EXPEDITION AND PROGRESS REPORT

The Status of a Rare and Recently Described Endemic Bird Species (the Príncipe Thrush *Turdus [Olivaceofuscus] Xanthorhynchus*) and a Search for an as yet Undescribed 'Owl'

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SUMMARY

São Tomé and Príncipe, in the Gulf of Guinea, are the oceanic islands with the highest concentration of endemic bird species in the world. In a combined area barely reaching 1000 km^2, 28 endemic species have been recognised. Recent evidence collected on the less explored Príncipe Island has suggested that two new endemic species may be added to the list. The phenotypically distinct São Tomé and Príncipe populations of the endemic Gulf of Guinea thrush *Turdus olivaceofuscus* are likely to constitute separate species; as supported by genetic evidence obtained form a single sample obtained in 2004. Additionally, there has been a growing suspicion that a small owl occurs in the lowland primary forest remnants. This possibility has been supported by anecdotes from parrot harvesters and by the recent recording of owl-like vocalisations. A five-week expedition to Príncipe Island was carried in November-December 2007 with the aim of clarifying the taxonomic and conservation status of these two potential new endemic species. We were able to mist-net three individuals of the thrush and obtain the first ever vocalisation recordings for this population. Genetic, phenotypic (morphometrics, colour of bare parts, feather coloration and patterning), and song data strongly support the split of the Gulf of Guinea thrush into two distinct species: the São Tomé thrush *T. olivaceofuscus* and the Príncipe thrush *T. xanthorhynchus*. In total, 18 thrushes were encountered from 177 point transect locations covering 12 sites (six in primary forest, three in secondary forest, three in plantations). Thrushes were restricted to primary forest. Overall densities were 0.06 birds/ha, with the highest densities found at the highest altitude site (0.22 birds/ha). We did not encounter thrushes in two out of the six primary forest sites and encounters were usually made above the altitude of 400 m a.s.l. This indicates that the area of occupancy of this species may be significantly lower than the remaining area of primary forest (c. 30 km^2). Conservation status of the São Tomé thrush will likely be of 'Least Concern' whereas the Príncipe thrush is at least 'Vulnerable', but will more likely be 'Endangered' or even ' Critically Endangered' pending further analyses and/or data collection. Regarding the putative owl, we heard and recorded its song in new sites. Vocalisations were always restricted to low altitude primary forest (< 200 m a.s.l), where they could be heard every night. Notes were in the same frequency range as notes of other scops owl species. We collected first-hand evidence from parrot harvesters corroborating two previously recorded anecdotes from harvesters finding 'owl-like' birds in tree holes. Although inconclusive, this evidence supports the need for further efforts in locating what will very likely be a new owl species. We recorded all species encountered in the 177 point transect locations, obtaining therefore a large dataset that will allow us to determine the distribution and habitat requirements of all Príncipe endemics. The Príncipe thrush, the Príncipe white-eye *Zosterops ficedulinus*, the Príncipe seedeater *Serinus rufobrunneus* and the putative owl were restricted to primary forest, whereas all other endemics were common in human-modified habitats.

INTRODUCTION

The Gulf of Guinea constitutes a spectacular, but overlooked, centre of endemism (Jones 1994; Gascoigne 2004). It comprises three oceanic islands (Príncipe, São Tomé, and Annobón), one land-bridge island (Bioko) and one ecological island (Mt. Cameroon), all part of the Cameroon line of volcanoes (Fig. 1). Birds are one of the groups for which endemism levels are truly impressive, with c. 33 endemic species present. This represents almost half of the endemic bird species of the very large Guinean Forests' hotspot (Bakkar *et al.* 1999). The high levels of bird endemism in the Gulf of Guinea are concentrated on São Tomé and Príncipe, the two largest oceanic islands, where up to 28 endemics have been recognised (Stattersfield *et al.* 1998; Jones & Tye 2006). This level of endemism in an area barely reaching 1,000 km^2 has no parallel in the world (for comparison, the 13 main islands of the Galápagos, with a total area of c. 8,000 km^2, have 22 endemic species).

Recent evidence gathered from the less-explored Principe Island suggests that the large number of endemic birds may further increase with the addition of two new species: a thrush and an owl, both of which are apparently restricted to the primary forests of the island (Melo 2007).

The case for a new thrush species depends only on the confirmation of the proposed splitting of the Gulf of Guinea Thrush (*Turdus olivaceofuscus*), endemic to São Tomé and Príncipe, into two single-island endemics (Melo 2007). This split was clearly supported by morphological and genetic data; nevertheless, because it was based on a comparison between 24 thrushes from São Tomé and a single individual from Príncipe – due to the rarity of the thrush in this island – it needed to be validated with additional samples.

The case for a new owl species is more shrouded in mystery, since no owl has ever been observed on Principe Island. Evidence that such an owl may indeed occur is based on two reports from parrot harvesters who saw, within tree holes, what fits the description of a small 'scops' owl. After exploring these forests, M. Melo identified an area where calls that very strongly resembles an owl can be heard every night. This call was recorded, and, although it has the same frequency as the calls of other scops owl species, it is clearly distinct from any known species.

This project aimed to clarify the taxonomic and conservation status of these two potential new endemic species, and to highlight the need to preserve a unique oceanic island lowland rainforest.

This report summarises the work done in São Tomé and Príncipe from November 2007 to January 2008, and presents some preliminary results. For the sake of clarity, methodological details are kept to a minimum; these will be detailed in scientific publications and in the meantime can be obtained directly from the authors.

OBJECTIVES

The main objectives of this project were:

i) to confirm the validity of up-grading the Principe thrush population to species status by increasing the genetic and phenotypic sampling and acquiring song recordings;
ii) to establish the current threat level of the Príncipe thrush population by estimating its population size distribution and density, and habitat requirements;
iii) to shed new light on the presence or otherwise of a new owl species, by collecting information from parrot harvesters, mapping the area where the owl-like song can be heard, and ultimately locating the species.

Additionally, we also set aside time for:
iv) outreach and capacity-building activities.

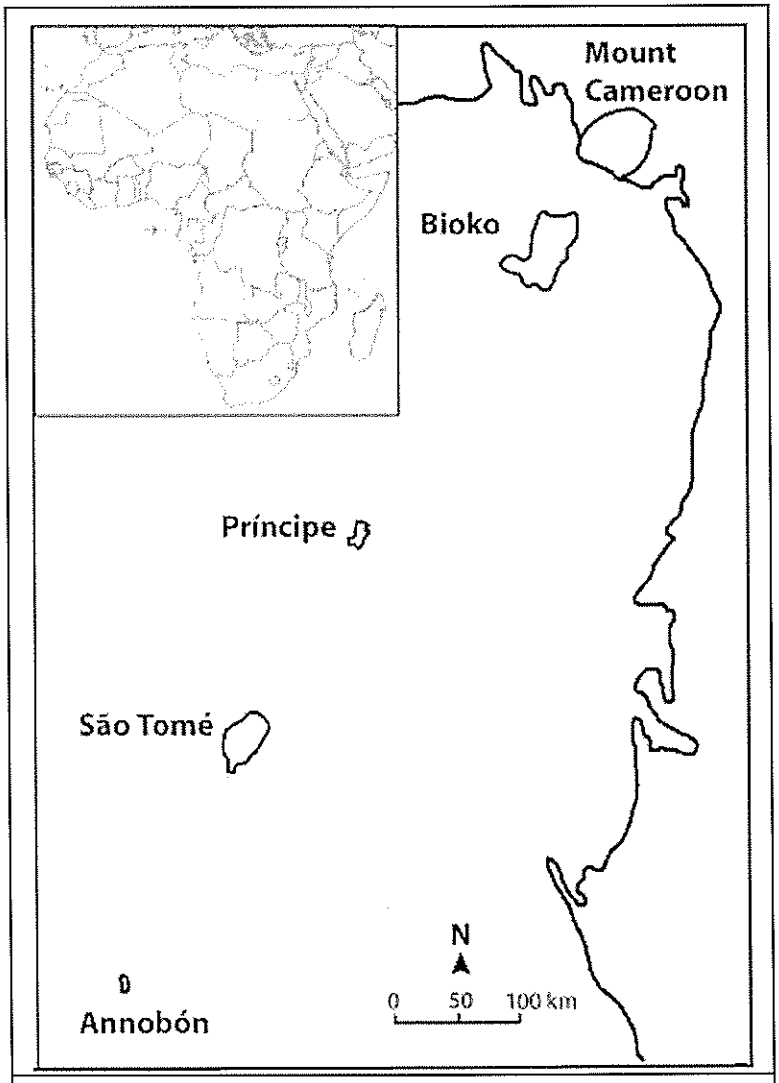

Fig. 1. Location of the islands of São Tomé and Príncipe in the Gulf of Guinea and in Africa (inset).

1. VALIDATING THE GULF OF GUINEA THRUSH SPLIT

The Gulf of Guinea Thrush *Turdus olivaceofuscus* is an endemic species of the islands of São Tomé and Príncipe. The two populations have been classified as two subspecies since 1924 (Sclater 1924), albeit the large morphological and colour differences between them suggests that each may constitute a distinct species (Fig. 2). For instance, the *olivaceofuscus* race from São Tomé has been considered more similar to the nominate race of *T. bewsheri* from the Indian Ocean Anjouan Island, on the other side of Africa, than to the neighbouring *xanthorhynchus* race from Príncipe (Naurois 1984). The population from Príncipe, initially described as a distinct species, is smaller than *olivaceofuscus*, the bill is shorter and it is yellow rather than black, the legs are pale instead of dark, and the breast and belly bars are larger and darker (Salvadori 1901).

The capture, in 2004, of one individual from the rare Príncipe population allowed the collection of a blood sample that was used to extract genetic material required to estimate the level of differentiation between the populations and infer their phylogenetic origins (Melo 2007). This study concluded that each population should be treated as a separate species because: i) genetic differentiation was well above levels documented for many congeneric species of birds (uncorrected genetic divergence: 6.8%; corrected: 10.4%); ii) genetic and phenotypic differentiation was similar or larger than between other *Turdus* species; iii) the monophyly of the two populations was not supported, suggesting that they may have derived from independent mainland colonisations; iv) the subsuming of the Príncipe and São Tomé populations within the same species was subjective, i.e., not based in new data.

Nevertheless, as the conclusion of the above study was based on a single sample from Príncipe, further validation with additional genetic and morphological sampling was required. The assignment of species rank to the Príncipe thrush would be further strengthened if its song – never recorded nor described – were found to be distinct from the song of the São Tomé thrush. It is well-established that song plays a major role in species recognition, and it is often the most diagnosable character to separate closely related species (Payne 1986).

Expedition achievements

During the course of this expedition we were able to capture with mist-nets three individuals of the Príncipe thrush. Two individuals were captured with the same net in Pico Mesa (altitude: 500 m), and the third was captured near the camp at the base of Pico Príncipe (altitude: 650 m). A fourth individual walked below a net close to the Ribeira Porco camp (altitude: 150m). Capture effort was restricted to the primary and secondary forest sites and totalled c. 2000 metre-hours. Overall capture rates were very low, typical of Príncipe forests. Out of the seven birds caught at Ribeira Porco, a site where 18 individuals had been captured in 2002 and 2003, we recaptured a Dhorn's thrush-babbler (*Horizorhinus dhorni*) from 2003.

At Pico Mesa we were able to record for the first time vocalisations from the Príncipe thrush.

Preliminary analyses of the genetic, phenotypic and vocalisation data are presented next. All the evidence supports assigning species rank to each of the two subspecies of *Turdus olivaceofuscus*. This conclusion is particularly strong has it is based on several independent data sources (Helbig et al. 2002)

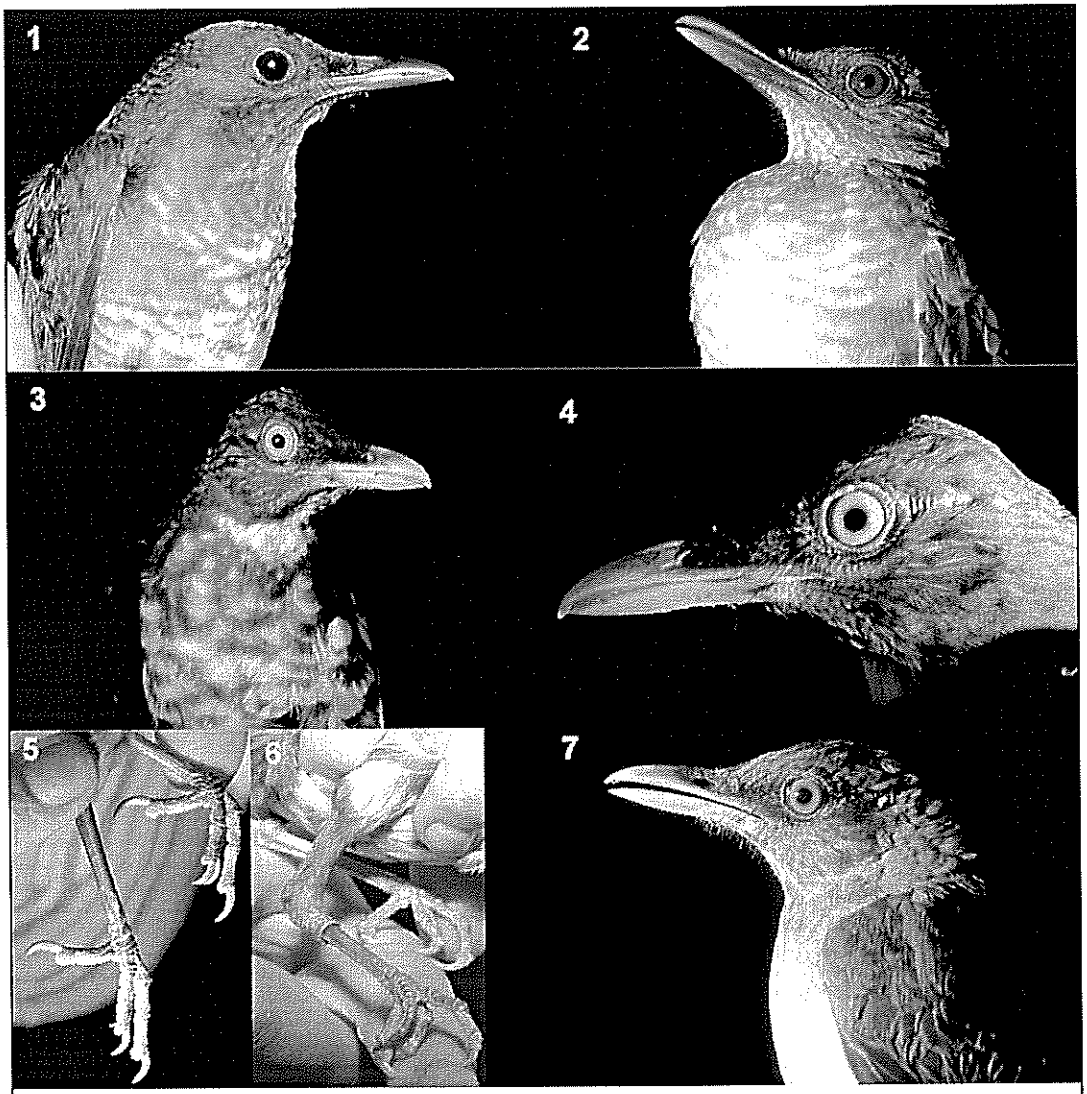

Fig. 2. The Gulf of Guinea thrush *T. olivaceofuscus* (1-4) and the African thrush *T. pelios* from nearby mainland (7). *T. olivaceofuscus* is currently classified into two very distinct subspecies: *olivaceofuscus* from São Tomé (1,2) and *xanthorhynchus* from Príncipe (3,4). The two subspecies differ in morphometry (see introduction and results) and in coloration. The bill of the nominate race is dark brown with variable extensions of light brown-dark yellow on its edges and tip, the iris is dark brown, and the feet are dark grey (5). This contrasts with the bright yellow bill, the almost white iris and the pale legs (6) of *xanthorhynchus*, which also has a yellow eye-ring. Additionally the patterns of the underparts differ, with *xanthorhynchus* presenting larger and more well defined bars (close to spots) and a clear white throat, and *olivaceofuscus* showing thin bars and a 'dirtier' throat. The barred underparts of the insular populations are not found on the mainland species most likely to be the closest relatives, which have either uniform (7) or streaked underparts. The 'eye-stripe' of naked skin present in this group is clearly visible on photo 4. Photos by M. Melo.

Genetic data

Methods

The four samples of the Príncipe thrush were analysed together with four samples of São Tomé thrush. Genetic analyses were based on the complete sequences of the mitochondrial genes NADH dehydrogenase subunit 2 (ND2; 1041 bp) and subunit 3 (ND3; 351 bp), and on 1000 bp of the mitochondrial cytochrome *b* (cyt *b*) gene.

Genetic data from this study could be directly compared with two existing datasets:

1. World *Turdus* dataset: a total of 2368 bp (ND2, ND3, cyt *b*) for 64 out of the 69 species making the *Turdus* genus (Voelker et al. 2007);
2. African *Turdus* dataset: a total of 1392 bp (ND2, ND3) for 16 African *Turdus* taxa (including species and subspecies), with special emphasis on the Olive thrush (*Turdus olivaceus*) species complex (Bowie et al. 2005).

Molecular phylogenies were estimated using Maximum Parsimony (MP) as implemented in PAUP* v4.10 (Swofford 2003), and model-based approaches (maximum likelihood, ML, and Bayesian inference, BI), as implemented in PHYML v2.4 (Guindon & Gascuel 2003) and MRBAYES 3.1 (Huelsenbeck & Ronquist 2001) respectively. We considered a priori that the model-based approaches were more adequate to deal with such large datasets and relatively large sequence divergences (more prone to saturation effects). In the same way, we considered that Bayesian inference was more appropriate for the analyses of the concatenated sequence data because, in contrast to current ML methods, it can take into account the fact that different regions (e.g., genes and codon positions) of the sequence data can follow different evolutionary dynamics. Clade support was assessed by non-parametric bootstrapping for MP and ML (Felsenstein 1985), and by the posterior probabilities for BI. Thresholds for support were set at 70 % for MP and ML bootstrap values (Hillis & Bull 1993) and at 0.95 for posterior probabilities from BI (Huelsenbeck & Ronquist 2001).

Uncorrected (percent divergence) and corrected pairwise genetic distances between taxa were calculated in PAUP*. The likelihood of different substitutions models were estimated with MODELTEST v3.7 and the best-fit models were selected using the Akaike Information Criterion (Akaike 1973).

Results and discussion

The two populations of the Gulf of Guinea thrush constituted distinct, reciprocally monophyletic, evolutionary lineages (Figs. 3&4). A sister relationship between the two lineages was supported in the 'World *Turdus*' dataset by MP and ML but not by BI (Fig. 3). Independently of the dataset used, none of the inference methods could determine the closest living relatives of the Gulf of Guinea thrush lineages, although BI did recover a clade grouping the African thrush (*T. pelios*) with the Gulf of Guinea lineages with a posterior probability of 0.94 (Fig. 3). BI placed the two African species, *T. bewsheri* and *T. libonyanus*, as a basal politomy, grouping all other taxa in a fully supported clade (Fig. 3). BI was also the only method that recovered a clade comprising all African species (except the basal ones), albeit with a posterior probability of 0.90.

Fig. 3. (next page). Bayesian phylogeny of the 'World *Turdus*' dataset based on the combined analysis of the mitochondrial ND2, ND3 and cyt *b* genes (consensus from 36,000 trees sampled during two independent Bayesian runs of 2 million generations). Colour and thickness of branches indicate clade support. Thick black lines: clade supported by all methods; medium black lines: clade supported by two methods; broken black lines: clade supported by ML only; grey lines: clade supported by BI only; broken grey lines: clade recovered with BI with a posterior probability in between 0.90 and 0.94. When not supported by all methods, non-supportive values indicated: MP bootstrap - below branch; ML bootstrap - above branch, bold; BI posterior probability - above branch, italic; clade not recovered: X.

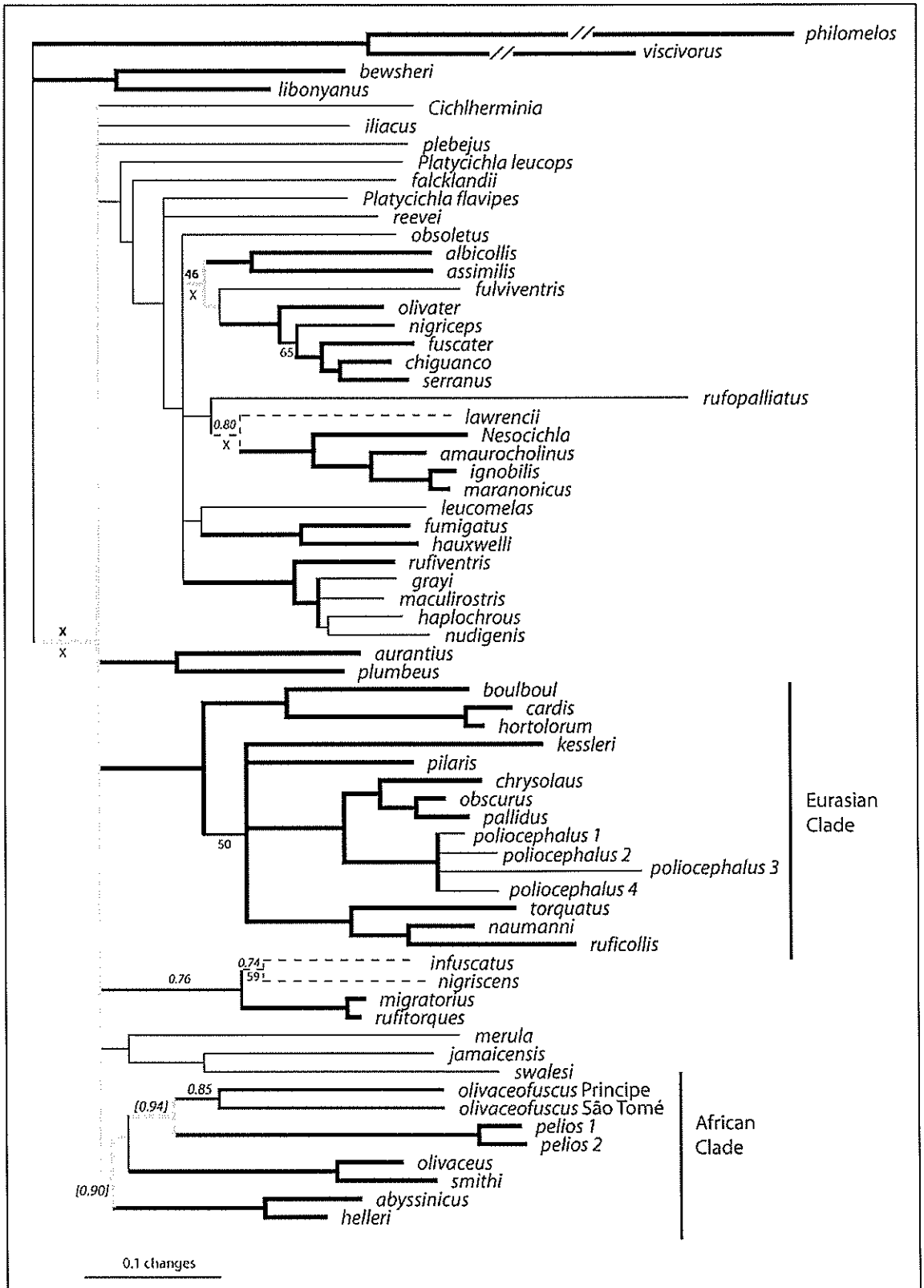

Fig. 3. Bayesian phylogeny of the 'World *Turdus*' dataset (detailed legend in previous page).

The lack of phylogenetic resolution is widespread over the entire *Turdus* genus, with the largest supported clade (by all methods) including most of the Eurasian species. This likely reflects several instances of fast radiation in this genus (Voelker *et al.* 2007).
The monophyly of the two lineages was not supported by any of the inference methods in the 'African *Turdus*' dataset (Fig. 4). As the 'World *Turdus*' dataset has more sequence data but a shallower taxon sampling close to the lineages of interest, it is at this stage impossible to conclude if the two lineages are monophyletic or not. Previously it has been shown that a paraphyletic relationship between both lineages is favoured over a monophyletic one by the Bayes factor hypothesis testing approach (Melo 2007).

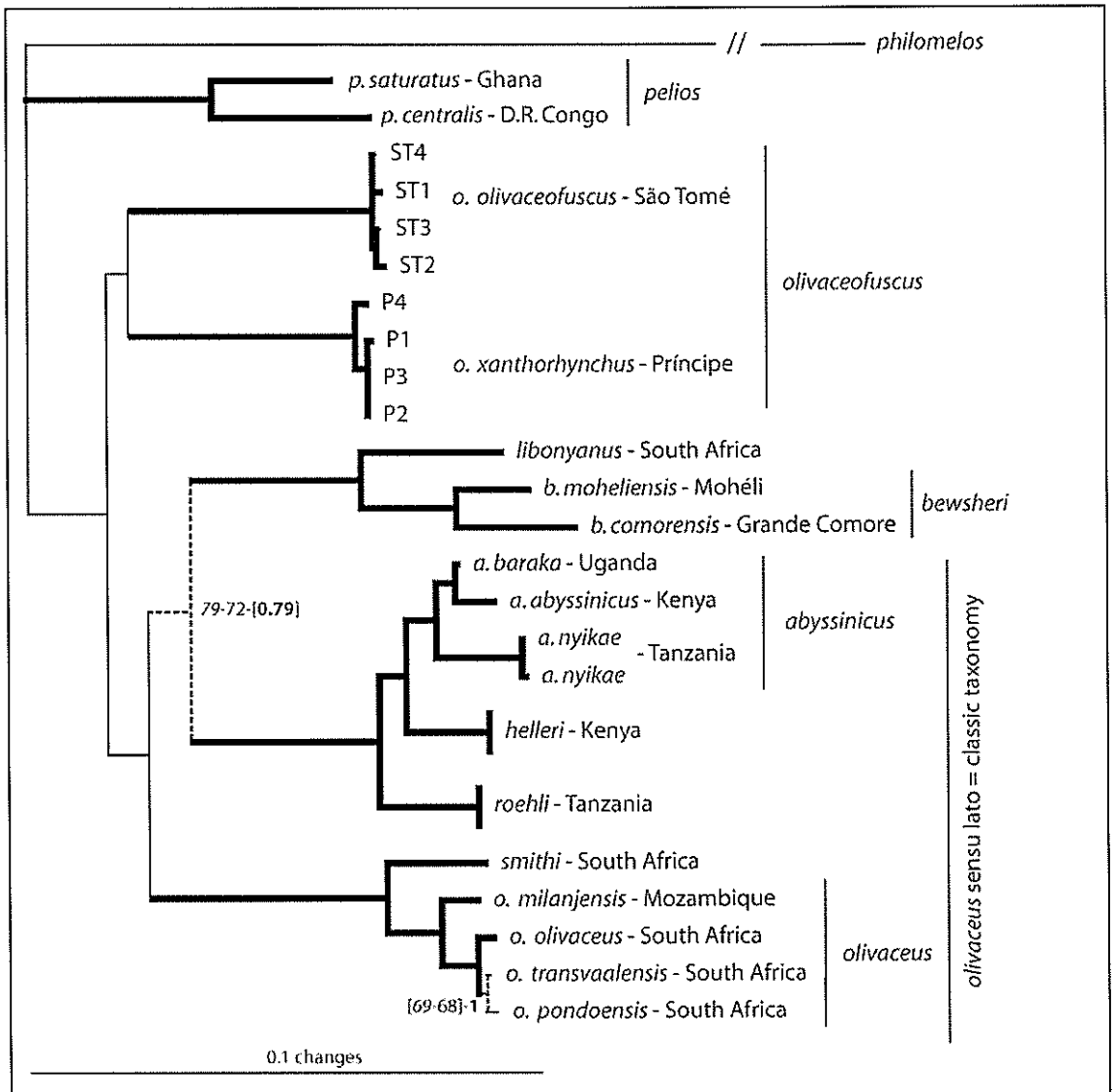

Fig. 4. ML phylogeny of the 'African *Turdus*' dataset based on a combined analysis of the mitochondrial ND2 and ND3 genes (TN+I+Γ model). BI topology was similar except in that the two Gulf of Guinea lineages were basal politomies. The two most MP trees placed the São Tomé lineage as basal to most of the other taxa. Thick lines: clades supported by all methods; broken lines: clades supported by some of the methods (support values indicated: MP-*ML*-BI).

Genetic divergence between the Gulf of Guinea thrush populations was large (cytochrome *b* uncorrected distance: 6.4%; TN+I+Γ corrected: 8.8%), and well above the very crude 2-2.5% barrier often associated with likely species rank in birds (Price 2007). A finer meta-analysis does confirm that the genetic distance between cytochrome *b* sequences was well within the range of the distances between pairs of sister species documented in birds (Fig. 5).

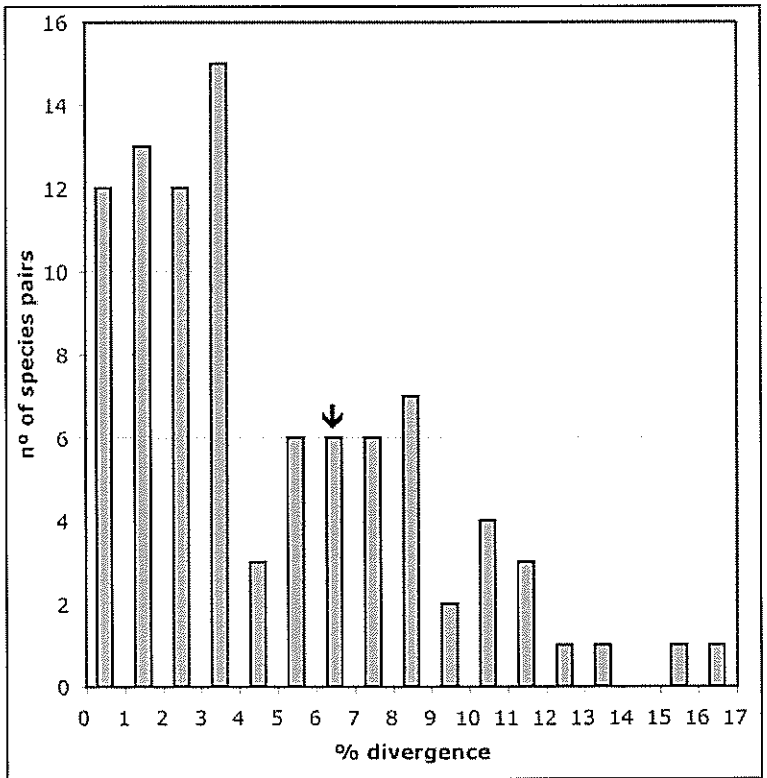

Fig. 5. Frequency histogram of mean cytochrome *b* genetic distances for pairs of sister species in birds. Adapted from Fig. 6 in (Johns & Avise 1998). Distances were calculated with the Kimura two-parameter model. With this correction, the divergence between the two Gulf of Guinea thrush lineages is of 6.8%, putting these lineages on the 25% most divergent species pairs.

Within the *Turdus* genus, genetic distances between the Gulf of Guinea lineages where typical of distances between species pairs (Fig. 6). Although in this analysis comparisons between sister species are a minority, the 8.8% divergence between the two Gulf lineages was on the second most common class, and close to the most abundant class (9-12%). It is also interesting to note that the São Tomé lineage was genetically (not meaning phylogenetically) closer to very distinct species from Jamaica (*T. plumbeus*), Peru (*T. fuscater*) and Puerto Rico (*T. swalesi*) than to the Príncipe lineage (which in turn was closer to *T. plumbeus*).

Comparing the distances with those between African taxa pairs is elucidative: distances between taxa that have been considered subspecies in classical taxonomy are separated from distances between taxa that have always been considered distinct species (Fig. 6), and the Gulf of Guinea lineages are part of this second group. It is also worth noting that divergence between the Gulf of Guinea populations was greater than between the phenotypically very distinct *T. bewsheri* and *T. libonyanus* (cyt *b* uncorrected distance: 5.4%; TN+I+Γ corrected: 7.0%).

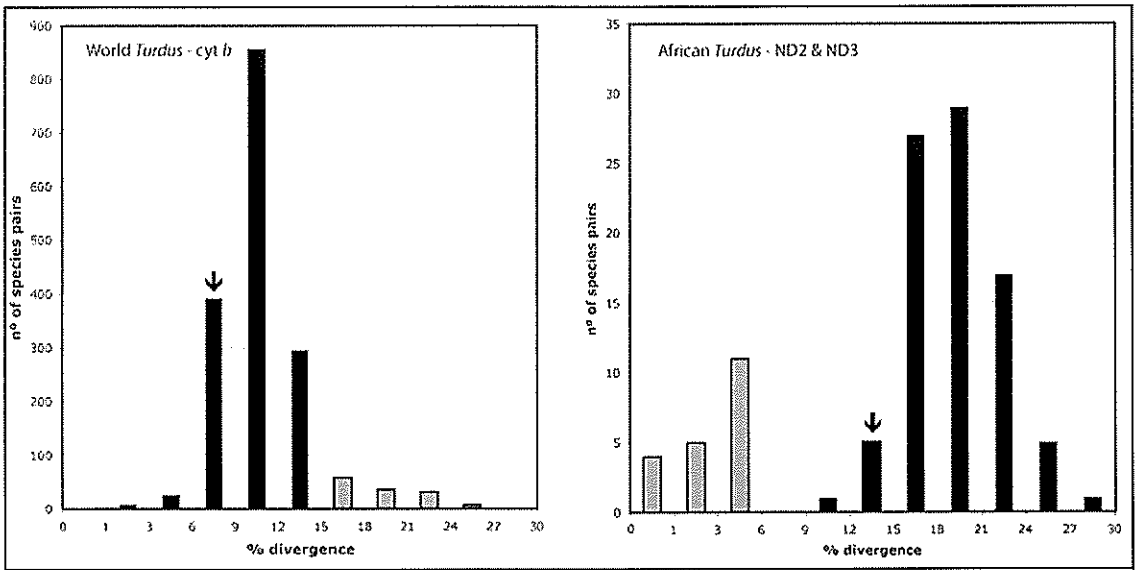

Fig. 6. Frequency histograms of pairwise genetic distances between 59 *Turdus* species (left) and between 15 pairs of African *Turdus* taxa (right). Arrows indicate where the distance between the Gulf of Guinea lineages lie. Grey columns on left graph correspond to comparisons with the three most divergent *Turdus* species (*philomelos*, *viscivorus*, and *rufopalliatus*). Grey columns on the right graph correspond to comparisons between taxa traditionally classified as subspecies; according to recent molecular studies (Bowie *et al.* 2005) all taxa that fall in the third column are valid species, except for the pair *T. a. abyssincus* and *T. a. nyikae* that diverge by 3.4%. Distances presented are corrected: TN+I+Γ for cyt *b*; GTR+Γ for the ND2-ND3 dataset.

The comparative evidence presented here confirms that the genetic distance between the Gulf of Guinea thrush lineages is typical of well-separated evolutionary lineages that constitute distinct species. Phylogenetic inference gave further support to the assignment of species rank to each population.

Phenotypic data

Methods

The level of phenotypic differentiation between the two thrush populations was assessed from a dataset comprising four individuals from Príncipe (1 female, 3 males) and 24 individuals from São Tomé (11 females, 13 males). All individuals were captured with mist-nets, ringed, measured and a blood sample was obtained. Sex was determined with a genetic-based protocol (Griffiths *et al.* 1998).

The following measurements were obtained: mass to the nearest 0.5 g with a Pesola spring balance; wing and tail length to the nearest 0.5 mm with a standard wing ruler; bill length, width and depth (height), tarsus length, longest toe length, and head + bill length to the nearest 0.1 mm with a digital calliper. These measurements were taken as follows: wing length (flattened), from the carpal joint to the tip of the longest primary; tail length, from the uropygial gland to tip of the central rectrix; tarsus length, from the tibiotarsus joint to the distal end of the tarsometatarsus when the foot is held to the leg; upper mandible length, from when the culmen enters the feathers of the head to tip; bill width and depth at the anterior end of nares; longest toe length (the middle anterior toe) from the base of the first phalange to the end of the third where it links with the claw; head + bill length from the back of the skull to the tip of the bill. The colour of the bill, leg and iris was recorded for all individuals.

Preliminary results are presented for descriptive purposes, from both a univariate and multivariate perspective. A principal component analysis (PCA) was used for the multivariate description of morphological differences. PCA was conducted in PRIMER version 5 (Primer-E Ltd) on standardised data with the components extracted from a covariance matrix.

Results and Discussion

In the field, two of the males were suspected to be juveniles due to the presence of light brown tips to their wing covers. This appears to have been confirmed by their small wing length and mass. Therefore, the Príncipe dataset consists of one adult female, one adult male, and two juvenile males. Nevertheless, apart from wing length and mass, the juveniles were not consistently smaller and were therefore treated with the adults for tarsus, bill and tail measurements.

PCA plots clearly separated the thrush populations of São Tomé and Príncipe (Fig. 7). As our main interest here was to determine if the two thrush populations are clearly morphologically distinct from each other (i.e., diagnosable) we pooled the data of each population (male, females, juveniles) and conducted separate t-tests for each variable. All measurements differed significantly between the two populations, except for wing and middle-toe length differences that were close to significance (P=0.06 and P=0.05, respectively). The almost significant middle-toe difference was on the direction of longer middle-toes in Príncipe birds. As Príncipe birds had significantly shorter tarsi, this resulted in Príncipe birds having larger feet in relation to their tarsi, which is in agreement with the field records of a bird whose movements are mostly restricted to the ground. Apart from the middle-toe, Príncipe birds were smaller than São Tomé birds for the other measurements, with the most diagnosable traits being bill length and width (Fig. 8).

The colour of the bill, iris and legs constituted synapomorphies: colour traits were shared among all individuals from the same population and different between populations. Differences were in agreement with the original description by Salvadori (1901).

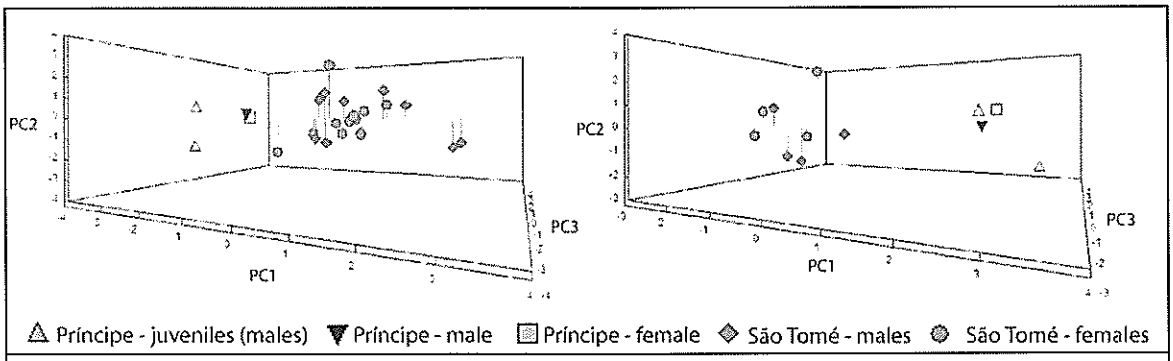

Fig. 7. Principal component plots of the Gulf of Guinea thrush populations (São Tomé and Príncipe) based on morphological measurements. Left: seven variables (mass; wing, tarsus, tail and bill length, bill width and depth); four Príncipe individuals, 21 São Tomé individuals; percent of variation explained: PC1 - 40%, PC2 - 20%, PC3 - 14%. Right: nine variables (extra: middle-toe and head + bill length), four Príncipe individuals, eight São Tomé individuals; percent of variation explained: PC1 - 48%, PC2 - 21%, PC3 - 14%.

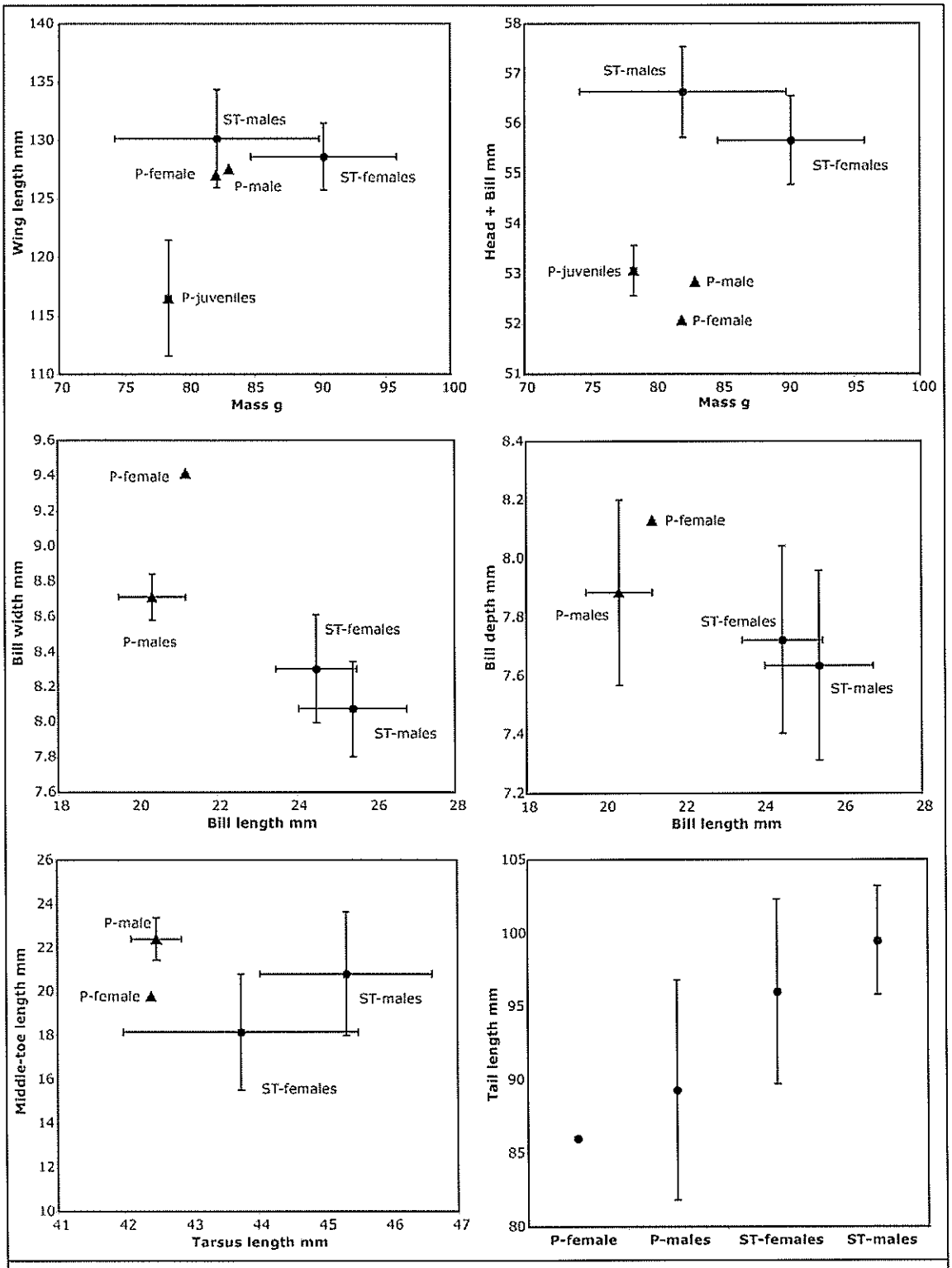

Fig. 8. Morphological measurements of the Gulf of Guinea thrush populations

Vocalisation data

Methods

Vocalisations were recorded with a Marantz PMD222 tape recorder with Type II 60 min tapes and a Sennheiser ME66K6 directional microphone.

Results and Discussion

We obtained the first ever recordings of vocalisations from the Príncipe thrush. All recordings were obtained at Pico Mesa. Recordings will be deposited at the Wildlife Sound Archive of the British Library.

All vocalisations that we heard and recorded appeared to constitute different types of contact calls rather than songs. This contrasts with São Tomé, where thrushes are very vocal and perform dawn songs during the same period we visited Príncipe. The calls that we recorded included a high-pitch whistle, a typical contact call of many thrush species, but also a very low frequency 'gurgling' (Fig. 9). Such call was never heard in thrushes from São Tomé, where both authors have a long field experience. According to Françoise Dowsett-Lemaire, one of the foremost experts in African ornithology and African bird songs in particular, no other African thrush has such call. This soft rolling vocalisation reminds calls of species from the genus *Alethe*, in particular *A. fuelleborni*, *A. poliophrys*, and *A. choloensis*, although in Alethes the calls are descending whereas in the thrush they are ascending; in Alethes, songs consist in a combination of two or three of these calls (F. Dowsett-Lemaire, *pers. comm.*).

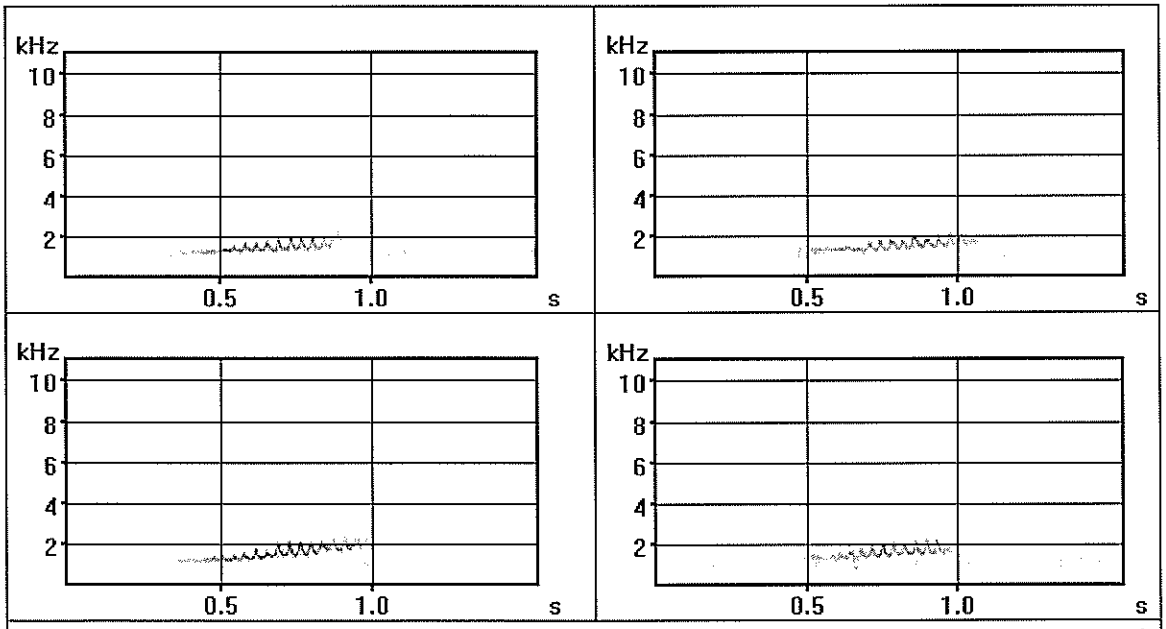

Fig. 9. Sonograms of the soft rolling call frequently emitted by Príncipe thrushes but unknown from São Tomé thrushes and any other African *Turdus* species.

In summary, the São Tomé and Príncipe populations of the Gulf of Guinea thrush are fully diagnosable in i) mitochondrial sequences, ii) morphological measurements, iii) colour of bare parts and plumage patterns, and iv) song. The level of divergence of all these traits is comparable or above the divergence that can be found between related *Turdus* coexisting in sympatry. Hence, each population should be assigned species rank (Helbig *et al.* 2002). This decision is supported by phylogenetic inference, which often favoured a paraphyletic (rather than monophyletic) relationship between both lineages. Splitting of the Gulf of Guinea thrush will simply require returning to the original nomenclature: on São Tomé it remains *T. olivaceofuscus* (Hartlaub 1852), and on Príncipe it returns to *T. xanthorhynchus* Salvadori 1901. The respective common names could indicate the island where each occurs.

2. CONSERVATION STATUS OF THE PRINCIPE THRUSH

The confirmation of the validity of the species status of the Principe thrush has very important conservation implications. Whereas the thrush population from São Tomé is common and present in most habitats with tree cover, from primary forest through to gardens in the capital city, the population from Príncipe has been rare since its discovery. A single specimen was collected in 1899 by the Italian naturalist Leonardo Fea who already considered it to be uncommon (Naurois 1984). Subsequent explorations could not find it, until 1928 when José Correia, a Portuguese collector working for the American Museum of Natural History, obtained four specimens. After Correia, the species was only rediscovered in 1997 (Sasha Lima *in litt*). Other records followed that showed its range to be confined to the southern third of the island where mature forest still occurs, from the highest peak (948 m) down to the southern coast. Hence its range might be less than 30 km^2. This is the area of highest conservation value, recently proclaimed a National Park albeit no concrete measures on the ground have so far been put in place. Thrushes on Príncipe are very tame – much more so than the birds from São Tomé – and may forage on the ground a few metres away from humans, but nevertheless sightings remain scarce.

Expedition achievements

Twelve sites were visited across Principe, six of these were within primary forest, and six were in secondary forest and plantations (Table 1, Fig.10). In total 18 thrushes were encountered from 177 point transect locations covering the 12 sites. At all points, data were gathered for all species present. Hence we obtained a large dataset on species distribution and density across the island. This will allow a detailed analysis of the habitat requirements of the different endemic species. Here we present preliminary results on the thrush.

Distance sampling

Methods

In order to assess the occurrence and density of the thrush across the island, distance sampling methodology was used (Buckland *et al.* (2001, 2004). In each study area, point transect locations were placed on a transect that was positioned independently of the local topography and intended to cover the range of available habitat in that area. Each location was at least 150m apart, a distance that was considered adequate to avoid double sampling the same areas of forest. On arrival at a point transect location, an initial five-minute settling down period was used to note locations, identities and cluster size of birds present. Thereafter, five minutes of actual survey time were allowed to measure distances (using a Leica laser rangefinder), confirm locations and identifications. At each point, the following habitat variables were measured (after the survey) within a 15m radius circle: number of canopy trees; maximum canopy height; canopy cover (by eye and using a mirror with a grid); number of fruiting and flowering trees; number of dead trees; abundance of creepers and of epiphytes; ground cover; slope.

Results and Discussion

The number of thrush records was insufficient to generate a species-specific detection function; instead, a detection function for the Gulf of Guinea Thrush on the island of São Tomé, previously obtained by M. Dallimer, was used as a surrogate, which allowed site-specific density estimates to be made for the thrush on Principe. Thrushes were only encountered in four primary forest sites. The overall density of thrushes in these sites was 0.06 (95% CI 0.03-0.13) birds/ha (Table 1), although densities were as high as 0.22 (95% CI 0.06-0.78) birds/ha at the high altitude Pico do Principe site. These densities compare with an overall density of 4.28 (95% CI 2.91-6.29) birds/ha for the São Tomé thrush in primary forest (Dallimer *et al.* unpublished data).

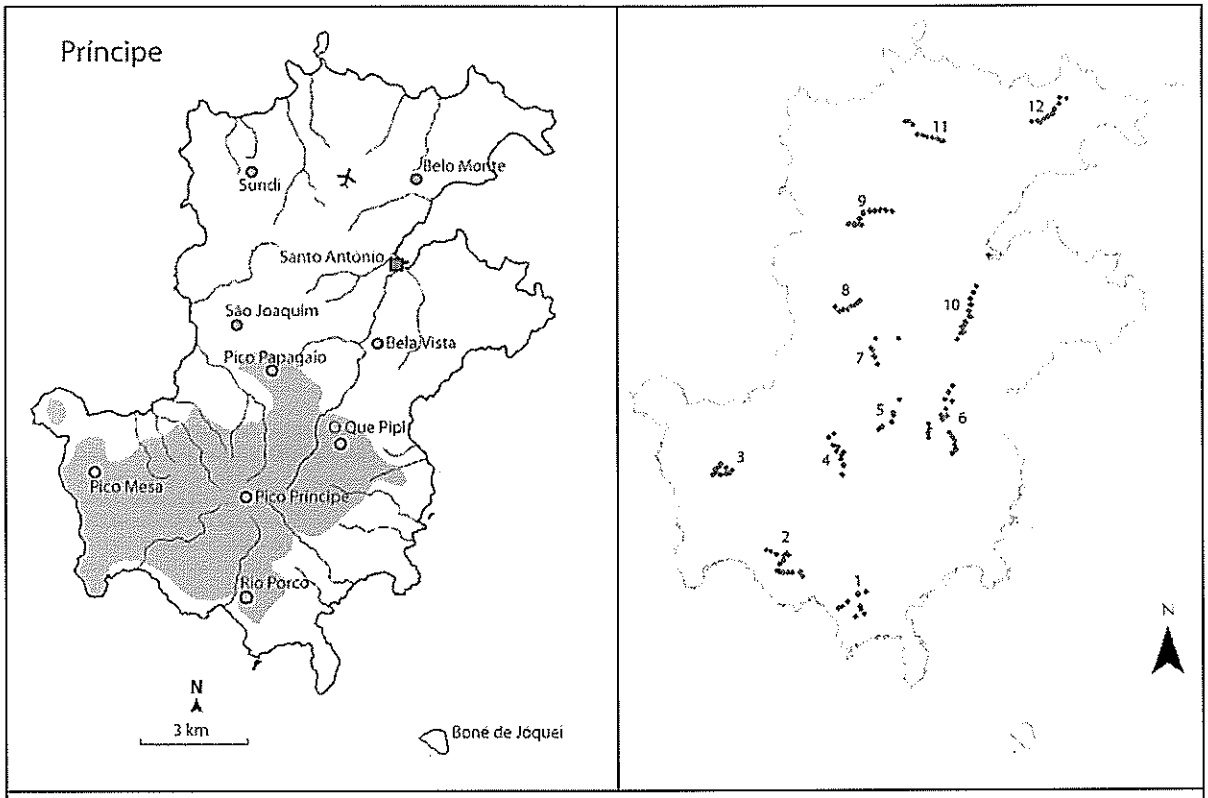

Fig. 10. Left: Landscape features (green dots), rural communities (orange dots) and the capital and only urban area (red square) of Príncipe Island close to the study sites (right). Shaded area represents the area of primary forest and old secondary forest. Right: location of point counts; number corresponds to sites on Table 1.

Table 1. Site-specific and overall density estimates for the Gulf of Guinea Thrush on the island of Principe across 13 forest sites. *: some points in a isolated patch of primary forest.

N	Site	Forest Type	Density (birds/ha)	Standard Error	Coefficient of Variation	Lower Confidence interval	Upper Confidence Interval
1	Rio Porco	Primary	0.12	0.09	74.50	0.03	0.48
2	Camp Tomé	Primary	0.00				
3	Pico Mesa	Primary	0.10	0.10	101.14	0.01	0.72
4	Pico do Príncipe	Primary	0.22	0.15	65.57	0.06	0.78
5	Boca do Inferno	Primary	0.00				
6	O Que Pipi	Primary	0.17	0.10	60.26	0.05	0.54
7	Morro Estanduarte	Secondary*	0.00				
8	Pico Papagaio	Secondary	0				
9	Ponta do Sol	Secondary	0.00				
10	Bela Vista	Plantation	0.00				
11	Ribeira Izé	Plantation	0.00				
12	Belo Monte	Plantation	0.00				
	Overall		*0.06*	*0.02*	*39.22*	*0.03*	*0.13*

Conservation status

Further data analysis is required in order to propose an estimate of the population size of the Príncipe thrush. The thrush was restricted to primary rainforest (area of c. 30 km^2) and was more abundant at altitudes above 400 m. In two of the primary forest sites (2: 'Camp Tomé' and 5: 'Boca do Inferno') the thrush was not detected. This evidence suggests that the thrush is not present continuously in all available primary forest, and its area of occupancy will therefore be under 30 km^2. It may occur in fragmented subpopulations, as the genetic divergence between the haplotype from the individual caught on the Pico do Príncipe (P4 on Fig. 4) and those caught on the southern forests suggests.

A very rough estimate of the population size of the Príncipe thrush can be obtained, for the purpose of this report only, using our overall estimates from the four sites were the thrush was reported and considering different area of occurrence scenarios (95% CI intervals are shown): 1 – 30 km^2: 90 to 390 birds; 2 – 20 km^2: 60 to 260 birds; 3 – 15 km^2: 45 to 195 birds. In the best case scenario (birds present in all primary forest at densities as high as the mean maximum densities we obtained) population size estimate is limited to 660 birds. It should also be noted that of four birds captured, two were juveniles and therefore the size of the adult population will be substantially smaller than the estimates presented here.

Regarding IUCN threat categories (IUCN 2001), at this preliminary stage of data analysis the Príncipe thrush qualifies as 'Vulnerable', under criteria D1 (less than 1000 mature individuals). The very restricted area of occupancy of this species may also warrant that it is classified as 'Vulnerable' independently of population size (Criterion D2: area of occupancy typically below 20 km^2). If the final population size estimate falls below 250 mature birds, the Príncipe thrush will be classified as 'Endangered'. It seems unlikely that the number of mature birds will be less than 50, the threshold where a species is considered 'Critically Endangered' based on population size alone.

It will be important to determine is there is evidence of a continuing decline (observed, inferred or projected) in either i) extent of occurrence, ii) area of occupancy; iii) area, extent and/or quality of the habitat, iv) number of locations or subpopulations, v) number of mature individuals. In contrast to the São Tomé thrush, the Príncipe thrush readily approaches people to within a couple of metres. For this reason, people that use the forest (hunters, parrot and snail harvesters) regularly hunt thrushes in an opportunistic manner. This pressure may explain why the thrush is restricted to the most inaccessible areas of the island, where the very sought after endemic giant land snail appears to be also restricted. It may also explain why we did not detect thrushes in the primary forest sites of 'Camp Tomé' and 'Boca do Inferno'. The former was close to an area were encounter rates were high in 2002 (King & Dallimer 2008); the latter was considered by snail harvesters to be an area were thrushes were abundant (but where neither thrushes nor live snails could be observed during this expedition). Although anecdotal, this evidence does suggest that the thrush population may be declining due to direct human impact, and it strongly supports the need to urgently assess the impact of snail harvesting both on the snail and on the thrush. If the decline is confirmed and the species is considered to occur at a single location (the southern forests of Príncipe) then it may be classified as 'Critically Endangered' under criteria B1a-b (extent of occurrence under 100 km^2) and maybe B2a-b (area of occupancy under 10 km^2). If the decline is confirmed, but the species is considered to be fragmented into subpopulations within its range, then it would be classified as 'Endangered' under criteria B1a-b (extent of occurrence under 5000 km^2) and B2a-b (area of occupancy under 500 km^2).

Whereas the Príncipe thrush will be listed in one of the three highest threat categories, the much more common São Tomé thrush will likely be listed as of 'Least Concern' (Fig. 11).

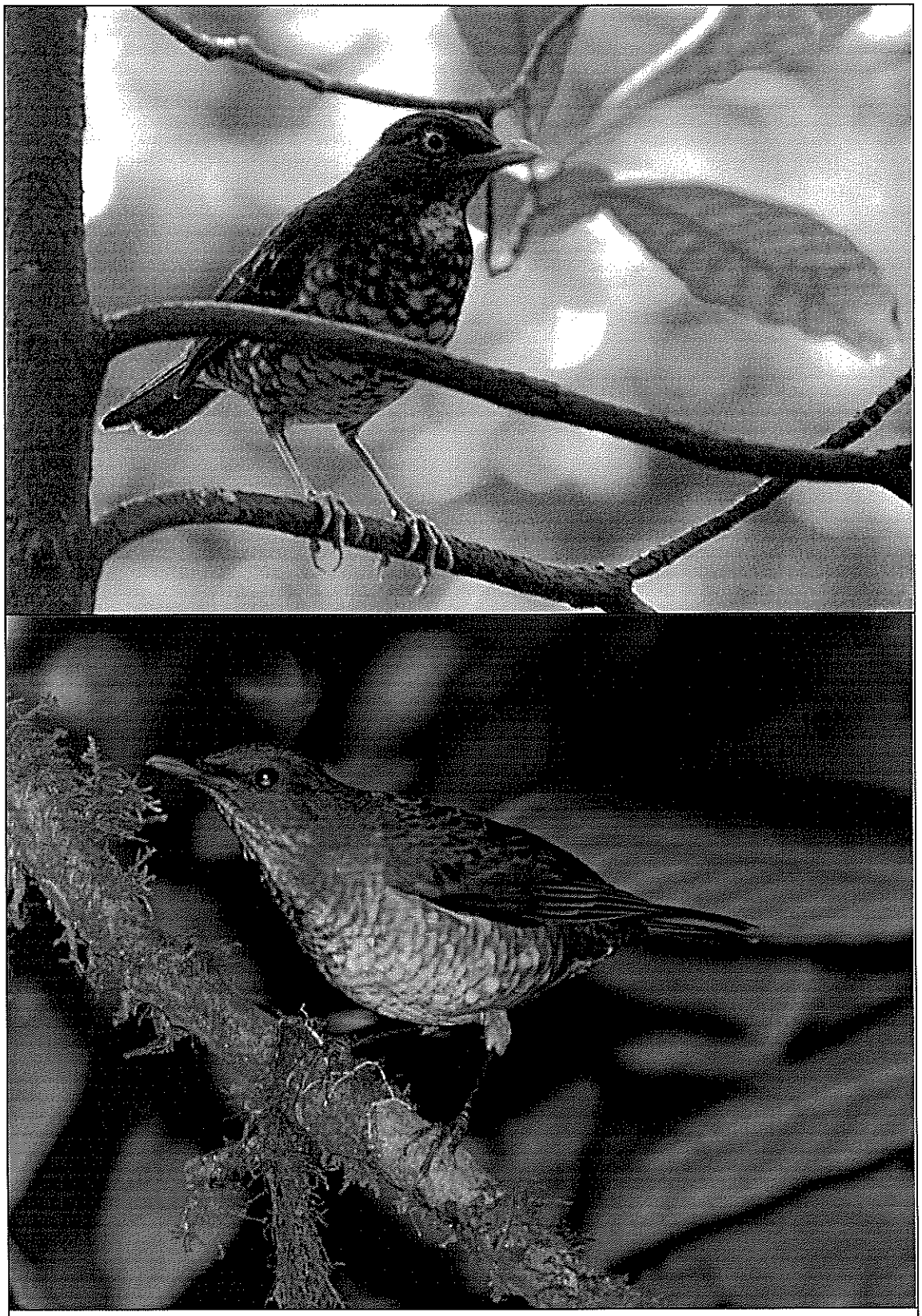

Fig. 11. With the data collected in this expedition, the 'near-threatened' Gulf of Guinea thrush *Turdus olivaceofuscus* will be split into two single-island endemics: top - the Príncipe thrush *T. xanthorhynchus* (photo: M. Dallimer); bottom - the São Tomé thrush *T. olivaceofuscus* (photo: Fábio Olmos). Conservation status of each new species differs, with the São Tomé thrush likely to be of 'least concern' whereas the Príncipe thrush is at least 'Vulnerable', but will more likely be 'Endangered' or even ' Critically Endangered' pending further analyses and/or data collection.

3. THE SEARCH FOR THE 'UNDESCRIBED' OWL

Expedition achievements

We heard the song of the putative scops owl at new sites and obtained new recordings. Playback experiments could not be performed due to a failure of the speaker system in the field. We collected first-hand evidence from parrot harvesters corroborating two previously recorded anecdotes from harvesters finding 'owl-like' birds in tree holes.

Methods

Vocalisations were recorded with a Marantz PMD222 tape recorder with Type II 60 min tapes and a Sennheiser ME66K6 directional microphone.

Results and Discussion

During the course of this expedition we heard the song of the putative owl only in primary forest below 250 m – lowest points in sites 1, 2, and 5 in Fig. 10. Previously, vocalisations had only been recorded in the Ribeira Porco area (site 1). Calls were often performed in duets. Although restricted to lowland primary forest sites, calls could be heard every night. On one occasion, we heard, for the first time, vocalisations during daytime – something the São Tomé scops owl (*Otus hartlaubi*) also does. Vocalisations mostly comprised of the repetition of an undulated note (Fig. 12), sometimes interspersed with a cat-like *'kee-a-ou'*. Notes are in the same frequency range as notes of other scops owls, and in particular of the neighbouring São Tomé and African (*O. senegalensis*) scops owls. This frequency range is very distinct from the calls emitted by frogs, as exemplified in the sonogram of the common endemic frog *Phrynobatrachus* [*feae*] *leveleve* (Fig. 12). Repetition rate of the note is high, at about one note per second. For comparison, typical repetition rate for the African scops owl is of one note every 6 seconds, whereas the São Tomé scops owl as a very slow rate of one note every 12 to 15 seconds.

The two anecdotes collected in 1998 (Melo 1998) on parrot harvesters finding an unknown bird in tree holes (where parrots also nest) fitting the description of scops owls were confirmed again. This time it was possible to meet one of the parrot harvesters that had seen the 'owl' (in 1998 he was living on São Tomé and could not be located). He pointed straight away to the São Tomé scops owl from the colour plates of the endemic birds of the Gulf of Guinea (Borrow & Demey 2001). He further said that the elders told him that it was a 'Kitóli', the name given in São Tomé to the São Tomé scops owl. Curiously he also suggested that some local knowledge existed on this elusive species as the elders said that the 'owl' destroys parrot eggs in order to occupy the nesting cavity.

The available evidence on the occurrence of an owl on Príncipe Island is solid enough to warrant further efforts to search for thespecies. It should be noted that two ornithologists have suggested independently that an owl may exist on Príncipe: when M Melo suggested this in 1998 (Melo 1998) he was unaware that the ornithologist René de Naurois had previously heard what he also thought to be an owl (Naurois 1983). As the vocalisations of this putative owl are distinct from other known owls, this species would very likely be a new addition to the world avifauna. Scops owls are species that can remain undetected for long periods. For example, the Anjouan scops owl (*O. capnodes*) was rediscovered in 1992 after 106 years of being undetected (Safford 1993). Its range is restricted to the primary forest, an area covering less than 10 km^2, and much more visited than the forests of Príncipe. Similarly, the Flores scops owls (*O. alfredi*) was rediscovered in 1994, 98 years after the last report (Widodo *et al.* 1999).

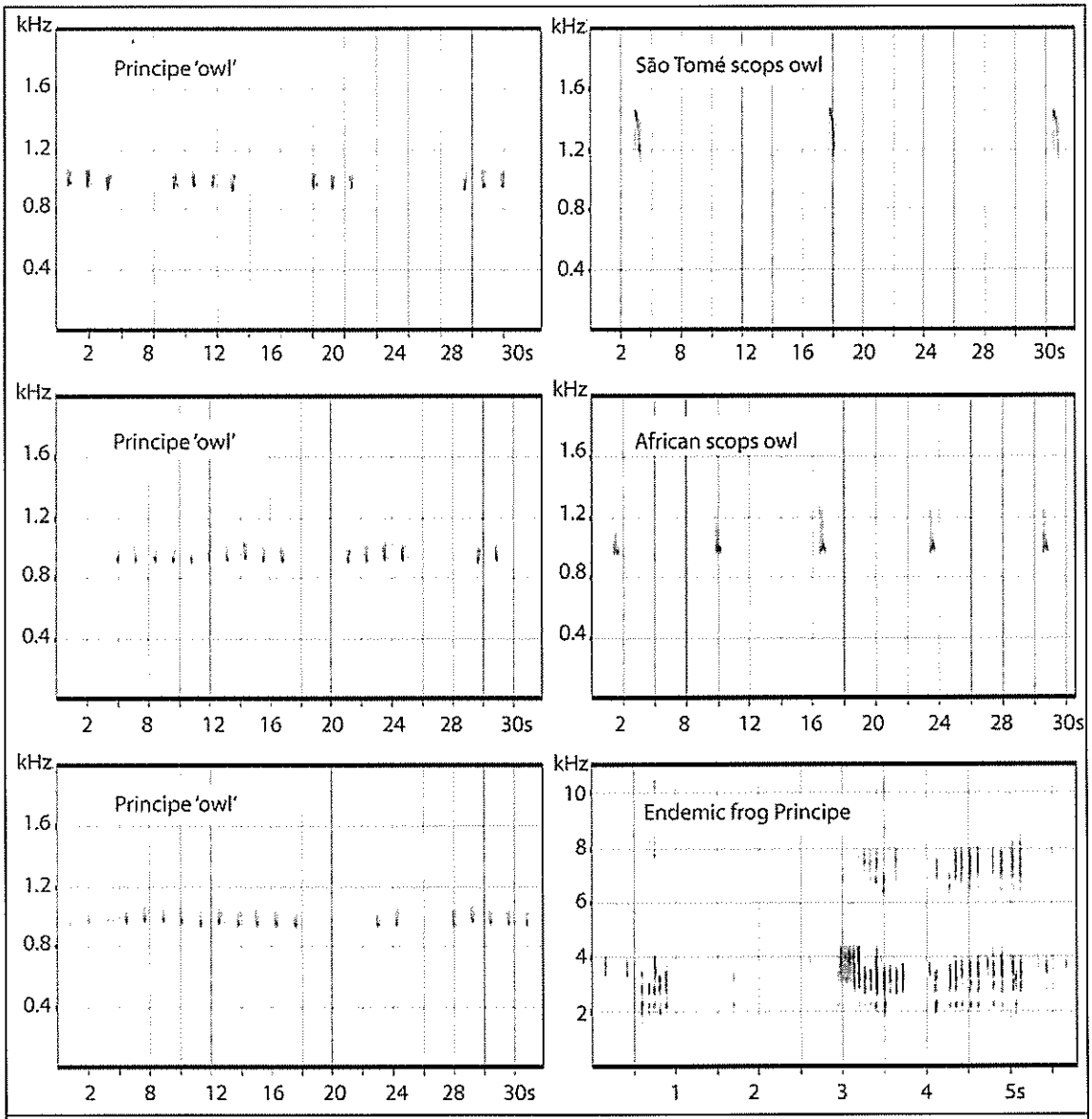

Fig. 12. Left: sonograms of the most common call of the putative owl from Príncipe. Right, top two graphs: sonograms from the São Tomé and African scops owls for comparison; notice similar frequency range. Right, bottom graph: sonogram from the endemic Príncipe frog *Phrynobatrachus leveleve*; notice the very distinct frequency scale.

4. OUTREACH ACTIVITIES

Expedition achievements

We concentrated our outreach activities on São Tomé Island where the institutions and associations linked to nature conservation are based. We had meetings with different government officials and conducted a six-day course on ornithology to members of a local association.

Official meetings

Below is a hierarchical scheme of the São Tomé and Príncipe institutions responsible for the environment and nature conservation in particular, with the name of theirs directors, whom we met both before and after the fieldwork.

Ministry of the Environment and Natural Resources

- Directorate of the Environment: Arlindo Ceita de Carvalho
    - Department of Nature Conservation: Victor Bonfim
        - Protected Areas: Salvador Sousa Pontes

A management plan for the recently established protected areas will be established during the course of this year. We were asked to actively contribute in setting up priorities related to the conservation of the endemic birds.

Ornithological course

We conducted an introductory course to ornithology to 11 members of the Association Monte Pico. This association is actively involved in conservation projects and is responsible for the management of the Botanic Garden and is often chosen by entities such as ECOFAC (European funded programme for the conservation of forests of Central Africa) to carry out projects on the ground. The association will play a major role in implementing management plans in the protected areas. One of the principal objectives of this association is to build local capacity in nature conservation by recruiting young members and investing on their training. This course was part of this goal. It will be followed up in August by a three-week long course conducted by Fábio Olmos and Longtong Turshak from BirdLife International and the Leventis Ornithological Institute (Nigeria).

The course took place during five sessions of five hours and a one-day fieldtrip (Fig. 13). It was held at the facilities of the Bom Sucesso Botanic Garden, allowing the course to combine theory and practice together at the same time. For example, the two days reviewing all endemic birds of São Tomé and Príncipe were spent outside with three mist-nets open. This allowed students to learn about mist-netting techniques and the value of capture-recapture studies, to observe birds, to handle birds, while discussing about each single endemic bird species of the country. Student interest was extremely high and their intuitive grasp of some of the more difficult concepts was striking. For example, the students were able to reconstruct by themselves the main steps required for the origin of new species on islands (isolation and adaptation).

The course was reported on the Monte Pico Association website (montepico.blogspot.com; entry of 28 February) and by a two minute television report on the 1pm News Service of the Portuguese Broadcast Services (RTP-Portugal, RTP-África, and RTP-International).

A pair of 8x42 binoculars was offered to the Association Monte Pico.

Ornithological course outline

Day 1. The Ornithological Interest of São Tomé and Príncipe.
1. The endemic birds.
- What is an endemic bird?
- STP as the country with the highest number of endemic birds in relation to area.
- Origin of endemic species: colonisation, isolation and adaptation to distinct environments - an introduction to evolutionary concepts: gene flow, population divergence, speciation.

2. Other bird species
- Native but not endemic
- Migrants and vagrants
- Exotic and Naturalised

Day 2. Adaptations to the island environment & notions of bird identification
1. Island adaptations (always with specific examples from the endemic avifauna).
- Giants and dwarfs
- Density compensation, loss of territoriality, decrease in sexual signals (colour loss).
- Tameness
- Low fecundity and high survival (comparing with related species from temperate regions)

2. Bird Identification
(started with a discussion on the traits each student considered more useful for bird ID)
- Body Size/Shape
- Bill shape: feeding types (fruits, seeds, nectar, insects...)
- Habitat
- Song

Day 3. Capture-recapture studies & the endemic birds of STP 1
1. Capture-recapture studies
- The value of such studies (estimating survival rates; tracking migrants)
- Introduction to mist-netting, ringing and measurement techniques

2. A review of the endemic birds of São Tomé
- What is a scientific name?
- Birds: Class Aves – Distinction between Passerines and Non-passerines
- For each endemic species: scientific name, local name, English name, French name; area and habitat of occurrence; identification issues; information of interest.

Day 4. Capture-recapture studies & the endemic birds of STP 2
1. Capture-recapture studies
- Net set-up by the students
- Bird handling by the students

2. A review of the endemic birds of São Tomé - end

Day 5. Field trip to Lagoa Amélia: observation and mist-netting
1. Birdwatching: use of binoculars, observations and song
2. Mist-netting: further practical training

Day 6. Bird Census Techniques and Review of the Course
1. Bird Census Techniques
- Distance Sampling: Point Counts
- Use of the GPS and RangeFinder

2. Review of the course contents: putting everything in place.

Fig.13. Ornithological course in São Tomé. Clockwise from top-left: open-air class; mist-net training; fieldtrip; part of the class at the entrance of the National Park.

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