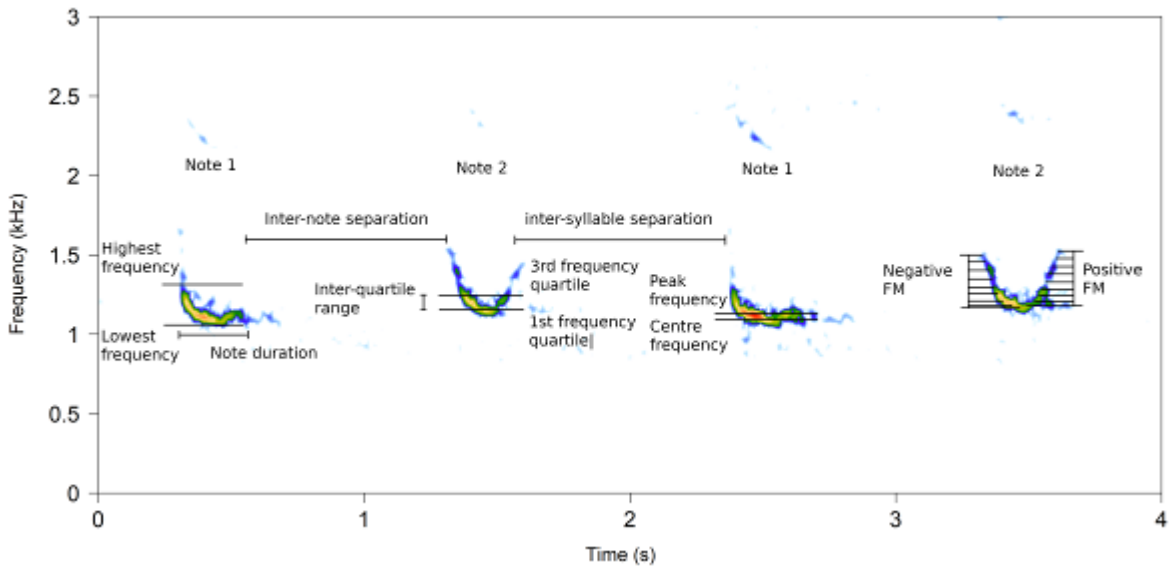


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503 Figure 1: Map of Central America and north of South America showing the sites of recordings of
 504 *Pharomachrus* species and subspecies used for the comparative analysis (Google® background).

505 Picture of *P. m. mocinno*, approximate body length 41 cm (picture reproduced with the
 506 authorization of Ricky Lopez).

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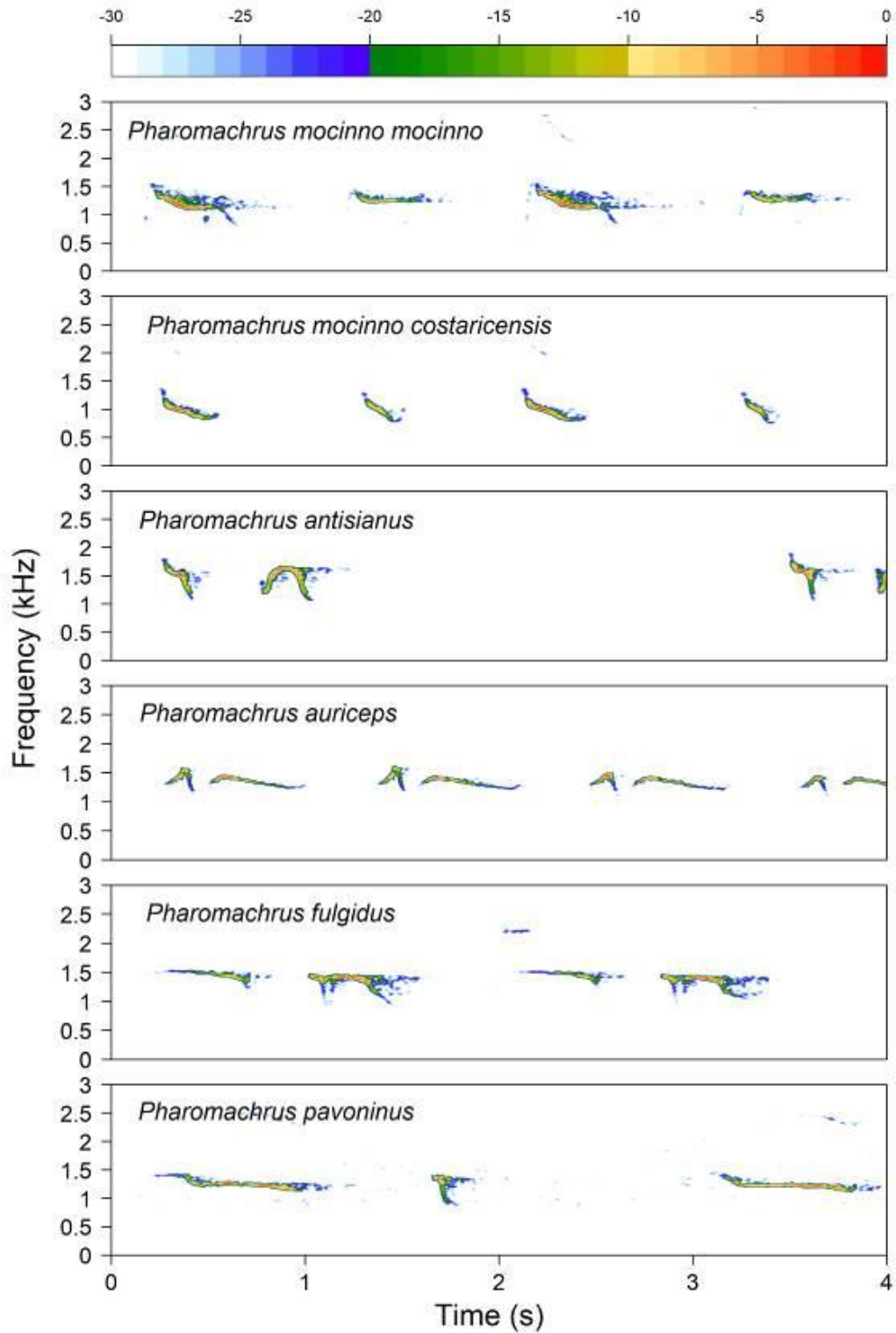
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509 Figure 2: Annotated spectrogram of a male territorial vocalization of *P. m. mocinno*, showing the
 510 time and frequency measurements (short-time Fourier transform parameters: Hann window made
 511 of 2048 samples and 87.5% of overlap between successive windows).

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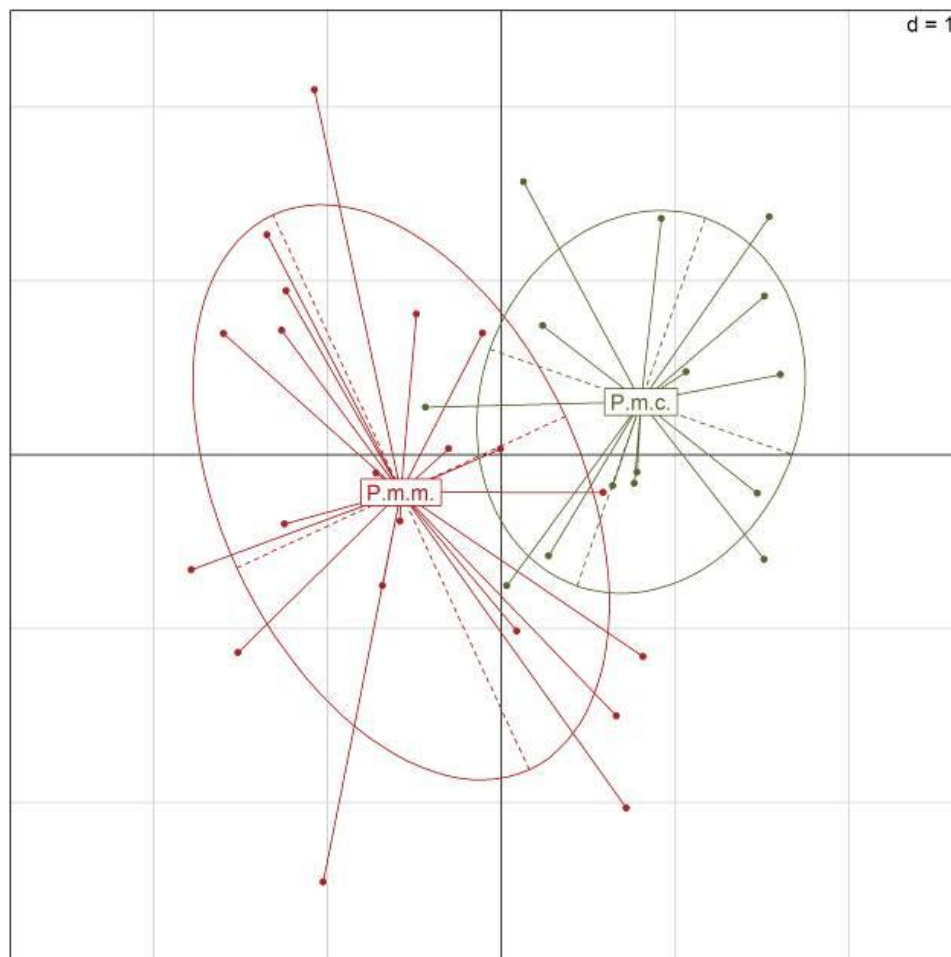


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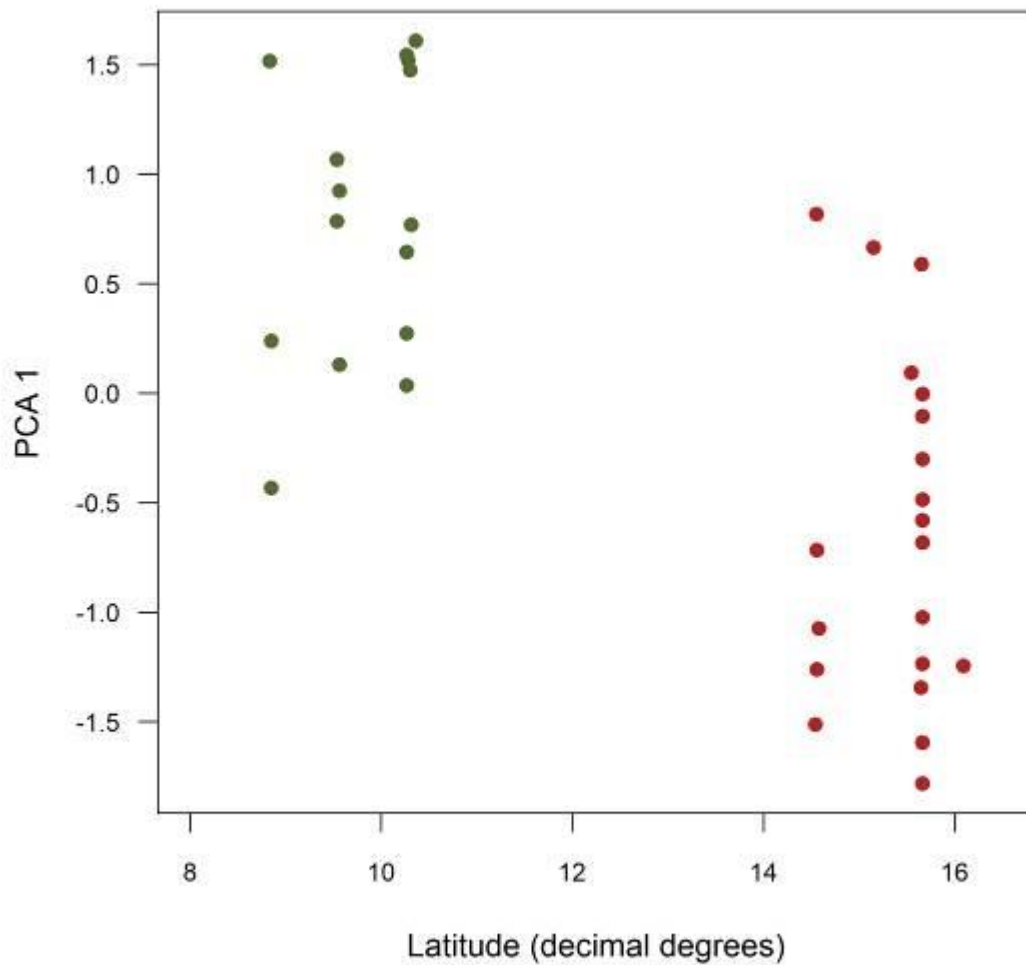
516 Figure 3: Spectrograms of the territorial vocalizations of *P. m. mocinno*, *P. m. costaricensis*, *P.*

517 *antisianus*, *P. auriceps*, *P. fulgidus* and *P. pavoninus* (Sound recordists in the same order: P.

518 Bolaños, L. Baptista, C. Marantz, V. Emanuel, M. Robbins, P. Boesman) (short-time Fourier
519 transform parameters: Hanning window made of 2048 samples and 87.5% of overlap between
520 successive windows). The vocalizations were aligned to fit into a 4 s window to allow temporal
521 comparison.
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524 Figure 4: Principal Component Analysis (PCA) projection showing the space defined by the two
525 first principal axes that explained 61.17% of the total variance. Each point corresponds to a single
526 individual. *Pharomachrus mocinno mocinno* (P.m.m.) individuals are indicated in red and *P. m.*
527 *costaricensis* (P.m.c.) individuals in green. The ellipses surround the centroid of each taxa and
528 delimit 67% of the vocalizations that are expected to be associated with each taxa.

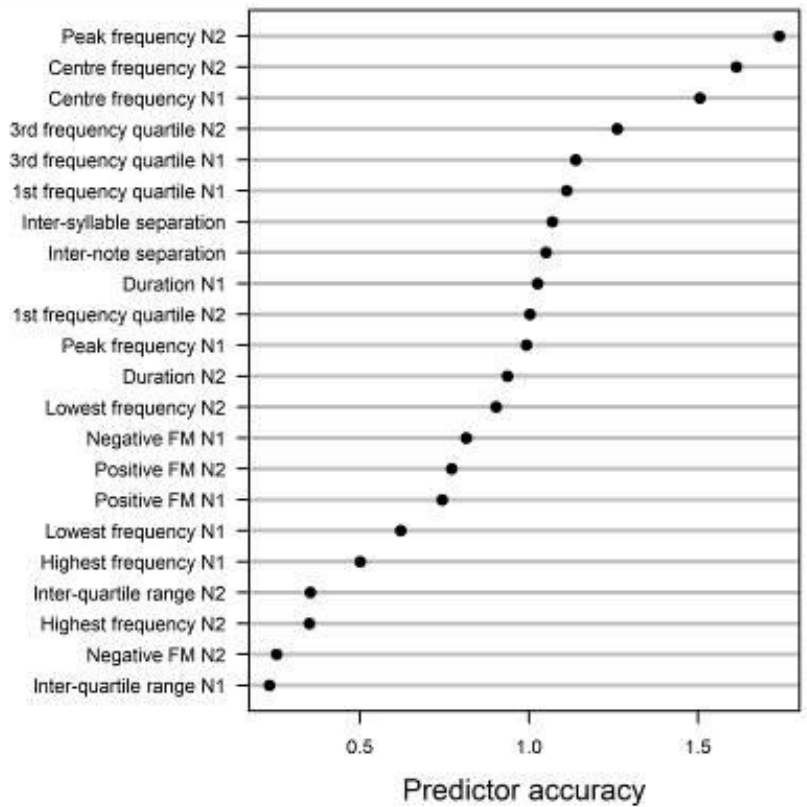


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530 Figure 5: Scores obtained from principal component analysis (PCA) based on 22 acoustic
 531 measurements of the song of *P. m. mocinno* (red dots) and *P. m. costaricensis* (green dots),
 532 plotted as a function of latitude (total individuals is 21 *P. m. mocinno* and 15 *P. m. costaricensis*).

533 The gap in latitude between 11° and 13° is a gap in the distribution of *P. mocinno* related to the
 534 lowlands of Nicaragua.

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537 Figure 6: Random Forest analysis for *Pharomachrus* taxa. Relative importance of the explaining
 538 variables based on the mean decrease Gini impurity criteria.

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548 Table 1: Characteristics of the territorial vocalization of *P. m. mocinno* and *P. m. costaricensis*
 549 (21 individuals for *P. m. mocinno* and 15 individuals for *P. m. costaricensis*). Mean \pm SD (range).

Acoustic Feature	<i>P. m. mocinno</i>	<i>P. m. costaricensis</i>
Inter note separation (s)	0.53 \pm 0.12 (0.08-0.65)	0.63 \pm 0.12 (0.41-0.82)
Inter syllable separation (s)	0.68 \pm 0.16 (0.38-1.01)	0.6 \pm 0.09 (0.4-0.73)
Note 1		
Duration (s)	0.38 \pm 0.09 (0.27-0.59)	0.3 \pm 0.05 (0.23-0.39)
Center frequency (Hz)	1094.8 \pm 99.4 (880.7-1205.9)	986.1 \pm 82.87 (865.9-1094.6)
Highest frequency (Hz)	1462 \pm 140.53 (1190-1728)	1409 \pm 182.28 (1147-1771)
Lowest frequency (Hz)	910.7 \pm 145.19 (678.8-1106)	723.6 \pm 65.75 (613-853.7)
First frequency quartile (Hz)	1051.5 \pm 102.98 (835.9-1169.1)	933.3 \pm 84.03 (816.5-1061.1)
Third frequency quartile (Hz)	1143.2 \pm 81.61 (954.3-1255.3)	1035.2 \pm 77.02 (905.6-1131.7)
Inter-quartile range (Hz)	91.76 \pm 55.9 (32.29-278.62)	101.88 \pm 40.22 (56.24-193.80)
Peak frequency (Hz)	1098 \pm 95.66 (889.3-1210.9)	991.4 \pm 84.23 (867-1109)
Negative FM (Hz)	1.65 \pm 0.43 (0.83-2.53)	1.5 \pm 0.33 (0.98-1.99)
Positive FM (Hz)	1.61 \pm 0.42 (0.95-2.49)	1.48 \pm 0.29 (1.03-1.99)
Note 2		
Duration (s)	0.34 \pm 0.09 (0.18-0.52)	0.31 \pm 0.06 (0.22-0.43)
Center frequency (Hz)	1164 \pm 84.66 (1006-1343)	987.6 \pm 67.21 (893.6-1100.5)
Highest frequency (Hz)	1439 \pm 129.81 (1212-1643)	1324 \pm 131.78 (1154-1679)
Lowest frequency (Hz)	970.8 \pm 135.94 (731.2-1196.5)	774.9 \pm 68.65 (689.7-913.3)
First frequency quartile (Hz)	1115.5 \pm 100.73 (943.9-1319.7)	931.2 \pm 81.95 (802.1-1065.3)
Third frequency quartile (Hz)	1210 \pm 81.61 (1072-1366)	1046.2 \pm 77.02 (970.3-1134.1)
Inter-quartile range (Hz)	94.55 \pm 55.91 (30.88-343.56)	114.95 \pm 40.22 (37.49-199.95)
Peak frequency (Hz)	1168 \pm 81.47 (1036-1354)	992.8 \pm 68.29 (881.2-1108.4)
Negative FM (Hz)	1.64 \pm 0.49 (0.63-2.53)	1.54 \pm 0.32 (1.01-2.1)
Positive FM (Hz)	1.66 \pm 0.49 (0.66-2.6)	1.54 \pm 0.3 (1.13-2.1)

550 Table 2: LDA confusion matrix used to classify the species belonging to *P. m. mocinno* or *P. m.*
 551 *costaricensis*, based on 22 acoustic measurements of the territorial vocalizations (21 individuals
 552 for *P. m. mocinno* and 15 individuals for *P. m. costaricensis*).

	<i>P. m. mocinno</i>	<i>P. m. costaricensis</i>
<i>P. m. mocinno</i>	92.86	0.07
<i>P. m. costaricensis</i>	13.1	86.9

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Table 3: RF confusion matrix used to classify the species belonging to *Pharomachrus* genus (total individuals is 21 for *P. m. mocinno*, 15 for *P. m. costaricensis*, 7 for *P. antisianus*, 6 for *P. auriceps*, 4 for *P. fulgidus*, 4 for *P. pavoninus*) on the basis of 22 acoustic features. Data mentioned in the text are underlined.

	<i>P. m. mocinno</i>	<i>P. m. costaricensis</i>	<i>P. antisianus</i>	<i>P. auriceps</i>	<i>P. fulgidus</i>	<i>P. pavoninus</i>	Class error
<i>P. m. mocinno</i>	<u>80.95</u>	14.29	0.00	0.00	4.76	0.00	0.19
<i>P. m. costaricensis</i>	13.33	<u>86.67</u>	0.00	0.00	0.00	0.00	0.13
<i>P. antisianus</i>	0.00	0.00	<u>100.00</u>	0.00	0.00	0.00	0.00
<i>P. auriceps</i>	0.00	0.00	0.00	<u>100.00</u>	0.00	0.00	0.00
<i>P. fulgidus</i>	25.00	0.00	0.00	0.00	<u>75.00</u>	0.00	0.25
<i>P. pavoninus</i>	0.00	0.00	0.00	0.00	0.00	<u>100.00</u>	0.00

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