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On the genetic distinctiveness of tailorbirds (Cisticolidae: *Orthotomus*) from the South-east Asian mainland with the description of a new subspecies

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Abstract

Background: The Cambodian Tailorbird (*Orthotomus chaktomuk*) was one of the most recent major ornithological discoveries in South-east Asia as it originated from lowland seasonally flooded scrub within the densely inhabited floodplain around the Tonle Sap, Mekong and Bassac rivers (Cambodia). The Cambodian Tailorbird is sister to the Dark-necked Tailorbird (*O. atrogularis*) with very limited genetics and biometric differentiation. Between 2004 and 2012, evidences of a new population of Ashy Tailorbird (*O. ruficeps*) in SE Cambodia/Vietnam accumulated but no museum specimens were ever reported. The Ashy Tailorbird currently consists of eight subspecies among which the closest geographical populations, *O. r. cineraceus* (SE Burma to Malay Peninsula, Sumatra, Bangka and Belitung) and *O. r. borneoensis* (Borneo), are allopatric. Subspecific identification of the Cambodian Ashy Tailorbirds individuals was not possible because of the limited differences in plumage among subspecies.

Methods: We inspected the *Orthotomus ruficeps* specimens housed at the Museum National d'Histoire Naturelle (Paris) and discovered five specimens of *O. ruficeps* collected by Louis Rodolphe Germain in 'Cochinchina' (corresponding to southern Vietnam) during the nineteenth century. We sequenced one mitochondrial locus and gathered biometric data from these specimens and compared them with other *Orthotomus* lineages.

Results: The Ashy Tailorbird population from SE Cambodia and Vietnam is distinct from the two geographically close subspecies *O. r. borneoensis* (1.7%) and *O. r. cineraceus* (1.3%). *O. chaktomuk* is nested within *O. atrogularis* in the mitochondrial gene tree. The SE Cambodia/Vietnam population of *O. ruficeps* is distinct from the two other subspecies in bill shape.

Conclusion: Our study described the biometric and molecular distinctiveness of a recently re-discovered population of Ashy Tailorbird in SE Cambodia and Vietnam and suggests that this population constitutes an independent evolutionary lineage that we describe here as a new subspecies. The newly described Cambodian Tailorbird is nested within the Dark-necked Tailorbird and the genetic divergence is much lower than initially described (0.4–0.7% vs 1.1–1.4%).

Keywords: *Orthotomus*, Differentiation, Subspecies, Taxonomy

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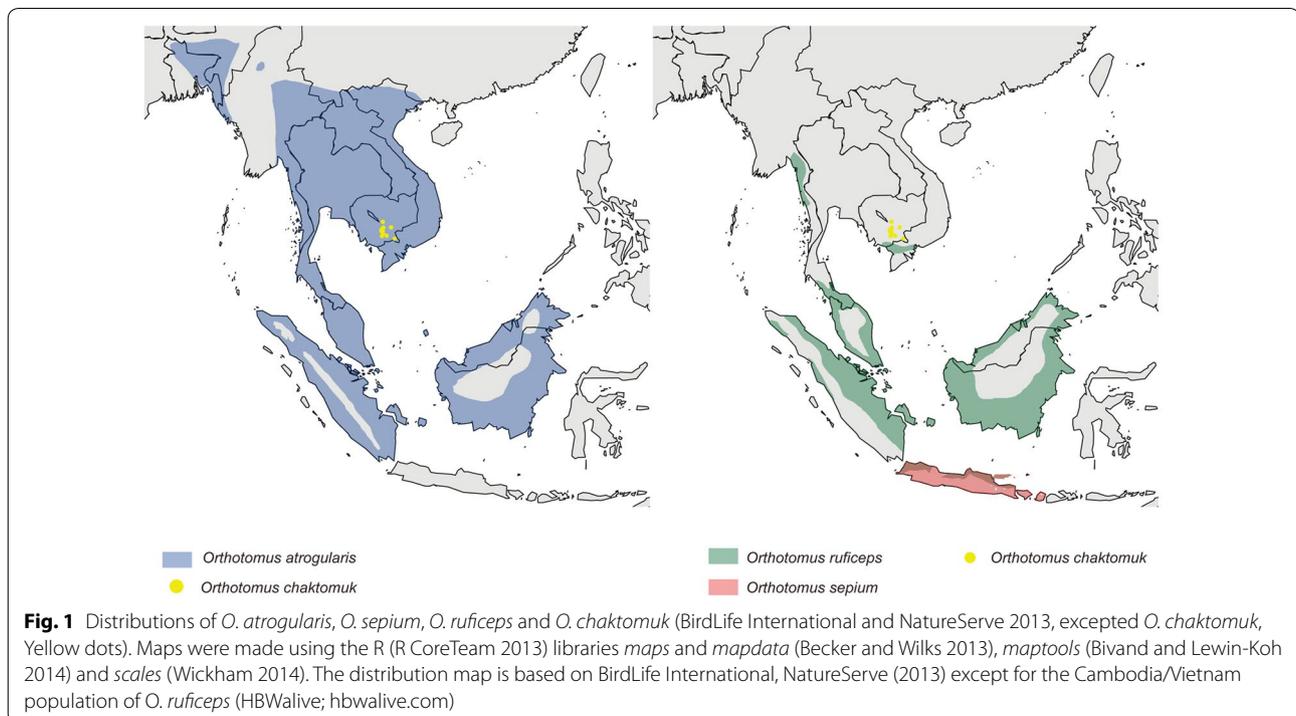


Background

South-east Asia has revealed several spectacular new species to science over the last three decades, mostly from the annamitic chain of Lao RDP and Vietnam, including a forest Bovidae (Saola, *Pseudoryx nghetinhensis*, Vu et al. 1993), the first representative of Caudata for Laos RDP (*Laotriton laoensis*, Stuart and Papenfuss 2002), a member of the Diatomyidae (*Laonastes aenigmaeus*, Jenkins et al. 2005), a lineage of rodents that was presumed to be extinct for the last 11 Myrs, and the Bare-faced Bulbul (*Pycnonotus hualon*, Woxvold et al. 2009), a highly distinct passerine for which a new genus was recently described (Fuchs et al. 2018).

Hence, it came with great surprise that the most recent major ornithological discovery in South-east Asian mainland, the Cambodian Tailorbird (*Orthotomus chaktomuk* Mahood et al., 2013), originated from lowland seasonally flooded scrub within the densely inhabited floodplain around the Tonle Sap, Mekong and Bassac rivers in southern Cambodia. This newly described taxon has been shown to be part of the *O. atrogularis*–*O. ruficeps*–*O. sepium* clade (Sheldon et al. 2012; Mahood et al. 2013). The Cambodian Tailorbird was sister to the Dark-necked Tailorbird (*O. atrogularis* Temminck, 1836), with limited genetic (1.1–1.4% divergence in the analysed mitochondrial locus, the NADH dehydrogenase II) and biometric (culmen/wing ratio in males) differentiation. Vocalizations and plumage characters appeared

to be slightly more divergent from its sister species *O. atrogularis* with the latter being somewhat intermediate between the plumage of *O. atrogularis* ssp. and *O. ruficeps*. The Ashy Tailorbird (*Orthotomus ruficeps* Lesson, 1830) currently consists of eight subspecies among which only one occurs on South-east Asian mainland, *O. r. cineraceus* Blyth, 1845 (SE Burma to Malay Peninsula, Sumatra, Bangka and Belitung; Fig. 1) (Dickinson and Christidis 2014). Further widely distributed subspecies occur on Borneo (*O. r. borneoensis* Salvadori, 1874) and Java (*O. r. ruficeps* Lesson, 1830). The remaining five subspecies are found in peripheric island of Sumatra (*O. r. baeus* Oberholser, 1912, Nias and Pagi Is.; *O. r. concinnus* Riley, 1927, Siberut, Sipura Is.), Java (*O. r. palliolatus* Chasen and Kloss, 1932, Kangean, Karimon Java Is.; *O. r. baweanus* Hoogerwerf, 1962, Bawean Is.) and Borneo (*O. r. cagayanensis* Riley, 1935, Cagayan Sulu Is.). Hence, no known populations of *O. ruficeps* were known to be distributed close to the range of *O. chaktomuk* until 2012 when the Ashy Tailorbird was detected near Kampot and Kep in SE Cambodia (Mahood and Martin 2013; Goes 2013). Further evidences of the occurrence of previously unknown *O. ruficeps* populations in the Mekong area of Vietnam were suggested by Buckton and Safford (2004) and Robson (2011). The localities where Ashy Tailorbirds were detected in Cambodia are only 20–30 km from the known populations in Vietnam. Yet, as Mahood and Martin (2013) stated, “Subspecific identification in Cambodia



is not possible because no specimens of the Mekong delta population have been collected; but due to its geographic isolation it is probable that this population represents an undescribed subspecies." As the Museum National d'Histoire Naturelle, Paris (MNHN) has an extensive collection of specimens from South-east Asia, we decided to inspect the *Orthotomus* specimens and discovered five specimens (Table 1) of *Orthotomus ruficeps* collected by Louis Rodolphe Germain, a veterinary surgeon in the French colonial's army during the 1860–1865 period, in 'Cochinchina' (corresponding now to Southern Vietnam). Inspection of the MNHN catalog indicated that one further individual was exchanged with H. von Berlepsch in 1892 and is now cataloged in the Senckenberg Museum (SMF 78404).

We here provide the first biometric data for the Cambodian/Vietnamese populations of *O. ruficeps* as well as preliminary genetic evidence for its distinctiveness from other *Orthotomus* populations in South-east Asian mainland. We also included in the genetic analyses three individuals of *O. atrogularis nitidus* Hume, 1874. This mainland subspecies was not included in the genetic analyses of Mahood et al. (2013) for the description of *O. chaktomuk*. Instead, only one individual of *O. a. atrogularis* from Singapore was included. Yet, several studies

now showed that the populations distributed south of the Isthmus of Kra are genetically very differentiated from populations distributed north of the Isthmus (Sheldon et al. 2009) and are even sometimes not directly related (e.g. Fuchs et al. 2015).

Methods

Molecular data

We extracted DNA from toe pads of nine specimens housed in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN, Table 2) using the Qiagen DNA Mini Kit (Qiagen, Inc), following the manufacturer's protocol or the modified protocol described in Zuccon and Ericson (2010) for the toe-pad samples. We obtained complete sequences for the mitochondrial NADH dehydrogenase II (ND2) gene by performing several overlapping PCR (size 200–250 bp) using specific primers designed in this study (Additional file 1: Table S1). The amplification profile was: initial denaturation 5 min at 95 °C, followed by 35–40 cycles of denaturation 30 s at 95 °C, annealing 30 s at 57 °C, extension 45 s at 72 °C, with a final extension of 5 min at 72 °C. PCR products were cleaned using QIAquick PCR Purification Kit (Qiagen) and run on an ABI Prism 3100 automated DNA sequencer (Perkin-Elmer Applied Biosystems).

Table 1 Details of the six specimens of *Orthotomus ruficeps* from southern Vietnam (original label mention is Cochinchina) collected by Louis Rodolphe Germain and representing, to our knowledge, the only specimens deposited in natural history museums

Collector	Catalog number	Sex	Collection date	Locality	Country
Germain L.R.	MNHN ZO 1866-573	Female	28 February 1864	'Cochinchina'	Vietnam
Germain L.R.	MNHN ZO 1882-738	Male		'Cochinchina'	Vietnam
Germain L.R.	MNHN ZO 1882-739	Female	21 April 1865	'Cochinchina'	Vietnam
Germain L.R.	MNHN ZO 1882-740	Female		'Cochinchina'	Vietnam
Germain L.R.	MNHN ZO 1882-742	Male	29 February 1864	'Cochinchina'	Vietnam
Germain L.R.	SMF 78404	Male		'Cochinchina'	Vietnam

The specimen in Senckenberg was previously cataloged at MNHN with the number MNHN ZO 1882-741; this specimen was not sequenced

Table 2 Details of the nine specimens of *Orthotomus* sequenced as part of this study

Species	Collector	Catalog number	Collection date	Locality	Country
<i>ruficeps</i>	Germain L.R.	MNHN ZO 1866-573	28 February 1864	'Cochinchina'	Vietnam
<i>ruficeps</i>	Germain L.R.	MNHN ZO 1882-738		'Cochinchina'	Vietnam
<i>ruficeps</i>	Germain L.R.	MNHN ZO 1882-739	21 April 1865	'Cochinchina'	Vietnam
<i>ruficeps</i>	Germain L.R.	MNHN ZO 1882-740		'Cochinchina'	Vietnam
<i>ruficeps</i>	Germain L.R.	MNHN ZO 1882-742	29 February 1864	'Cochinchina'	Vietnam
<i>atrogularis</i>	Germain L.R.	MNHN ZO 1882-728	13 April 1867	'Saigon, Cochinchina'	Vietnam
<i>atrogularis</i>	J. Delacour/P. Jabouille	MNHN ZO 1929-1463	27 February 1928	Phu-Qui	Vietnam
<i>atrogularis</i>	J. Delacour/P. Jabouille	MNHN ZO 1939-913	25 January 1930	Hoi-Xuan	Vietnam
<i>atrogularis</i>	J Roche/J. Thiney	MNHN ZO 1989-120	17 December 1986	Trang	Thailand

Our new sequences were analysed together with all ND2 sequences published by Sheldon et al. (2012), Mahood et al. (2013) and Lim et al. (2014). Newly generated sequences have been deposited in Genbank (Accession numbers: MH507590-MH507598).

Analyses

Gene tree reconstruction of the unique mitochondrial haplotypes was performed using Bayesian inference (BI), as implemented in MRBAYES 3.2 (Ronquist et al. 2012). We used the *nst=mixed* option such that model uncertainty is taken into account during the phylogenetic reconstruction, and incorporated rate variation using the *gamma* setting. Four Metropolis-coupled MCMC chains (one cold and three heated) were run for 10×10^6 iterations with trees sampled every 10^3 iterations.

We used PHASE v2.1.1 (Stephens et al. 2001), as implemented in DNASP 5.0 (Librado and Rozas 2009), to infer the alleles for the two nuclear loci (TGFB2 and MUSK) sequenced by Mahood et al. (2013). We used the recombination model and ran the iterations of the final run 10 times longer than for the initial runs. We considered the output of the long final PHASE run as the best estimate of haplotypes. We used TCS 1.21 (Clement et al. 2000) to reconstruct a 95% statistical parsimony network for two nuclear loci.

We used a Bayesian implementation of the general mixed Yule-coalescent model (bGMYC 1.0; Reid and Carstens 2012) to delimit species with our molecular data (speciation probability smaller than 0.05). This implementation incorporates gene tree uncertainty by sampling over the posterior distribution of sampled gene trees. We obtained a posterior distribution of ultrametric gene trees of the unique *Orthotomus* haplotypes using BEAST v1.8 (Drummond and Rambaut 2007) under GTR+G model, a strict clock model and a Yule process for the tree model. We calibrated the tree by using the ND2 rate estimated by Lerner et al. (2011). We ran MCMC for 10^7 iterations with sampling of parameters and trees every 10^3 iterations. The first 10% of the samples were removed as the burnin period. We analysed 100 trees sampled randomly from the posterior distribution and used the default setting in bGMYC. We ran the MCMC chains for 5×10^4 iterations, with a burn-in of 4×10^4 iterations, and sampled parameters every one hundred iterations. All taxa that are not part of *Orthotomus* were pruned from the ultrametric trees prior the bGMYC analyses.

Biometry

Measurements were taken on specimens housed in the MNHN and the Swedish Museum of Natural History (NRM) from *O. sericeus* ($n=12$), *O. atrogularis* ($n=50$), *O. ruficeps* ($n=23$) and *O. sepium* ($n=10$) for five

characters: culmen length (to skull insertion), bill width at posterior edge of nostril, bill height at posterior edge of nostril, tarsus length (both sides) and wing chord (both sides). All measurements were taken by JF using a wing-rule (measurements to the nearest 0.5 mm) and a caliper (measurements to the nearest 0.1 mm) (data available in Additional file 1: Table S2). The average measurement was taken for the tarsus length and the longest measurement was taken for the wing length for the further analyses. We performed a principal component analysis in R, using the *prcomp* function, on all taxa. We also estimated the culmen length/wing length ratio to compare our results with those of Mahood et al. (2013).

Results

Molecular data

We obtained the entire ND2 sequences for all *Orthotomus* specimens (Fig. 2). The Bayesian Inference ND2 tree of the 66 unique *Orthotomus* haplotypes (107 individuals) yielded a tree with congruent topology to those previously reported by Sheldon et al. (2012) and Mahood et al. (2013). Among the five Vietnamese individuals of *O. ruficeps*, we identified two haplotypes (differing by 2 transitions) that were sister to the two other *O. ruficeps* (*O. ruficeps borneoensis* and *O. ruficeps cineraceus*) subspecies, although these relationships were not strongly supported (Posterior probabilities PP: 0.75). The uncorrected genetic distances between the Vietnamese *O. ruficeps* and *O. ruficeps borneoensis* and *O. ruficeps cineraceus* were 1.7% and 1.3%, respectively. These genetic distances are similar to the one observed between *O. ruficeps borneoensis* and *O. ruficeps cineraceus* (1.3%). The sister-species relationship between *O. ruficeps* ssp. and *O. sepium* was strongly supported (PP: 1.0). *O. chaktomuk* was nested within *O. atrogularis*, the two taxa forming a strongly supported clade (PP: 1.0). Among the four newly sequenced *O. atrogularis* individuals, MNHN ZO 1989-120, collected in Trang (Southern-Thailand), clustered with the individual collected in Singapore (PP: 0.75). The relationships of *O. a. nitidus* MNHN ZO 1882-728, 'Cochinchina', were unresolved whereas *O. a. nitidus* MNHN ZO 1929-1463, collected in Phu-Quy, and MNHN ZO 1939-913, collected in Hoi Xuan (northern Vietnam), were sister to part of the clade that includes all *O. chaktomuk* individual (PP: 0.96). Genetic distance between *O. a. nitidus* MNHN ZO 1929-1463/ZO 1939-913 and *O. chaktomuk* was 0.4–0.7%.

The allele network for the two nuclear loci sequenced by Mahood et al. (2013) indicated extensive sharing of alleles among *O. ruficeps*, *O. chaktomuk* and *O. a. atrogularis* for TGFB2 and sorted, but intermingled haplotypes, for MUSK (Additional file 2: Fig. S1), suggesting that the three taxa could not be differentiated using these two loci.

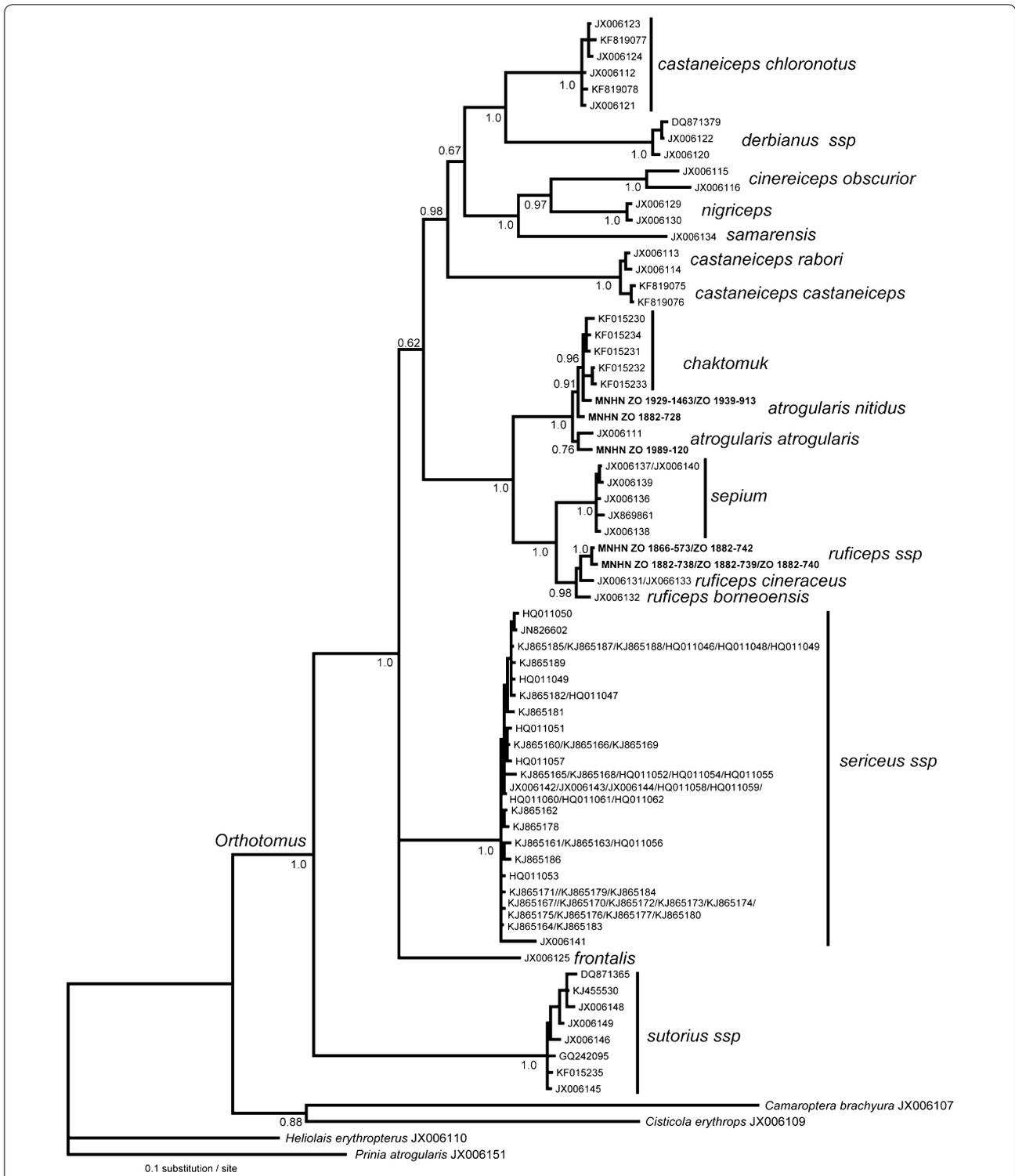


Fig. 2 The 50% majority rule consensus rule tree resulting from the Bayesian analysis of the ND2 gene (only unique haplotypes are included). Numbers close to nodes refer to posterior probabilities greater than 0.95. Number close to nodes refer to Genbank accession numbers excepted for the newly sequenced individuals (MNHN ZO codes)

Results from the molecular species delimitations methods indicate that eleven species could be recognized in *Orthotomus*, as opposed to the thirteen recently proposed (Sheldon et al. 2012; Mahood et al. 2013; Additional file 2: Fig. S2). This estimate is possibly an underestimate as *O. sepium* and *O. ruficeps*, two undisputed species, were considered conspecific (speciation probability: 0.55; average uncorrected percent ND2 distances: 3.2%; Sheldon et al. 2012). *O. chaktomuk* and *O. atrogularis* were considered conspecific (speciation probability: 0.77) as were all *O. ruficeps* individuals (speciation probability: 0.90–0.91).

Biometric data

The boxplots indicated that the three analysed *O. ruficeps* lineages could be distinguished from each other based on bill height. *O. r. borneoensis* could be further distinguished from continental populations based on culmen length, and the Cambodian/Vietnamese population could be distinguished from the two other lineages based on bill width (Table 3, Fig. 3). Tarsus and wing lengths do not allow discriminating between the different taxa. The boxplots of the culmen/wing ratio indicated that the Vietnamese *O. ruficeps* had the lowest ratio of all analysed taxa, whereas *O. a. atrogularis* had the highest (Fig. 3).

Results from the Primary Component Analysis indicated that the *O. ruficeps* collected in Vietnam could be

discretely differentiated from individuals collected in Borneo (*O. r. borneoensis*) and Malay Peninsula (*O. r. cineraceus*) (Fig. 4). Most *O. ruficeps* subspecies could be separated on Axis 2. No differentiations could be made between the two *O. atrogularis* taxa (Fig. 4).

Discussion

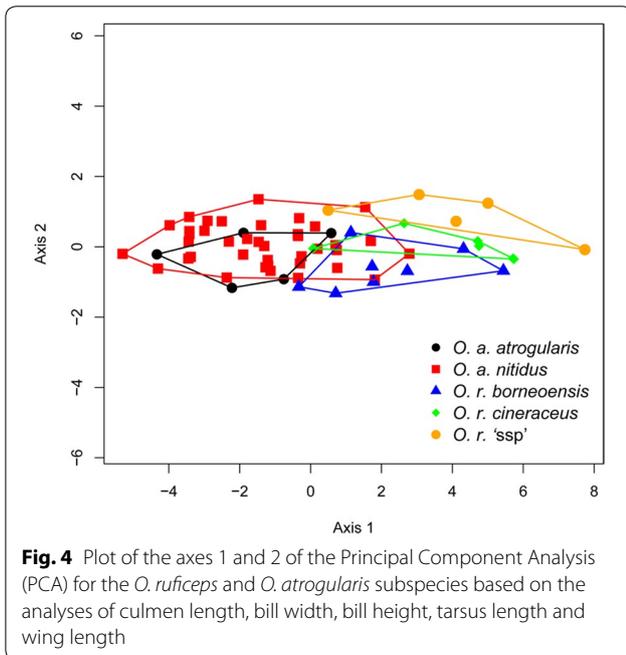
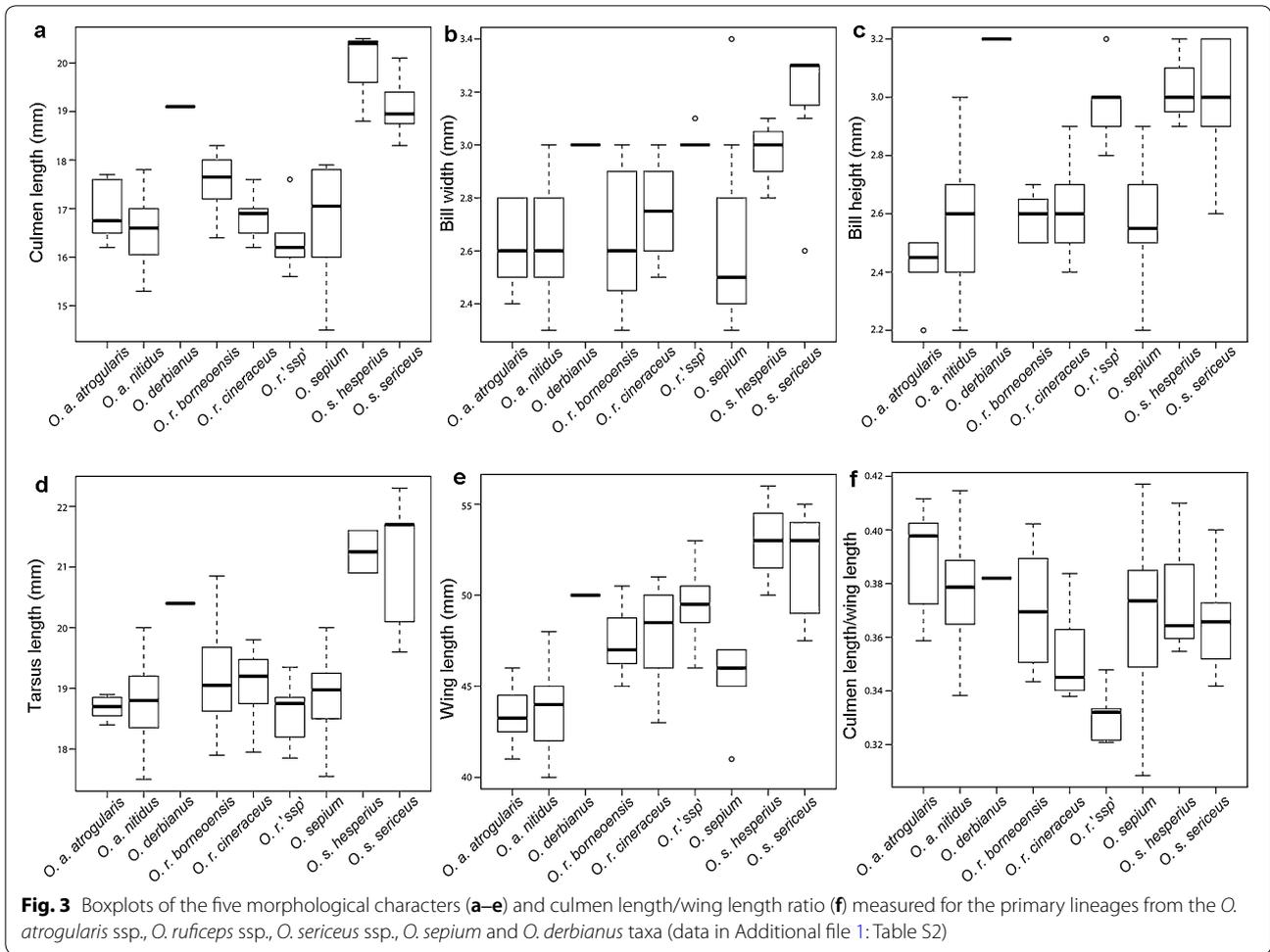
Re-discovered Ashy Tailorbird population

Our analyses include for the first time individuals from a recently re-discovered allopatric population of Ashy Tailorbird (*O. ruficeps*) in SE Cambodia and Vietnam and highlighted the existence of discrete genetic and biometric characters when compared to other *O. ruficeps* populations. This population of Ashy Tailorbird (*O. ruficeps*) from SE Cambodia and Vietnam is between 500 km (assuming oversea dispersal) or 1000 km (assuming no over sea dispersal) from closest populations. This large geographic distance, coupled to the inferred poor dispersal capability of tailorbirds (small rounded wings) as well as the genetic and biometric differences, suggests that the Cambodian/Vietnamese population constitutes an isolated population and an independent evolutionary lineage worth of description. We consider that the subspecies we could not sample (*O. r. ruficeps*, Java; *O. r. baeus*, Nias and Pagi Is.; *O. r. concinnus*, Siberut, Sipura Is.; *O. r. palliolatus*, Kangean, Karimon Java Is.; *O. r. baweanus*, Bawean Is.; *O. r. cagayanensis*, Cagayan Sulu Is.) are highly unlikely

Table 3 Average/standard deviation and minimum–maximum values for six biometric characters for *Orthotomus* lineages

Lineage	Culmen length (mm)	Bill width (mm)	Bill height (mm)	Tarsus length (mm)	Wing chord (mm)	Culmen length/wing chord
<i>r. borneoensis</i>	17.55/0.62 (16.4–18.3) (n=8)	2.65/0.26 (2.3–3.0) (n=8)	2.59/0.08 (2.5–2.7) (n=8)	19.18/0.95 (17.9–20.85) (n=8)	47.44/1.8 (45–50.5) (n=8)	0.37/0.022 (0.34–0.40) (n=8)
<i>r. cineraceus</i>	16.87/0.51 (16.2–17.6) (n=9)	2.74/0.16 (2.5–3.0) (n=10)	2.62/0.16 (2.4–2.9) (n=10)	19.06/0.66 (17.95–19.8) (n=7)	48.0/2.70 (43–51) (n=9)	0.35/0.017 (0.34–0.38) (n=8)
<i>r. 'ssp.'</i>	16.38/0.76 (15.6–17.6) (n=5)	3.02/0.04 (3.0–3.1) (n=5)	2.98/0.15 (2.8–3.2) (n=5)	18.6/0.58 (17.85–19.35) (n=5)	49.5/2.57 (46–53) (n=5)	0.33/0.011 (0.32–0.35) (n=5)
<i>a. atrogularis</i>	16.92/0.62 (16.2–17.7) (n=6)	2.62/0.16 (2.4–2.8) (n=6)	2.42/0.12 (2.2–2.5) (n=6)	18.68/0.21 (18.4–18.9) (n=5)	43.42/1.72 (41–46) (n=6)	0.39/0.02 (0.36–0.41) (n=6)
<i>a. nitidus</i>	16.56/0.60 (15.3–17.8) (n=40)	2.63/0.17 (2.3–3.0) (n=41)	2.57/0.19 (2.2–3.2) (n=41)	18.73/0.65 (17.5–19.9) (n=42)	43.84/1.85 (40–48) (n=44)	0.38/0.018 (0.34–0.41) (n=40)
<i>s. sepium</i>	16.78/1.10 (14.5–17.9) (n=10)	2.65/0.35 (2.3–3.4) (n=10)	2.57/0.19 (2.2–2.9) (n=10)	18.92/0.69 (17.55–20) (n=10)	45.65/1.80 (41–47) (n=10)	0.37/0.03 (0.31–0.41) (n=10)
<i>s. hesperius</i>	19.9/0.95 (18.8–20.5) (n=3)	2.97/0.15 (2.8–3.1) (n=3)	3.0/0.15 (2.9–3.2) (n=3)	21.25/0.49 (20.9–21.6) (n=3)	53.0/3.0 (50–56) (n=3)	0.38/0.03 (0.355–0.376) (n=3)
<i>s. sericeus</i>	19.08/0.57 (18.3–20.1) (n=8)	3.16/0.26 (2.6–3.1) (n=7)	2.98/0.25 (2.6–3.2) (n=5)	21.1/1.04 (19.6–22.3) (n=9)	51.94/2.88 (47.5–55) (n=9)	0.365/0.018 (0.34–0.40) (n=8)
<i>derbianus</i>	19.1 (n=1)	3.0 (n=1)	3.2 (n=1)	20.4 (n=1)	50 (n=1)	0.38 (n=1)

The morphological characters are culmen length, bill width at nostril, bill height at nostril, tarsus length (average of the left and right tarsus when possible) and wing chord (longest measurement of the left or right wing). All characters but wing length were measured to the nearest 0.1 mm. Wing length was measured to the nearest 0.5 mm



to be more closely related to the Cambodian/Vietnam populations than populations from continental South-east Asia based on described phylogenetic patterns (e.g. Sheldon et al. 2009; Lim et al. 2014; Fuchs et al. 2015).

Based on the phylogenetic pattern, biometrics differences and distribution data, we recognize a new subspecies for the *Orthotomus* population distributed in Southern Vietnam and Cambodia that we name “*O. ruficeps germaini* Fuchs Zuccon subsp. nov”.

Holotype

Adult Female Muséum National d’Histoire Naturelle, Paris MNHN ZO-1882-739. Collected by Louis Rodolphe Germain on the 21 April 1865 in ‘Cochinchina’, corresponding now to southern Vietnam.

Diagnosis

Based on available specimens, the Ashy Tailorbird subspecies from Vietnam could not be diagnosed by plumage characters from other Ashy Tailorbird subspecies we examined.

Madge (2018) mentioned that *O. r. borneoensis* has a slightly darker head than *O. cineraceus* and that subspecies differs by the darkness of on the upperparts but we could not recover this trend on the specimens we examined. As for other *O. ruficeps* subspecies, there is sexual dimorphism in *O. ruficeps*: males have a tawny chin and grey throat whereas females have a white chin and throat washed with gray. One male (MNHN ZO 1882-738) has white streaks on throat suggesting that it could be a first-year bird. The new taxon could be diagnosed by culmen length, bill height from other *O. ruficeps* subspecies and by plumage, bill width, and wing length from the sympatric *O. atrogularis* (Fig. 5). The new subspecies differs from the recently described and possibly parapatric *O. chaktomuk* by cheeks and thighs colours (Mahood et al. 2013). A genetic divergence of 1% from *O. r. cineraceus* and 1.1–1.3% from *O. r. borneoensis* was recovered at the analysed mitochondrial locus.

Description of holotype

We used the colour designations from Smithe (1975; corresponding number in parentheses) when relevant. Forehead, lores, ear-coverts and legs TAWNY (38), rectrices, primaries, secondaries, crown and nape CINNAMON BROWN (33) back and rump DARCK NEUTRAL GRAY (83), flanks and sides LIGHT NEUTRAL GRAY (85) chin, throat, and abdomen white and washed with MEDIUM NEUTRAL GRAY (84). The mitochondrial ND2 locus was sequenced for the holotype (Genbank Accession Number MH507592).

Measurements of holotype

Left/right tarsus: 18.1/18.3 mm, left/right wing-chord: 50.5/50 mm, culmen length: 16.2 mm, bill-width at anterior end of nostril 3.0 mm, bill-depth at anterior end of nostril 3.0 mm.

Paratype. MNHN ZO 1866-573 (Fig. 6)

Adult female collected by Louis Rodolphe Germain (collector number 170) on the 28 February 1864 in 'Cochinchina', corresponding now to southern Vietnam. Measurements: left/right tarsus: 17.7/18.0 mm, left/right wing-chord: 46/46 mm, culmen length: 16.0 mm, bill-width at anterior end of nostril 3.0 mm, bill-depth at anterior end of nostril 2.9 mm. The mitochondrial sequence of ND2 of the paratype MNHN ZO 1866-573 (Genbank Accession Number: MH507590) differs by two substitutions from that of the holotype.

Paratype. MNHN ZO 1882-742

Adult male collected by Louis Rodolphe Germain (collector number 170) on the 29 February 1864 in 'Cochinchina', corresponding now to southern Vietnam. Measurements: left/right tarsus: 19.2/19.5 mm, left/right wing-chord: 53/52 mm, culmen length: 17.6 mm, bill-width at anterior end of nostril 3.0 mm, bill-depth at anterior end of nostril 3.2 mm. The mitochondrial sequence of ND2 of the paratype MNHN ZO 1882-742 (Genbank Accession Number: MH507594) differs by two substitutions from that of the holotype and is identical to that of the paratype MNHN ZO-1866-573.

Etymology

We dedicate this subspecies to the initial collector Louis Rodolphe Germain (1827–1917), a veterinary surgeon in the French colonial's army during the 1860–1865 period, in 'Cochinchina'. He made important zoological collections in Vietnam and later in New Caledonia, including new species of birds (Germain's Peacock-Pheasant *Polyplectron germaini*, Germain's Swiftlet *Aerodramus germani*) and mammals (Indochinese Lutung *Trachypithecus germaini*).



Fig. 5 Photos of the five specimens held at MNHN in ventral (a), dorsal (b) and lateral (c) views. From left to right: MNHN ZO 1966-573 (paratype), MNHN ZO 1938-738, MNHN ZO 1938-739 (holotype), MNHN ZO 1938-740, MNHN ZO 1938-742 (paratype)



Fig. 6 Comparisons of *O. r. germaini* with other *Orthotomus* taxa in ventral (a), dorsal (b), and lateral (c) views. Upper specimens (from left to right) *O. r. germaini* MNHN-ZO 1866-573 (paratype), MNHN-1882-739 (holotype); *O. r. cineraceus* MNHN ZO-1881-518, MNHN ZO-2011-465; *O. r. borneoensis* MNHN-ZO 1860-827, MNHN ZO-1860-824; *O. sepium* MNHN-ZO 2011-463. Lower specimens (from left to right): *O. a. nitidus* MNHN-ZO 1882-728, MNHN-ZO 1929-1463, MNHN-ZO 1939-913, MNHN-ZO 1997-161; *O. a. atrogularis* MNHN-ZO 1989-120

Other specimens examined and not part of the type-serie
 Three other specimens are known for *O. r. germaini*:
 MNHN ZO 1882-738 and MNHN ZO 1882-740 all

collected by Louis Rodolphe Germain in ‘Cochinchina’.
 One further specimen ‘MNHN ZO 1882-741’ was part of
 a set of 88 specimens donated to Hans von Berlepsch in

1892 by the MNHN. This specimen is now in the Senckenberg Museum (Frankfurt am Main, SMF 78404).

Vocalisations

We only found one vocalization on Xeno-Canto (<http://www.xeno-canto.org/>) that could apply to *O. r. germani*, a call (XC306137) from Cái Bè, Tiền Giang, Vietnam (Latitude: 10.3282°, Longitude: 105.9659°, 24 January 2016) collected by Nick Talbot.

Distribution and conservation

This new subspecies appears to be restricted to Southern Vietnam (specimen data, vocalisations) and possibly south-east Cambodia (Goes 2013; Mahood et al. 2013; Mahood and Martin 2013). Southern Vietnam and Cambodia hold several mangroves that are under threat and recognition of this population as an endemic taxon could enhance conservation plans and understand macroecological patterns of biodiversity. Clearly, thorough inventories of these areas are crucial to understand in more details the distribution of *O. ruficeps germani*.

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999), and hence the new name contained herein is available under that Code from the electronic edition of this article. This published work and the nomenclatural act it contains have been registered in ZooBank with the ZooBank Life Science Identifier urn:lsid:zoobank.org:pub:0D7F54E5-F467-4D43-8404-A936F644279E. The electronic edition of this work was published in a journal with ISSN 2053-7166, and has been archived and is available from the digital repository <http://www.ncbi.nlm.nih.gov/pmc/>.

What is the Cambodian Tailorbird?

Our analyses revealed that the Cambodian Tailorbird haplotypes are nested within *O. a. atrogularis*. Noticeably, the genetic divergence between *O. chaktomuk* and *O. atrogularis* is now 0.7%, as opposed to the initial 1.4% in the initial species description (no *O. a. nitidus* were included in the original genetic analyses; Mahood et al. 2013). This genetic distance is lower than the genetic divergence observed among populations of the White-rumped Shama (*Copsychus saularis*) distributed in Singapore and China (Sheldon et al. 2009). The Cambodian Tailorbird is barely distinguishable from *O. nitidus atrogularis* or *O. ruficeps cineraceus* species based on biometric data although culmen/wing ratio was slightly lower (Mahood et al. 2013). The Cambodian/Vietnamese *O. ruficeps* population has the lowest culmen wing ratio in our biometric data. The

most distinctive *O. chaktomuk* feature is its plumage which is, in fact, intermediate between *O. atrogularis* and *O. ruficeps*. *O. ruficeps germani* currently inhabits the Mekong area of Vietnam (Buckton and Safford 2004; Robson 2011) whereas *O. chaktomuk* inhabits evergreen dense scrubs of the Mekong and Tonle Sap floodplain that is just further north on the same floodplain system. Hence, we here hypothesize that *O. chaktomuk* could refer to a hybrid population, not necessarily first generation between *O. ruficeps germani* and *O. atrogularis nitidus*. If this hypothesis is correct, then from the current data available, hybridization is or was possibly asymmetrical as the mitochondrial DNA is always 'atrogularis like' or particular combinations of backcrosses are not viable or fertile. In favor of this hypothesis is the intermediate plumage and somewhat intermediate culmen/wing ratio where the *O. chaktomuk* culmen/wing ratio is intermediate and closer to *O. atrogularis*, and the restricted distribution of *O. chaktomuk*. We notice that our ratios for all taxa are slightly different than those reported by Mahood et al. (2013) and we attribute the differences to the operator effect. Evolutionary implications for this putative hybrid zone could either be a stable hybrid or tension zone or despeciation process where non-differentiated taxa remerge (e.g. *Phoenicurus phoenicurus*, *Corvus corax*; Webb et al. 2011; Hogner et al. 2012). Alternatively, *O. chaktomuk* could represent a very recent offshoot of *O. atrogularis* that differentiated very recently and not enough time passed for the mtDNA to coalesce. This scenario is also possible as genetic distances are not always indicative of reproductive isolation. Yet, the molecular species delimitation method is a good approximation of the number of *Orthotomus* species recognized in previous studies with dense sampling (Sheldon et al. 2012; Lim et al. 2014). The only exception implies the species pair *O. ruficeps/O. sepium*, which have a limited genetic divergence (3.2%, Sheldon et al. 2012) but their speciation probability was only 0.55. In contrast, the probability that *O. chaktomuk* was distinct at the species-level from the *O. atrogularis* was much lower (speciation probability 0.77). The limited nuclear data available do not allow favoring or rejecting any of the hypotheses as they are lacking for the two taxa that would be involved in the hybridization (*O. atrogularis nitidus* and *O. ruficeps germani*). Furthermore, the fact that all these taxa are closely related may result in the fact that most taxa would not be monophyletic in the nuclear loci or hold distinctive substitutions that could detect hybrids with few intronic loci. Hence further individuals and genomic approaches using the isolation with migration model may be needed to resolve the question. If *O. chaktomuk* indeed represents a distinct taxon, its interaction from a genetic point of view with its sympatric (*O. atrogularis nitidus*) or parapatric (*O. ruficeps germani*) should be further studied.

From a conservation point of view, we argue that the fact that whether *O. chaktomuk* constitutes a ‘good’ species or in fact refers to a hybrid population should have no impact. Indeed, the area where *O. chaktomuk* occurs should be protected either to preserve the patterns of evolution (species) and/or the underlying processes (speciation or hybridization).

Conclusion

Our analyses characterized the biometric and molecular distinctiveness of a recently re-discovered population of Ashy Tailorbird in SE Cambodia and Vietnam. These diagnosable differences, coupled to a strict allopatric distribution with other *O. ruficeps* populations distributed in South-east Asia suggest that the Cambodian/Vietnamese population constitutes an independent evolutionary lineage worth of description. Concerning the Cambodian Tailorbird, our analyses revealed that this species is in fact nested within the Dark-necked Tailorbird and that the genetic divergence is much lower than initially described. Because of its phylogenetic position, intermediate biometry, plumage and distribution when compared to *O. a. nitidus* and the Cambodian/Vietnamese population of *O. ruficeps*, we hypothesize that the name *O. chaktomuk* refers to a hybrid population, hybrid swarm or the results of the introgression between *O. a. nitidus* and *O. ruficeps* and is not a valid species. Final confirmation of this hypothesis would nevertheless need the analyses of more individuals and genome scale data.

Additional files

Additional file 1: Table S1. Primer pairs used in the amplification and sequencing of the ND2 gene. **Table S2.** Measurement details for the specimens included in the biometric analyses.

Additional file 2: Figure S1. 95% statistical parsimony network obtained for the TGFb2 and MUSK loci. Black dots represent unsampled or extinct haplotypes. Circle size is proportional to haplotype frequency. Colour codes are: *O. atrogularis* (Blue), *O. chaktomuk* (green), *O. r. borneoensis* (yellow), *O. r. cineraceus* (orange). **Figure S2.** Summary of bGMYC species delimitation using the mitochondrial data set. The tree is the maximum clade credibility tree from BEAST within the *Orthotomus* tailorbirds. The heat map is a sequence-by-sequence matrix in which cells are coloured by the posterior probability such that the corresponding sequences are conspecific, with increasing probability represented by light yellow colours. The scale is given on the right of the tree. The analyses would support the recognition of eleven species within *Orthotomus*.

Authors' contributions

JF and DZ conceived the study, analysed the data and wrote the manuscript. JF collected the biometric data. DZ performed laboratory work. Both authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Newly generated data have been deposited in Genbank (www.ncbi.nlm.nih.gov). Biometric data analysed in this study are listed in Supplementary Table 2 (Additional file 1: Table S2). All specimens examined and measured are deposited in the collection of the Muséum National d’Histoire Naturelle, Paris (MNHN) and Swedish Museum of Natural History (NRM).

Consent for publication

Not applicable.

Ethics approval and consent to participate

No animals were captured and sampled by the authors as part of this study.

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