

Multiple lines of evidence support the recognition of a very rare bird species: the Príncipe thrush

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Keywords

allopatric speciation; conservation; endemic; Gulf of Guinea; mitochondrial DNA; systematics; *Turdus*.

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Editor: Jean-Nicolas Volf

Received 10 December 2009; revised 30 April 2010; accepted 5 May 2010

doi:10.1111/j.1469-7998.2010.00720.x

Abstract

The Gulf of Guinea thrush *Turdus olivaceofuscus* is endemic to the islands of São Tomé (nominate *olivaceofuscus*) and Príncipe (subspecies *xanthorhynchus*). Relationships between the two island taxa, originally described as two different species, are uncertain. This problem has been difficult to resolve due to the scarcity of information from Príncipe birds. A focused effort to find birds from Príncipe resulted in new observations, the first records of its song, and in the capture of four individuals, which provided new data for analyses. We obtained additional data from museum specimens. Our analyses indicate that the two populations differ substantially in size, bill shape and bill, eye and leg coloration as well as in several plumage characteristics. In addition, *xanthorhynchus* utters a low call of a type not previously recorded in the genus *Turdus*. Genetic evidence corroborates the phenotypic evidence: both taxa constitute clearly independent evolutionary lineages (2368 bp from the mitochondrial markers ND2, ND3 and cytochrome *b* (*cyt-b*) from four individuals of each population). Genetic divergence between the taxa (*cyt-b*: uncorrected: 6.4%; corrected: 8.8%) suggests that they may have been isolated for over 4 Myr. These results support the split of *T. olivaceofuscus* into two species: São Tomé thrush *T. olivaceofuscus* and Príncipe thrush *Turdus xanthorhynchus*. The latter is a very rare species, restricted to the most inaccessible parts of Príncipe Island. Phylogenetic inference favoured the African thrush *Turdus pelios* as the closest living relative to the Gulf of Guinea species.

Introduction

The islands within the Gulf of Guinea constitute a spectacular centre of bird endemism (Collar & Stuart, 1988; Stattersfield *et al.*, 1998; Jones & Tye, 2006; Melo & Jones, in press). This archipelago comprises three oceanic islands (Príncipe, São Tomé and Annobón) and one land-bridge island (Bioko), arranged along the Cameroon fault line (Fig. 1). The highest levels of bird endemism in the Gulf of Guinea are concentrated on São Tomé and Príncipe, where up to 28 endemics have been recognized (Melo & Jones, in press). Of these, only five species are shared between both islands, 150 km apart, three of which have distinct subspecies on each island: the Gulf of Guinea thrush *Turdus olivaceofuscus*, Príncipe white-eye *Zosterops ficedulinus* and Príncipe seedeater *Serinus rufobrunneus*. A similar pattern has been described for angiosperm plants, where just 16 of 176 Gulf of Guinea endemics are shared between islands

(Figueiredo, 1994). These patterns suggest that isolation between islands has played a major role in promoting diversification within this archipelago.

The two subspecies of *T. olivaceofuscus* (São Tomé: *olivaceofuscus*, Príncipe: *xanthorhynchus*) conform to the general pattern of isolation among Gulf of Guinea islands. The thrush populations are the most phenotypically distinct of all the shared endemic pairs, with the Príncipe taxon originally described as a full species (Salvadori, 1901). The two species were lumped together at least since Sclater (1924), in what appears to have been a subjective taxonomic decision not based in any new data or study. Recently, some authors have speculated that the original classification is the correct one (Sinclair & Ryan, 2003; Collar, 2005). Nominate *olivaceofuscus* has been judged to be phenotypically closer to the nominate race of *Turdus bewsheri* from Anjouan (Comoro Islands), in the Indian Ocean, than to the neighbouring *xanthorhynchus* (de Naurois, 1984), which differs from

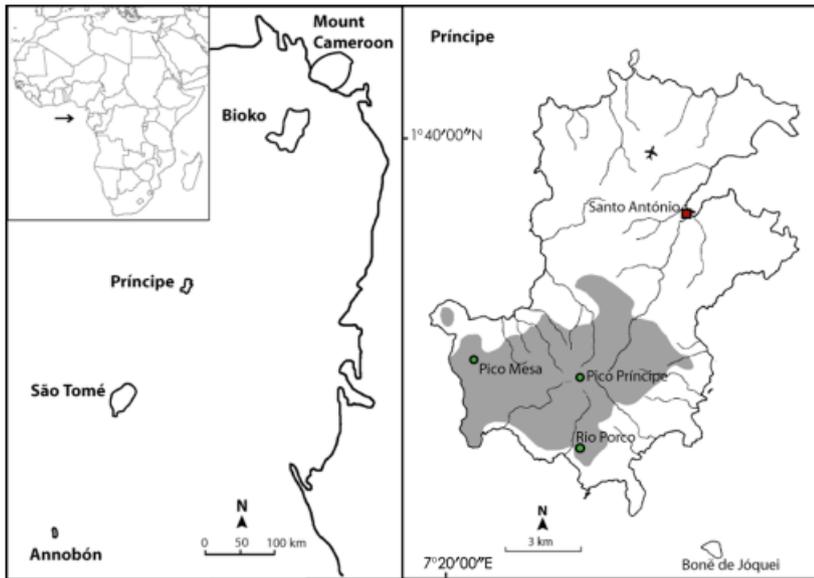


Figure 1 Left: Location of the islands of São Tomé and Príncipe in the Gulf of Guinea relative to Africa (inset). Right: Príncipe Island – shaded area: primary forest and old secondary forest to which the thrush is restricted; dots: thrush sampling locations; square: Santo António, the capital and only urban area of the island.

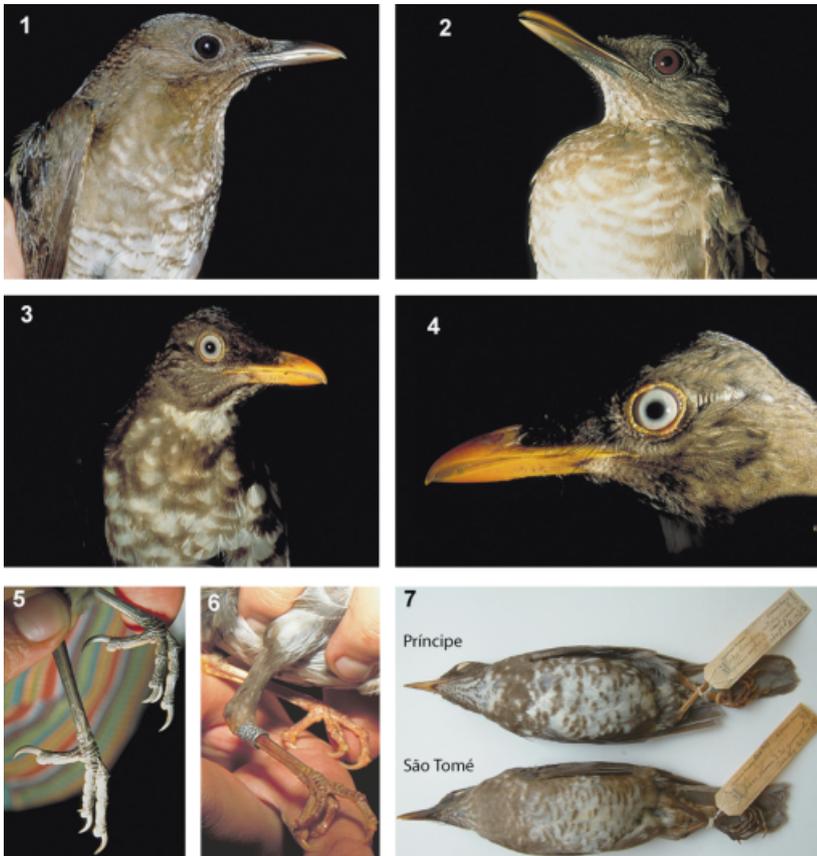


Figure 2 Phenotypic differences between the two taxa in the Gulf of Guinea thrush: *olivaceofuscus* from São Tomé (1,2,5,7) and *xanthorhynchus* from Príncipe (3,4,6,7). The barred underparts of the insular populations (7) are not found on the mainland species, which have either uniform or streaked underparts. Photos 1–6 by M. M., photo 7 by N. J. C.

olivaceofuscus in a suite of plumage and morphometric characteristics (Fig. 2 & ‘Results’).

In Africa, the genus *Turdus* is a good example of a group where high levels of geographic variation have made the definition of species boundaries difficult (Keith & Urban,

1992; Clement & Hathway, 2000; Bowie *et al.*, 2005; Collar, 2005). In the case of the Gulf of Guinea thrush, the ability to resolve species boundaries has been compromised by the lack of data on the thrush from Príncipe (*xanthorhynchus*). Whereas the thrush on São Tomé (*olivaceofuscus*) is

common and widespread, the Príncipe thrush has rarely been seen since its discovery. The single type specimen was collected in 1899 by the Italian naturalist Leonardo Fea, who regarded the form it represented to be uncommon (de Naurois, 1984); four additional specimens were collected in 1928 (Amadon, 1953), and after that, the bird was not encountered until 1997 (Jones & Tye, 2006). Subsequent records have suggested that the thrush is confined to the southern third of Príncipe where mature forest still occurs (King & Dallimer, 2008; Dallimer *et al.*, in press).

Deciding whether an allopatric population should be given a subspecific or a specific status might appear to be a relatively trivial issue, especially in light of the widely accepted view that there is a continuum between intraspecific evolutionary change and speciation (de Queiroz, 2005). However, incorrect classifications can lead to incorrect inferences with respect to evolutionary history and have serious conservation implications. To attribute subspecific status to the two island forms of thrush assumes that they are sister taxa, with the corollary that one island was colonized from the other. Considering their levels of phenotypic differentiation, however, an equally likely hypothesis is that each thrush population derives from independent colonization from the mainland. This has been shown to be by far the dominant process of diversification in the Gulf of Guinea (Melo & Jones, in press); moreover, *Turdus* thrushes are capable over-water dispersers (Voelker *et al.*, 2009). From a conservation perspective, as a subspecies of *T. olivaceofuscus*, the Príncipe thrush's rarity is obscured by the São Tomé thrush's abundance, which currently leaves *T. olivaceofuscus sensu lato* in the IUCN category 'Near Threatened' (BirdLife International, 2000).

The taxonomic position of *T. olivaceofuscus sensu lato* has been difficult to ascertain because both taxa differ strikingly in phenotype from any putative mainland relatives (the African thrush *Turdus pelios* or members of the olive thrush *Turdus olivaceus* complex). The most obvious differences from these mainland forms are in size and colour, with the insular thrush being one of the largest African *Turdus* (Christy & Clarke, 1998), and with barred underparts quite unlike the uniform or streaked underparts of its putative mainland relatives. This divergence in morphology has led to the suggestion that the Gulf of Guinea thrush and the morphologically similar Comoro thrush *T. bewsheri* – endemic to three Indian Ocean islands of the Comoros archipelago on the other side of the African continent – are representatives of an old mainland lineage now extinct (de Naurois, 1984; Keith & Urban, 1992). Alternatively, the distinctiveness of *T. olivaceofuscus* and *T. bewsheri* could represent a parallel retention of a juvenile barred plumage in the adults of insular *Turdus* (de Naurois, 1984; Herremans, 1990; Jones & Tye, 2006).

The objectives of this study were: (1) to clarify the relationships between the Príncipe and São Tomé populations of *T. olivaceofuscus*, with the aim of determining whether they constitute distinct species; (2) to determine the phylogenetic affinities of the two Gulf of Guinea thrush taxa.

Methods

Morphometrics and colour patterns

The level of phenotypic differentiation between the São Tomé and Príncipe thrush populations was assessed using two distinct datasets: one from individuals captured in the field and the other from museum specimens (supporting information Table S1). The field dataset consisted of four individuals from Príncipe (one female, three males) and 24 individuals from São Tomé (13 males, 11 females); sex was determined using a genetic-based protocol (Griffiths *et al.*, 1998). Two males from Príncipe were suspected of being young birds, owing to the presence of light brown tips in their wing-coverts, typical of juvenile *Turdus*. The museum dataset consisted of four individuals from Príncipe (three males, one female) and 10 from São Tomé (six males, four females), all held at the American Museum of Natural History, New York; sex was determined by gonad inspection at the time of collection. As with the living sample, two Príncipe males were suspected of being juveniles, owing to the small size of one and to the presence of light brown tips in the wing-coverts of the other.

The following measurements were obtained: mass (field dataset only), wing and tail length, bill length, width and depth (height), tarsus length, longest toe length (field dataset only) and head + bill length. We analysed each dataset separately and abstained from making direct comparisons between the results of each because: (1) each dataset was measured by different researchers; (2) some metrics had to be taken in slightly different ways in museum specimens and in live birds; (3) some metrics have little repeatability between researchers (details in supporting information). The colour of the bill, leg and iris was recorded for all individuals (for the museum specimens from the collector's label notes).

A principal component analysis (PCA) was used for the multivariate description of morphometric differences. PCA was conducted in R v2.9.0 (<http://www.r-project.org>) on log-transformed data with the components extracted from a covariance matrix. As the sample size was small and our main aim was to determine whether the two thrush populations are distinct from each other (i.e. diagnosable) in morphospace, we pooled the data for each population and conducted separate *t*-tests for each variable.

Vocalizations

We obtained the first-ever recordings of vocalizations from the Príncipe thrush. All recordings (up to eight individuals) were obtained at Pico Mesa (Fig. 1) in December 2007. Recordings are deposited at the Wildlife Sound Archive of the British Library and can be accessed in the supporting information. Spectrograms were generated and analysed with AVISOFT-SASLAB PRO version 4.3 (R. Specht, Berlin, Germany). See supporting information for methodological details.

Genetic evidence: laboratory procedures

Four Príncipe thrushes were caught and sampled: one in December 2004 (Ribeira Porco, Fig. 1) and three in December 2007 (two: Pico Mesa, one: Pico Príncipe, Fig. 1). These samples were analysed together with four samples of the São Tomé thrushes collected in the field (supporting information Table S1). Genetic analyses were based on complete sequences of 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. Sequences from one individual from São Tomé (sample *olivaceofuscus*2, supporting information Table S1) were already available (Bowie *et al.*, 2005; Voelker *et al.*, 2007; GenBank accession numbers DQ081009, DQ081055, DQ910973). The protocols used for the amplification and sequencing of the ND2, ND3 and *cyt-b* genes are described in detail in the supporting information. Sequences have been deposited in GenBank (accession numbers GU257923–GU257943).

Genetic evidence: phylogenetic inference

Genetic data from this study were directly combined with two existing datasets:

1. A worldwide *Turdus* dataset: a total of 2368 bp (ND2, ND3 and *cyt-b*) for 65 out of the 72 species comprising the genus *Turdus* (Voelker *et al.*, 2007). We excluded from this dataset five species for which sequence data were limited (DNA amplification from museum skins).
2. An 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).

The congruence of the phylogenetic signal of the different markers was demonstrated previously for both datasets (Bowie *et al.*, 2005; Voelker *et al.*, 2007) and, therefore, all analyses were performed on the concatenated dataset. 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 RAxML v7.0.4 (Stamatakis, Hoover & Rougemont, 2008) and MRBAYES 3.1 (Huelsenbeck & Ronquist, 2001), respectively. Uncorrected (percent divergence) and corrected pairwise genetic distances between taxa were calculated in PAUP*.

Results

Morphometrics

In the field dataset, the two males from Príncipe suspected of being young birds had a smaller mass and wing length. Therefore, the Príncipe field dataset was treated as comprising one adult female, one adult male and two juvenile males. In the museum dataset, only one of the two suspected juveniles from Príncipe was treated as juvenile on the basis of its very short wing.

PCA plots clearly separated the thrush populations from São Tomé and Príncipe, both for the field and the museum datasets (Fig. 3). In the field dataset, 'head + bill' and 'longest toe' length were obtained for only eight out of the 21 birds captured on São Tomé – hence, we present PCA plots for the reduced dataset (eight variables) and for the complete dataset (six variables). Príncipe birds have a smaller body size and bill length, but a larger bill width and depth; Príncipe birds also have longer middle toes (field dataset data). Excluding the putative juveniles from Príncipe did not alter the results (supporting information Fig. S1).

A univariate approach confirmed the multivariate patterns (supporting information Tables S3 and S4, Fig. S2). In the field dataset, 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. All pooled measurements from each population (male, females, juveniles: except for wing and mass) differed significantly ($P < 0.03$) between the two populations, except for middle-toe length (t -test: $P = 0.05$, d.f. = 10) and bill depth differences (t -test: $P = 0.08$, d.f. = 5). Príncipe birds were smaller than São Tomé birds in typical body size indicators (mass, wing, tarsus) and also in tail, head + bill and bill length. Bill width and depth were larger in Príncipe birds. Together with a shorter bill, this results in the stouter bill shape of Príncipe birds. The nearly significant middle-toe difference was in the direction of longer middle toes in Príncipe birds. As Príncipe birds had significantly shorter tarsi, this resulted in them having larger feet in relation to their tarsi, probably reflecting the fact that their movements are more restricted to the ground than are those of São Tomé birds (see 'Vocalizations and other behaviours'). The most diagnosable traits separating birds from the two populations were bill length and width (supporting information Fig. S2). In the small museum dataset, significant differences were detected for tarsus (t -test: $P = 0.005$, d.f. = 7), bill length (t -test: $P < 0.001$, d.f. = 10) and head + bill (t -test: $P < 0.001$, d.f. = 10).

Colour patterns

Plumage and bare-part colour differences between nominate *olivaceofuscus* and *xanthorhynchus* are considerable. Both taxa are dull olive-brown on the head and upperparts. Underneath, they share a general pattern of dark and pale barring, but the distribution and emphasis of this pattern are distinctly different. In *olivaceofuscus*, the throat is dusky-brown with off-white streaking, with the upper to mid-breast dusky-brown in a broad band that breaks down on the lower breast and belly into off-white with narrow dusky-brown barring. In *xanthorhynchus*, the throat is off-white with dark brown streaking, ending in an off-white crescent-shaped patch on the uppermost breast, with a narrow dark-brown breast-band that breaks down on the lower breast and belly into off-white with coarse dark-brown scaling. The former pattern is

subtle, the latter bold. This distinction is further emphasized by the dusky bill and legs and brown eye of *olivaceofuscus* contrasting with the bright yellow bill, dull yellow legs, bluish-white eye and narrow yellow eye-ring of *xanthorhynchus* (Fig. 2).

Vocalizations and other behaviour

All vocalizations heard and recorded from Príncipe thrushes appeared to constitute different types of contact calls rather than songs. This contrasts with São Tomé, where thrushes are very vocal and were singing at dawn during the period we visited Príncipe. The silent behaviour of thrushes on Príncipe may be a sampling artefact if the breeding, and hence singing, period of the São Tomé and Príncipe thrushes differ, although data from other species have shown a very good concordance between breeding periods for species in the same feeding guild between the two islands (Jones & Tye, 2006). The calls that we recorded on Príncipe included not only a high-pitched whistle, which is a typical contact call of several thrush species, but also a very low-frequency ‘gurgling’ (Fig. 4). Such a call has never been heard in São Tomé thrushes, despite the long experience there of M. M. and M. D.; indeed, according to F. Dowsett-Lemaire (pers. comm.), no other African thrush has such a call. This soft rolling vocalization evokes similarities to the calls of species from the unrelated genus *Alethe* (Muscicapidae), in particular *Alethe fuelleborni*, *Alethe poliophrys* and *Alethe choloensis*, although in alethes, the calls are descending, whereas in the Príncipe thrush, they are ascending.

The two populations also differ in non-vocal behaviour, with the Príncipe thrush being much more confined to the ground and understorey than the São Tomé thrush, much tamer and much more restricted in habitat utilization (Dallimer *et al.*, in press).

Genetic evidence

Sequence variation for the eight samples of *T. olivaceofuscus* was much higher between the São Tomé and Príncipe populations than within. For ND2, 82 out of the 1041 bp were variable, 78 of which were parsimony informative. There were only two variable sites among the four São Tomé individuals and five among the four Príncipe individuals sequenced; none was parsimony informative. There were nine to 11 non-synonymous mutations between populations, none within São Tomé, and in Príncipe the most divergent of the four samples (P4, Pico do Príncipe) had a single amino-acid substitution. For *cyt-b*, 70 out of 1000 bp were variable, 43 of which were parsimony informative;

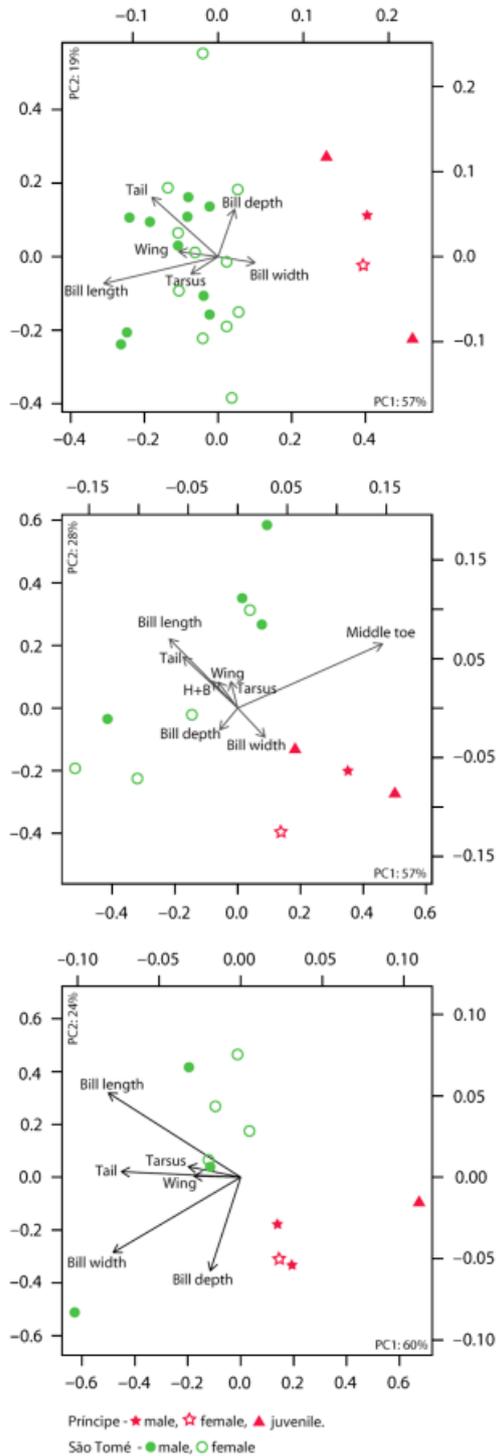


Figure 3 Principal component plots based on morphological measurements from individuals of the Gulf of Guinea thrush populations (São Tomé and Príncipe). Top: field dataset, six variables; four Príncipe individuals, 21 São Tomé individuals. Middle: field dataset, eight variables; four Príncipe individuals, eight São Tomé individuals. Bottom: museum dataset, six variables: four Príncipe individuals, seven São Tomé individuals. Percentage of the variance accounted for by PC1 and PC2 is indicated. H + B, head plus bill length. H + B not shown in the museum PC plot because it was not available for one of the Príncipe specimens (its inclusion does not change the plot, the direction of its eigenvector being almost coincident with that of bill length).

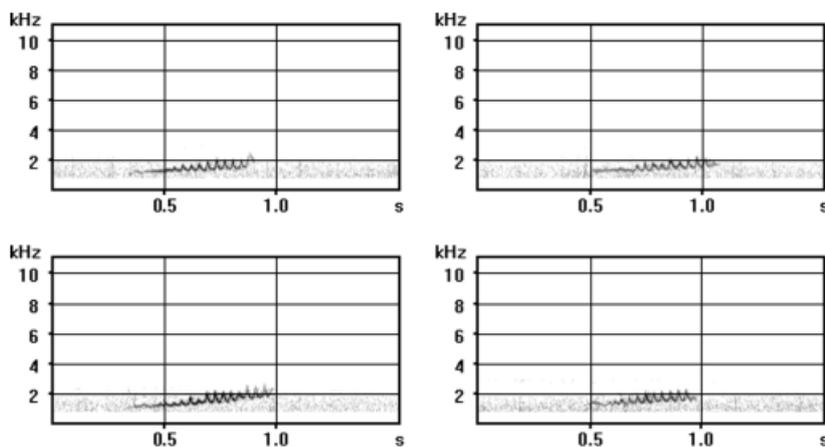


Figure 4 Sonograms of the soft rolling call frequently emitted by Príncipe thrushes, but unknown from São Tomé thrushes or any other African *Turdus* species.

there were four variable sites within São Tomé and five within Príncipe, none being parsimony informative. There were 11–13 non-synonymous mutations between populations, between one and three in Príncipe, and between zero and two within São Tomé. For ND3, 23 out of 351 bp were variable, 18 of which were parsimony informative. There were four variable positions within São Tomé (one informative) and two within Príncipe (not informative). There were between four and six non-synonymous mutations between populations, and only one sample had an amino-acid replacement in both Príncipe and São Tomé.

Genetic divergence between the Gulf of Guinea thrush populations was large, with *cyt-b* distances (uncorrected distance: 6.4%; TN + I + Γ corrected: 8.8%) in the top 25% of the range of distances between pairs of sister species documented in birds (Johns & Avise, 1998, supporting information Fig. S3). Within the genus *Turdus*, the 8.8% divergence between the two Gulf lineages was close to the most common frequency class (9–12%), even though pairwise comparisons between sister species are a minority in this *Turdus* dataset (supporting information Fig. S4). Interestingly, distances between African taxa that have been considered subspecies in classical taxonomy are considerably shorter than distances between taxa that have always been considered distinct species (supporting information Fig. S4), and the Gulf of Guinea lineages fall in this second group.

Phylogenetic inference revealed that the two populations of the Gulf of Guinea thrush constitute two clearly distinct evolutionary lineages, with the branches separating both lineages being much longer than the branches separating most sister species (Figs 5 and 6). The independence of both lineages was further highlighted by the fact that a sister species relationship between them was not always recovered or supported. A sister relationship was supported by maximum MP and ML in the ‘World *Turdus*’ dataset (Fig. 5, and supporting information Figs S5, S6), but only by ML in the ‘African *Turdus*’ dataset (Fig. 6). For this dataset, MP recovered a paraphyletic relationship between the two lineages, with the Príncipe lineage sister to *T. pelios* (supporting information Fig. S7).

A close relationship between the Gulf of Guinea lineages and the African thrush *T. pelios* was favoured by most methods in both datasets (Figs 5 and 6). The only exception was the MP tree of the ‘World *Turdus*’ dataset that recovered, without support, a sister relationship with the *Turdus abyssinicus*–*Turdus helleri* clade (supporting information Fig. S6). Poor phylogenetic resolution was widespread across the ‘World *Turdus*’ dataset, with phylogenetic inference being highly dependent on the taxon set used. For example, when only one sample of *T. pelios* was used, this species joined the largely South American clade (as in Voelker *et al.*, 2007) and the Gulf of Guinea lineages were sister to the *T. olivaceus*–*Turdus smithi* clade, a relationship supported by a Bayesian posterior probability of 0.95 (supporting information Fig. S9).

Discussion

Relationships between the Gulf of Guinea thrush populations

The defining feature of the speciation process is the evolution of reproductive isolation between diverging populations (Coyne & Orr, 2004). In the case of allopatric populations, we are left with the task of establishing the likelihood of two allopatric populations interbreeding if they met. Proxies to estimate this likelihood are phenotypic and genetic divergence, the latter being indicative of time since divergence and therefore of the level of genetic incompatibilities that may have accumulated, and the former being indicative of the likelihood of recognizing foreign individuals as potential mates and of the potential inferiority of hybrids that inherit an intermediate phenotype. Interpretation of the meaning of the levels of genetic and phenotypic differentiation between allopatric populations can be guided by comparisons of the divergence observed between sympatric species of the same group, and concordance between these proxies provides valuable evidence of the specific status (Helbig *et al.*, 2002).

In the case of the Gulf of Guinea thrush, the large genetic distance between the two populations is indicative of a very

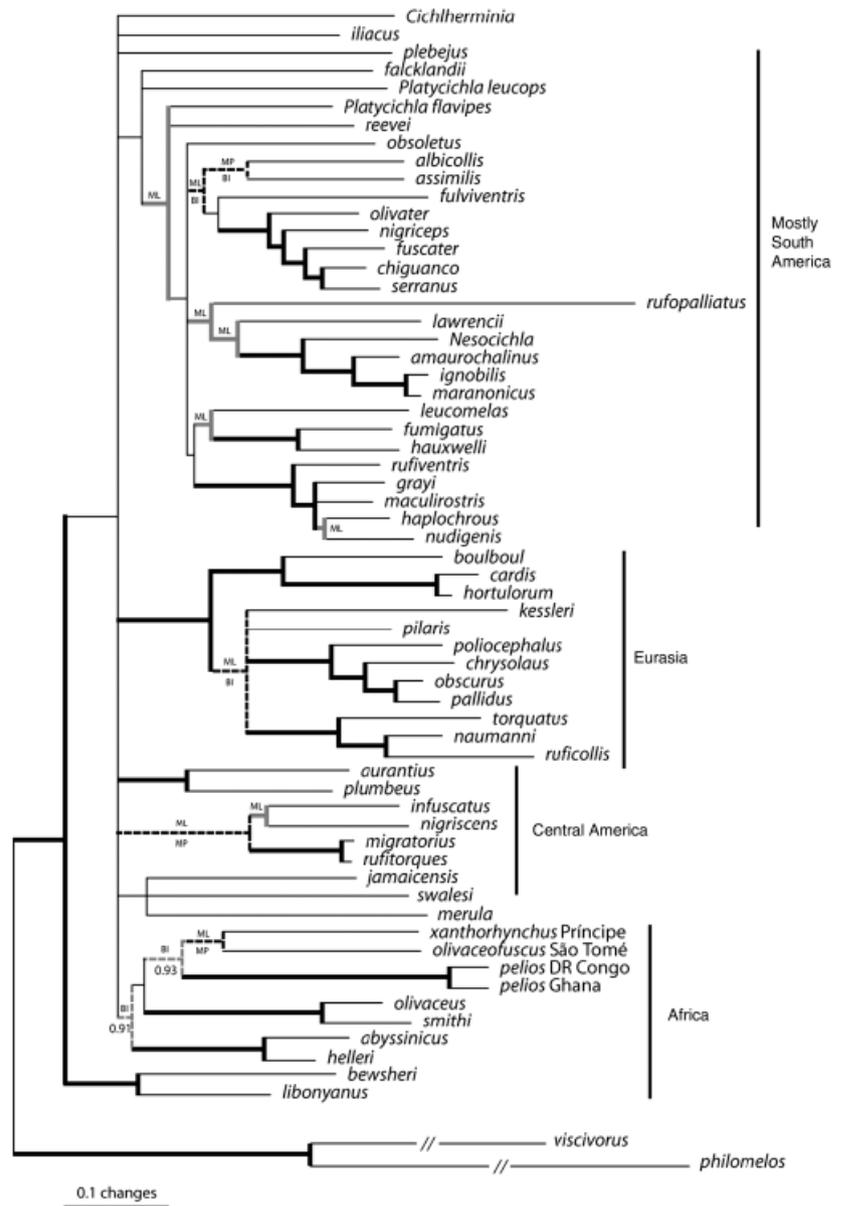


Figure 5 Bayesian phylogeny of the ‘World *Turdus*’ dataset based on the combined analysis of the mitochondrial ND2, ND3 and *cyt-b* genes (consensus from 36 000 trees sampled during three independent Bayesian runs of 3 million generations with a 20% burn-in period; data partitioned by gene and codon position). Thick black lines: clade supported by all methods; thick broken lines: clade supported by two methods; thick grey lines: clade supported by a single method. When not supported by all methods, supportive methods indicated: MP – maximum parsimony; ML – maximum likelihood; BI – Bayesian inference. Maximum likelihood and maximum parsimony trees are presented in supporting information Figs S5 and S6.

long period of isolation between them; for illustrative purposes, using the general, but apparently robust avian *cyt-b* substitution rate of 2%/Myr (Weir & Schluter, 2008) would date the split between the two Gulf of Guinea lineages at 4.4 Myr ago. Several unambiguous *Turdus* species show smaller genetic distances. Similarly, the two Gulf of Guinea taxa show levels of phenotypic differentiation in the same order of magnitude as other congeneric African thrushes, with the population from São Tomé being more distinct from the Príncipe population than it is from the more distantly related *T. bewsheri* from Anjouan (Comoro Islands). Of special significance is our observation that the Príncipe thrush possesses a vocalization not known in any other African thrush. Also of interest is its much more silent behaviour in comparison with its vocal neighbour on São

Tomé. If confirmed, the silent behaviour of the thrush on Príncipe may have evolved as its numbers declined to the very low densities of the present (Dallimer *et al.*, in press): a positive relationship between frequency of vocalizations and bird densities has been documented extensively in the Turdidae family (Collar, 2005). A similar situation has been described in the thrush *Myadestes lanaiensis* on Molokai in the Hawaiian Islands, which became increasingly silent as it grew rarer and more thinly distributed (Wakelee & Fancy, 1999).

Cumulatively, the phenotypic and genetic differences between the two populations of the Gulf of Guinea thrush represent a significant degree of differentiation, easily enough to qualify them as separate species under the criteria developed by Helbig *et al.* (2002) and the scoring system

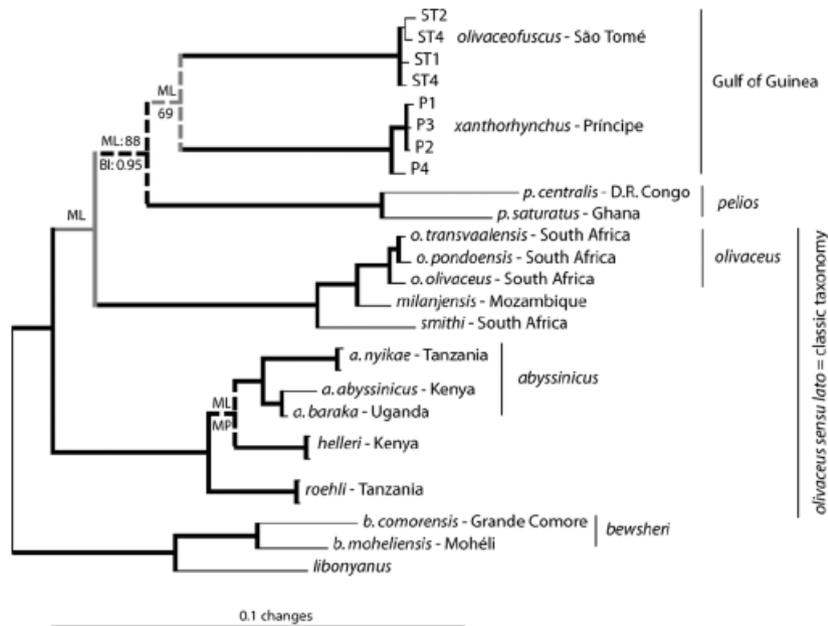


Figure 6 Bayesian phylogeny of the 'African *Turdus*' dataset based on a combined analysis of the mitochondrial ND2 and ND3 genes (consensus from 36 000 trees sampled during three independent Bayesian runs of 3 million generations with a 20% burn-in period; data partitioned by codon position). Maximum likelihood topology was identical. The two most parsimonious trees recovered a paraphyletic relationship between the Gulf of Guinea lineages (supporting information Fig. S7) Thick black lines: clades supported by all methods; thick grey line: clade supported by a Bayesian posterior probability of 0.95.

used by Collar (2006). Splitting of the Gulf of Guinea thrush simply requires returning to the original nomenclature: São Tomé thrush *T. olivaceofuscus* and Príncipe thrush *Turdus xanthorhynchus*. The conservation implications of this revised classification are important. As a race of the widespread São Tomé thrush, the conservation status of the rare Príncipe thrush was hardly noticed; as a distinct species, it qualifies for the highest IUCN threat level: 'Critically Endangered' (Dallimer *et al.*, in press).

Our results join an increasing body of molecular evidence highlighting that evolution on the neighbouring islands of Príncipe and São Tomé has proceeded mostly independently, even in the few cases where links were assumed – this includes the detection of cryptic speciation in passerines (*Zosterops*; Melo & Jones in press) and in frogs (*Phrynobatrachus*, where taxa formerly treated as subspecies diverge by 21% in *cyt-b*; Uyeda, Drewes & Zimkus, 2007). Taken together, these results further increase the levels of endemism across several taxonomic groups of the islands of Príncipe and São Tomé, placing them among the highest in the world relatively to island area.

Phylogenetic relationships with mainland taxa

Model-based phylogenetic inference methods favoured the African thrush *T. pelios* as the closest living relative of the

two islands' endemics. This makes sense from a biogeographic perspective, as *T. pelios* is the only thrush species occurring on the neighbouring mainland and on the island of Bioko (Collar, 2005). This result also suggests that the recent placement of the African *T. pelios* within a largely South American clade (Voelker *et al.*, 2007) is likely to be incorrect.

Lack of a phylogenetic resolution was a problem across the 'World *Turdus*' phylogeny owing to long terminal branches with very short internal branches, probably reflecting several cases of old rapid radiation events in this genus (Voelker *et al.*, 2007, 2009; Nylander *et al.*, 2008). That the Gulf of Guinea thrushes are found to be sister to *T. pelios* only when two different subspecies of *T. pelios* (rather than a single one) are used reinforces the notion that lack of support is related to the long branches of terminal taxa and short basal nodes, the inclusion of an additional taxon having helped shorten such distances. The large genetic divergence between the two *T. pelios* subspecies, responsible for increasing the phylogenetic resolution within the African taxa, suggests that this polytypic species may be a complex of several distinct species, and hence warrants a detailed phylogeographic study. Such additional taxon sampling may be sufficient to resolve the relationships between the African taxa, but additional molecular data, from slower-evolving nuclear introns in particular, will be required to confirm and clarify some of the relationships within the genus *Turdus*.

Acknowledgements

We thank Eng. S. Pontes, from the Department of Nature Conservation of São Tomé and Príncipe, for arranging permits. On Príncipe we are grateful to A. Salvaterra and K. Salvaterra, R. Delpont and P. Bosman of the Bom-Bom Island Resort and the Society for Conservation and Development. On São Tomé, we are grateful to A. Gascoigne, B. Loloum and Luis Mário. Sr. Pedro, Bikegila and Sátiro were indispensable guides. R. Prÿs-Jones supported our grant applications. P. Sweet kindly allowed access to specimens in his care at the American Museum of Natural History, New York. R. Covas provided measurements from the museum specimens. F. Dowsett-Lemaire kindly gave her expert advice on the song recordings. The fieldwork was funded by the University of Edinburgh's Davis Expedition Fund, the British Ecological Society and the British Ornithologists' Union. This study was in part supported by a Fellowship from the Portuguese Fundação para a Ciência e a Tecnologia to M.M. (SFRH/BD/6396/2001) and by NSF DEB-0613668 to R.C.K.B. and G.V. This is publication number 1220 of the Texas Cooperative Wildlife Collection. We are thankful for the helpful comments of two anonymous reviewers.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Taxon sampling.

Table S2. Molecular markers and primers used.

Table S3. Summary statistics of morphometric data (Field).

Table S4. Summary statistics of morphometric data (Museum).

Table S5. Loadings of the principal components of the morphometric data.

Figure S1. Principal components plots of the morphometric datasets excluding putative juveniles from Príncipe.

Figure S2. Bivariate plots of morphological measurements from field-captured individuals.

Figure S3. Frequency histogram of mean cytochrome-*b* genetic distances for pairs of sister species in birds.

Figure S4. Frequency histograms of pairwise genetic distances between 59 *Turdus* species and between 15 pairs of African *Turdus* taxa.

Figure S5. Maximum-likelihood tree of the World *Turdus* dataset.

Figure S6. Maximum parsimony trees of the World *Turdus* dataset.

Figure S7. Maximum parsimony trees of the African *Turdus* dataset.

Figure S8. Bayesian Inference tree of the World *Turdus* dataset with a single sample of *T. pelios*, and support information for all methods (BI, ML, MP).

Figure S9. Photos of the Príncipe and São Tomé thrushes in the wild.

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