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

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

Abstract

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

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

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

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

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

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

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

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

Material and methods

Acoustic analysis

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

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

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

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

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

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

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

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

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

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

Molecular analyses

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

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

Results

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

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

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

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

Molecular analyses

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

Discussion

Acoustic difference between *Pharomachrus* taxa

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

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

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

Integrative taxonomy of the Resplendent Quetzal and implications for conservation

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

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

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

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

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

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

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

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

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

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Figure 1: Map of Central America and north of South America showing the sites of recordings of *Pharomachrus* species and subspecies used for the comparative analysis (Google® background). Picture of *P. m. mocinno*, approximate body length 41 cm (picture reproduced with the authorization of Ricky Lopez).

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

515

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.

522

523

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.

529

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.

535

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

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

553

554

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

555