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Research article

Tracing the colonization history of the Indian Ocean scops-owls (Strigiformes: Otus) with further insight into the spatio-temporal origin of the Malagasy avifauna

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Abstract

Background: The island of Madagascar and surrounding volcanic and coralline islands are considered to form a biodiversity hotspot with large numbers of unique taxa. The origin of this endemic fauna can be explained by two different factors: vicariance or over-water-dispersal. Deciphering which factor explains the current distributional pattern of a given taxonomic group requires robust phylogenies as well as estimates of divergence times. The lineage of Indian Ocean scops-owls (Otus: Strigidae) includes six or seven species that are endemic to Madagascar and portions of the Comoros and Seychelles archipelagos; little is known about the species limits, biogeographic affinities and relationships to each other. In the present study, using DNA sequence data gathered from six loci, we examine the biogeographic history of the Indian Ocean scops-owls. We also compare the pattern and timing of colonization of the Indian Ocean islands by scops-owls with divergence times already proposed for other bird taxa.

Results: Our analyses revealed that Indian Ocean islands scops-owls do not form a monophyletic assemblage: the Seychelles Otus insularis is genetically closer to the South-East Asian endemic O.
**Background**

The island of Madagascar is considered a biodiversity hotspot with an intriguing endemic fauna [1]. The origin of the island’s peculiar and highly unique fauna can be explained by two contrasting processes: vicariance when Madagascar became separated from the African landmass approximately 165 mya and from India approximately 88 mya [2,3], or over-water dispersal from the African, Australian and Eurasian landmasses. In near proximity to Madagascar are several archipelagos, which have different geological histories. The eastern islands in the Seychelles archipelago are of granitic origin and likely broke off from India when India drifted northwards [4], while the western portion of the Seychelles is comprised of recent atolls. In contrast, the volcanic Comoros archipelago is of relatively recent age (0–11 mya), hence the only plausible explanation for the colonization of its biota is by over-ocean dispersal from Africa, Australia, Madagascar or Eurasia.

The origin, timing and modes (vicariance versus over-water dispersal) of colonization by animals of Madagascar and in some cases the surrounding Comoro islands have been the focus of several research programs [5-10]. Given the antiquity of the break-up of Indo-Madagascar from Africa, which considerably pre-dates the first known fossil records of most modern families or genera of animals, in particular vertebrates, it is now assumed that the majority of extant groups arrived on Madagascar via over-water dispersal [11]. From recent phylogenetic studies, as well as traditional taxonomy, it is inferred that most of the Malagasy avifauna originated from African ancestors, although colonization events from Eurasia and Australia have also been documented for flying organisms such as bats and birds [12-16].

Interpreting the origin of certain components of the Malagasy avifauna is in many cases difficult when based on current taxonomical classifications alone as several genera of birds are shared between Madagascar, Africa and Eurasia. Among these are scops-owls of the genus *Otus*. As currently defined, *Otus* is present in five biogeographic areas (Indo-Malaya, Afrotropics, Nearctic, Neotropics, Palearctic) [17]. However, monophyly of *Otus* (sensu [17]) is uncertain; some molecular studies suggested that the New World *Otus* (*Megascops*-including *Otus flammeolus*, see [18]) which differ from Old World *Otus* by song type, are genetically more closely related to the widespread owl genera *Strix* and *Bubo* [19,20] whereas the African White-faced Owl (*O. leucotis*) has closer affinities with the genus *Asio* [20]. The above-mentioned taxa excluded, *Otus* species have their center of diversity in Eurasia (26 species); secondary radiations occur in the Indian Ocean (six or seven species) and Africa (four species). To our knowledge, the earliest known *Otus* fossil, a distal end of the right humerus, is from western Kenya and dates from the Miocene (16.5–18.5 mya; [21]). This partial fossil is morphologically close to *O. senegalensis*, but its relationship among members of the genus is not clear. The western Indian Ocean taxa are often thought to constitute a superspecies (*rutillus* group) of five or six species [22]: *O. capnodes* from Anjoan, *O. mayottensis* from Mayotte, *O. moheliensis* from Mohéli, *O. pauliani* from Grande Comore, and *O. rutillus/O. madagascariensis* from Madagascar (see [23] for a discussion about phylogeography and taxonomic status of the two Malagasy forms), and *O. insularis* from the granitic Seychelles. The latter species shows apparent affinities, based on vocalization data, to the Indonesian *O. magicus* [24]. Most of the western Indian Ocean taxa are poorly known: specific status has been proposed only within the last twenty years for *Otus*...
pauliani, O. capnodes, O. madagascariensis and O. mayottensis using both biometric and vocalization data [25-28], while O. moheliensis was first described in 1998 [29].

The evolutionary and biogeographic history of the Indian Ocean Otus taxa has not been the focus of a phylogenetic study. In this paper, we use two nuclear introns (myoglobin intron-2 and TGFβ2 intron-5) and four mitochondrial protein coding-genes (ND2, ND3, ATP6, cytochrome-b) in order to propose a first multi-locus phylogeny of scops-owls and to track their colonization history of the western Indian Ocean islands in space and time. We additionally compare the biogeographic affinities of Malagasy scops-owl species to those of other Malagasy avian lineages which have been the focus of recent genetic studies [12,13,16].

**Results**

**Sequence properties**

We obtained between 680 and 726 bp (Otus lettiaussurien sis and Aegolius acadicus, respectively) for the myoglobin intron-2 resulting in a final alignment of 749 bp. Among the 749 bp, 151 were variable (20%) and 56 were parsimony informative (7.5%). Maximum Likelihood (ML) analyses yielded one tree (-ln = 2198.81) that slightly differs topologically from the 50% majority consensus rule tree obtained from the Bayesian analyses (-ln = 2442.98). We obtained between 561 and 593 bp (O. leucotis and O. pembanensis/O. senegalis) Allele 1, respectively for the TGFβ2 intron-5 resulting in a final alignment of 605 bp. Three individuals were found to be length-variant heterozygotes: O. leucotis possesses a CCT duplication in a region with a CCT pattern in all other species, O. rutilus (FMNH 431150) possesses a one base pair deletion (G) in position 171 of the alignment, and O. senegalis possesses a one base pair insertion (A in position 559 of our alignment; this insertion was also found in the two O. pembanensis individuals sequenced). The two O. senegalis alleles also differ by two further mutations; these two alleles clustered together as the sister-group to O. pembanensis in a ML analysis (tree not shown). Therefore, we use the consensus sequence (the two single nucleotide polymorphisms were coded using the appropriate IUPAC code) from the two O. senegalis alleles for further phylogenetic analyses. Only the alleles without the insertion/deletion were included in the phylogenetic analyses for O. leucotis and O. rutilus, as the insertion/deletion events were autapomorphic in both cases. Among the 601 base pairs retained for the analyses, 163 were variable (27%) and 71 were parsimony informative (11.8%). ML analyses yielded one tree (-ln = 2131.71) that slightly differs from the 50% majority consensus rule tree obtained from the Bayesian analyses (-ln = 2179.36).

The topologies obtained from the nuclear loci were very similar to each other, delineating the primary clades without achieving resolution at the tips (see Additional Files 1 and 2). The 50% majority consensus rule tree obtained from the Bayesian analyses (Figure 1, -ln = 4638.29) and Maximum Parsimony strict consensus tree (105675 equally parsimonious trees of 412 steps, CI = 0.82, RI = 0.86) of the two concatenated nuclear loci provided a well-resolved topology for inter-generic relationships as well as some resolution of relationships among the primary Otus lineages but failed to provide resolution among members of the Indian Ocean radiation.

The concatenated mitochondrial sequences retained for analyses were 2983 bp long (1047 bp for ND2, 684 bp for ATP6, 351 bp for ND3 and 901 bp for cytochrome-b) and correspond to the positions 5246 to 6281 (ND2), 9240 to 9923 (ATP6), 10776 to 11120 (ND3), and 15011 to 15911 (cytochrome-b) of the Gallus gallus mitochondrial genome sequence [30]; GenBank accession number X52392). The ATP6 and cytochrome-b sequences contained no insertions, deletions and stop-codons in the reading frame. The ND2 sequence of Aegolius acadicus exhibits a two-codon insertion (CAACACC) just before the stop codon. All the ND3 sequences exhibited the pyrimidine insertion (T for O. capnodes, C for all other species analyzed) previously reported for several clades of birds [31]; this extra-nucleotide was removed before phylogenetic analyses. Partitioning the gene by codon positions significantly improved the fit of models to the data for all four mitochondrial loci, as inferred from the Bayes Factor (BF) values (BFND2 = 826.3, BFACTP6 = 681.7, BFND3 = 319.1, BFCytb = 1048.8). Mitochondrial gene trees were very similar to each other (ND2: ML -ln = 10556.45, BI partitioned by codon position = 10161.23 – Additional File 3; ATP6: ML -ln = 6550.30, BI partitioned by codon position = 6232.87 – Additional File 4; ND3: ML -ln = 3266.29, BI partitioned by codon position -ln = 3146.47 – Additional File 5; cytochrome-b, ML -ln = 8623.79, BI partitioned by codon position -ln = 8128.06 – Additional File 6), albeit levels or resolution varied among genes. As expected, no conflict was detected between the individual evolutionary histories of the mitochondrial gene trees (as inferred from posterior probabilities). The 50% majority-rule tree obtained from the concatenated analyses of the mitochondrial genes (partitioned by gene and codon position: -ln = 27725.63, Figure 1) was very similar in terms of topology and number of supported nodes to the MP strict consensus tree (two equally most parsimonious trees of 5922 steps, CI = 0.42, RI = 0.60).

**Phylogenetic results**

The individual trees obtained from the two nuclear introns and mitochondrial data sets were very similar to each other and no incongruence was detected between the
nuclear and mitochondrial topologies (Figure 1), according to the criteria defined in the Material and Methods section. Further, there was usually strong congruence for nodal support among the different methods. Most of the nodes present in the 50% majority-rule consensus tree resulting from the Bayesian analyses performed on the concatenated data set (partitioned by gene and codon position- 14 partitions, -ln = 4638.29) were very well supported in the parsimony analyses too (two equally most parsimonious trees, 6340 steps, CI = 0.44, RI = 0.62).

The African species O. ireneae was recovered as the sister-taxon of all remaining Otus sensu stricto scops-owls. All the western Indian Ocean taxa clustered in a clade that also contains the Eurasian O. scops, the São Tomé endemic O. hartlaubi, the Pemba Island endemic O. pembaensis, the African mainland O. senegalensis, the Philippine taxa O. mirus and O. longicornis, as well as the Indo-Malayan O. sunia. The western Indian Ocean taxa were not recovered as a monophyletic lineage since the Seychelles O. insularis was more closely related to the Indo-Malayan O. sunia than to any other taxon occurring in the western Indian Ocean region. Uncorrected-p mitochondrial distances among members of the Indian Ocean taxa/O. sunia clade range between 4.6% (between O. capnodes and O. rutilus) and 7.0% (O. sunia and O. rutilus), with a mean of 5.3% (s.d. = 0.7%). Most of the relationships between the Comorian and Malagasy taxa (capnodes, mayottensis, mohe- liensis, pauliani, rutilus) did not receive statistical support and short inter-nodes characterized most of the branches among these lineages. We attribute this lack of resolution to rapid speciation events ('hard polytomy') rather than a lack of sufficient character sampling ('soft polytomy').

Figure 1
Fifty percent majority-rule consensus tree resulting from the Bayesian mixed-model analyses of the nuclear (left, arithmetic mean, -ln = 4638.29) and mitochondrial (right, arithmetic mean, -ln = 27725.63) data sets. Values close to nodes represent MP bootstrap percentages and BI posterior probabilities. Grey blocks highlight Indian Ocean taxa. Species between quotes indicate samples for which geographic origin is unknown (captive individuals). Branch lengths of the outgroup (Tyto alba) were reduced by a scale of two for graphical purpose.
Fifty percent majority-rule consensus tree resulting from the mixed-model analyses of the concatenated data set (14 partitions, arithmetic mean, $-\ln = 32195.91$). Values close to nodes represent MP bootstrap percentages and BI posterior probabilities. Note the occurrence of color morphs in most of the *Otus* sensu stricto species. Colors for *Otus* taxa names refer to geographic distribution (green: South-East Asia, red: Africa and Blue: Indian Ocean Islands). The genera *Ptilopsis* (African White-faced Owl) and *Megascops* (New World Screech Owls) refer to taxa that were previously included in *Otus*. Species between quotes indicate samples for which geographic origin is unknown (captive individuals). The branch length of the outgroup (*Tyto alba*) was reduced by a scale of two for graphical purpose. Pictures were modified from [32].
because 1) we sampled several genes with different evolutionary properties resulting in a final alignment of more than 4300 bp and, 2) the nodes above and below the polytomy received bootstrap percentages of 70% or more or posterior probabilities of 0.95 or greater. The remaining Otus species clustered in a second large clade. Within this latter clade, O. letitia ussuriensis (eastern Russia) did not cluster with another O. letitia sample from Laos, but with the Philippine taxon O. megalotis, suggesting that further work with more complete geographic sampling is needed to address the evolutionary history of the O. letitia/O. megalotis species complex.

**Dating analyses**

The biogeographic history inferred from the topology of the concatenated analyses is intriguing as several faunistic exchanges involving the Indian Ocean islands and Indo-Malaya region occurred (split O. mirus-O. longicornis from the Indian Ocean taxa and split between O. insularis and O. sunia). These faunistic exchanges imply either multiple colonization events of the western Indian Ocean islands or one re-colonization of the mainland by O. sunia, about 0.25–0.30 mya. The main radiation of western Indian Ocean island taxa (2.5 mya, 95% HPD = 1.2–4.0 Table 1) occurred soon after the initial colonization event (3.6 mya, 95% HPD = 1.8–6.0). Our analyses revealed that O. hartlaubi, endemic to the African Atlantic island of São Tomé, and O. pembaensis, restricted to Pemba Island off the east African coast, have strong affinities with the African mainland species O. senegalensis. A very close relationship between O. senegalensis and O. pembaensis is further supported by the fact that these two taxa share a one nucleotide insertion in the TGFβ2 locus. The position of O. hartlaubi in the mitochondrial and concatenated tree renders this insertion paraphyletic. Yet, it is worth noting that the two alleles of O. senegalensis differ in length at this site. Considering that the first colonizers of São Tomé and Pemba were probably in small numbers when compared to the continental and widely distributed O. senegalensis, we regard the discrepancy between the mitochondrial and nuclear trees as being due to the different effective population size of the markers (the mitochondrial genome has an effective population size that is one fourth of the nuclear genome) and random processes (coalescence). The basal split within Otus sensu stricto occurred about 11.7 mya (95% HPD = 6.0–19.0) and the divergence between the two primary clades about 9.3 mya (95% HPD = 4.7–15.1, Figure 2).

**Discussion**

**Phylogeny of Otus sensu stricto**

Previous studies [18,20] highlighted that several species usually assigned to Otus (Ptilopsis leucotis, Megascops) are not directly related to Otus. Our analyses, using independent samples and additional genes, confirmed the lack of direct relationships among these three lineages. Given that these results were previously suggested, we do not discuss them further.

Our analyses based on nuclear and mitochondrial sequence data provide the first phylogenetic hypothesis on the diversification of Otus sensu stricto. We suggest that Otus consists of at least three primary lineages. The first lineage consists of the ‘relictual’ Sokoke Scops-owl (O. ireneae), endemic to the coastal forests of Kenya and Tanzania. This species is sometimes considered to form a superspecies with the patchily distributed Sandy Scops-owl (O. ictrorhynchus) [22], the only African Otus species we were not able to sample.

The second major lineage is restricted to South-East Asia and consists of O. spilocephalus, the O. babkamoena-O. letitia-O. lempiji superspecies, as well as Otus lettia ussuriensis and O. megalotis. As inferred from our results, the current taxonomy within this group appears to be problematic as O. lettia lettia and O. l. ussuriensis are not recovered as sister-taxon. O. l. ussuriensis, restricted to Sakhalin, Ussuriland and North-East China, is sometimes considered to be related to the Japanese Scops-owl (O. semitorques), based on voice, plumage and iris color data [22]. We could not include the latter species in the present work, but highlight here that this relationship needs to be further tested using molecular data.

The third major lineage includes all the remaining Otus species we sampled and is divided into two subclades that consist of 1) O. scops, O. senegalensis, O. hartlaubi and O. pembaensis, and 2) O. longicornis, O. mirus, O. mayottensis, O. rutillus, O. capnodes, O. moheliensis, O. pauliani, O. insularis and O. sunia. Relationships among these taxa are discussed below.

**Phylogeny and origin of the western Indian Ocean Otus**

Data accumulated in recent years on the biology and distribution of the western Indian Ocean scops-owls [26-29] together with our analyses based on DNA sequences of six
loci, shed new light on the evolutionary history of these birds. The phylogeny we propose here is in full agreement with recent work based on phenotypic characters suggesting that *O. pauliani*, *O. capnodes*, *O. moheliensis* and *O. mayottensis* represent distinct evolutionary lineages [25,28,29]. In addition we find good support for Asian biogeographic affinities of the western Indian Ocean islands *Otus* spp., and highlight that they may constitute a paraphyletic assemblage.

The Indo-Malayan *O. sunia* formed a well-supported clade with *O. insularis* from the granitic Seychelles, which was very closely related to the Indian Ocean lineage, the latter forming a sequentially paraphyletic assemblage. The nested position of *O. sunia/O. insularis* within the Malagasy-Comorian clade, supported by a posterior probability of 0.98 (but not by MP bootstrap percentage), suggests a recent re-colonization of the mainland from an island-distributed ancestor. Yet, we also acknowledge that posterior probabilities could be misleading when short internodes/polytomies are involved [33], which is the case here, and we await the implementations of reversible-jump Markov chain Monte Carlo algorithms to explore these aspects [33].

It is also worth noting that in a similar biogeographic comparison of a different group of birds, non-monophyly of western Indian Ocean taxa was also retrieved by Warren et al. [13]: the Indo-Malayan bulbul *Hypsipetes madagascariensis* was nested within the western Indian Ocean taxa, suggesting a similar scenario to the one we present for *Otus*. Colonization of the mainland from an island-distributed ancestor is generally regarded as unlikely because: 1) mainland taxa are considered more competitive than island taxa and, 2) insular populations are smaller and produce fewer emigrants compared to those on continents [34,35]. However, empirical cases of continental re-colonization from islands are accumulating [16,36-38], including cases from Madagascar [39]; this suggests that islands can also act as colonization sources for continental faunas. Data on the phylogeographic structure within the Indo-Malaya *O. sunia* complex may help to decipher which hypothesis (multiple colonizations of the Indian Ocean islands or re-colonization of the mainland) best explains the current pattern. Indeed, if strong and ancient phylogeographic structure occurs within *O. sunia*, we would expect two independent colonizations of the western Indian Ocean islands. In contrast, if genetic data indicate weak differentiation among *O. sunia* populations and patterns of population expansion from western to eastern Indo-Malaya, this would support the hypothesis of re-colonization of the mainland from the Seychelles Islands.

**Vocalisations as a tool to infer evolutionary relationships among scops-owls**

The genus *Otus* is fairly homogeneous in plumage relative to many bird genera. However, the Indian Ocean taxa show strong differences from each other in structural, plumage and vocal characters [26,28]. Considering, for example, only the songs of the Comorian taxa (which inhabit islands as close as 50 km from each other), *O. pauliani* gives a very long series of *chaw* notes repeated at about 2/sec; *O. moheliensis* a sequence of hisses; *O. capnodes* a high-pitched whistled *peeooee*; and *O. mayottensis* a series usually of 3–11 deep, single hoots. Intuitively, these differences may be used to argue against close evolutionary relationships; however, our data indicate that the relationship is indeed close in all cases, as could be predicted from the islands’ proximity to one another. This study confirms that the vocal and morphological differences are indeed associated with distinct evolutionary lineages, but suggests that they are not related in any simple or obvious way to the evolutionary distance between these lineages, and therefore must be used with caution in identifying affinities between taxa (or lineages). Therefore, we highlight here that the close relationship suggested by Marshall [24] between *O. insularis* and *O. magirus* should be further tested using molecular data, especially if we consider the considerable distance between the two areas (over 6000 km).

**Comparison with the biogeographic history of other avian lineages that colonised the Indian Ocean islands**

The geographic and temporal origins of certain western Indian Ocean island bird taxa have received attention in recent years [e.g. [5,9,10,13,16,40-45] this study] (Table 2).

Our estimate (3.6 mya, 95% HPD: 1.8–6.0) for the timing of colonization of the Indian Ocean islands by *Otus* coincides with estimates of at least seven other lineages of birds (Table 2), suggesting that the Indian Ocean islands avifauna was highly enriched at that time. The period associated with these multiple independent colonizations corresponds with the emergence of the volcanic islands of the Comoros archipelago (Mohéli 5 mya, Anjouan 11.5–3.9 mya, Mayotte 11.5–7.7 mya and Grande Comore 0.5 mya; [50,51], thus providing possible stepping stones for dispersal between Africa and Madagascar. This possibility is also highlighted by the fact that several species or populations with African biogeographic affinities (*Streptopelia capicola, Turtur tympanistria, Turdus beauforti*) [53-55], colonized the Comoros islands but the colonization of Madagascar has not yet been achieved.

All faunal exchanges that unambiguously involve Madagascar and Indo-Malaya, or Madagascar and the Seychelles occurred during the last 3.5 mya [[13,49], this study]
(note that the biogeographic history of the Philepittinae, Vangidae and the Sturnidae genus Hartlaubia are still uncertain or ambiguous). Warren et al. [48] hypothesised for members of the genus Zosterops that the colonizations could have been favored by dramatic sea-level shifts that occurred during the last 2.5 mya, which would have allowed the emergence of currently submerged landmasses between the Seychelles and Madagascar [56-58], implying a ‘stepping-stone’ model. Our divergence dates estimates are slightly older than those of Warren et al. [49], although confidence intervals are largely overlapping. This biogeographic hypothesis fits the three unambiguous described cases involving Madagascar and Indo-

Table 2: Summary of divergence dates and geographic origins involving Comorian and/or Malagasy taxa (Note that dating methods and calibration points vary among the studies).

| Taxa                     | Geographic origin | Date estimate          | Reference            |
|--------------------------|-------------------|------------------------|----------------------|
| Philepittinae            | Africa or Indo-Malaya | 41.2 ± 2.5 mya     | [40,41]              |
| Vangidae                 | Africa or Indo-Malaya | (1) 19.7 mya, (16.8–27.0); (2) 28 ± 4.0 mya | [10,42]              |
| Bernieridae              | Africa             | 25.2 mya (21.4–31.7)  | [42]                 |
| Streptopelia picturata/Nesoenas mayeri | Africa | 20.8 ± 4.1 mya | [43]                 |
| Coracina cinerea         | Australasia       | 18.8 ± 2.2 mya       | [16,43]              |
| Hartlaubius auratus      | Africa or Indo-Malaya | 12.9–17 mya        | [44]                 |
| Ispidina madagascariensis | Africa            | 13.5 ± 2.6 mya       | [9,45]               |
| Corythornis cristata     | Africa             | 5.5 ± 1.1 mya        | [9,45]               |
| Indian Ocean Dicrurus    | Africa             | 4.7 mya (95% CI: 2.7–7.4) | [46]               |
| Motacilla fliviventris   | Africa             | 4.5 ± 0.3 mya        | [47]                 |
| Otus                     | Indo-Malaya       | 3.6 mya (95% HPD: 1.8–6.0) | This study         |
| Nectarinia souimanga clade | Africa          | 1.9–3.9 mya          | [48]                 |
| Nectarinia notata clade  | Africa             | 1.5–3.5 mya          | [48]                 |
| Zosterops borbonicus lineage | Indo-Malaya | 1.8 mya              | [49]                 |
| Indian Ocean Hypsipetes  | Indo-Malaya       | 0.6–2.6 mya          | [13]                 |
| Zosterops maderaspatanus lineage | Africa    | 1.2 mya              | [49]                 |
| Anas                     | Australasia       | No divergence date   | [50]                 |

mya: million years ago

Subfossil remains of three extinct small owl species have been described from the Mascarene Islands (La Réunion, Mauritius, Rodrigues). Based on certain osteological features, these three species have been included in their own genus, Mascarenotus, which has been suggested to be derived from Otus [59]. The relationships of Mascarenotus with respect to the other owl lineages still needs clarification, as this genus could represent a recent and derived off-shot of the western Indian Ocean lineage, possibly a first off-shot of the colonization process from the Sey-
chelles to more westerly regional islands or even an unrelat

d lineage of owls.

Whereas the continental biogeographic affinities of cer

tain western Indian Ocean island bird taxa seem largely

d resolved, the timing of colonizations or faunal affinities
among volcanic islands are less well understood and the
only aspect that is emerging is the absence of a common
pattern, whatever the initial geographic origin. Indeed, all
the studies that have been conducted so far indicate explo-
dive diversification and a lack of resolution among the
Comoros islands species. These data indicate that once the
initial colonization was successful on any of the islands in
this archipelago, dispersion and then diversification be-
 tween nearby islands occurred quickly and randomly.
The lack of biogeographic structure at the archipelago
scale may thus be partly explained by the geographical
arrangement of islands and the short inter-islands dis-
tances (maximum distance between two islands in the
Comoros archipelago is 90 km) that probably favored col-
onization by alternative routes.

One final factor that could hide common patterns of
diversification is unequal rates of extinction and recoloni-
 zation across lineages. Indeed, not all lineages would face
the same risk of extinction on islands as their characteris-
tics (ecological requirements, population size) often con-
siderably differ. For example, middle-sized birds, like
owls (70–120 g), may be more prone to extinction on
islands than small birds, like sunbirds (12–15 g) [60]. Yet,
even if uneven extinctions rates occurred amongst these
lineages, it can be concluded that the overall diversifica-
tion pattern in the Indian Ocean islands is star-like.

Conclusion

Our analyses revealed the occurrence of multiple synchro-
nous colonization events of the Indian Ocean islands by
scops-owls, at a time when faunistic exchanges involving
Madagascar was common as a result of lowered sea-level
that would have allowed the formation of stepping-stone
islands. Patterns of diversification that emerged from the
scops-owls data are: 1) a star-like pattern concerning the
order of colonization of the Indian Ocean islands and 2)
the high genetic distinctiveness among all Indian Ocean
taxa, reinforcing their recognition as distinct species.

Methods

Taxonomic sampling

We obtained tissue samples from all western Indian
Ocean Otus taxa, as well as samples from several Indo-
Malayan and Afrotropical species (Table 3), focusing on
as many super-species complexes as possible (sensu [22]).
We obtained tissues for seven of these super-species com-
plexes. We were unable to obtain samples of the distinct-
tive O. rufescens and O. sagittatus, as well as representatives
of four super-species with localised and distant distribu-
tions relative to the western Indian Ocean (brooki/angeli-
nae from Sumatra/Java/Borneo; mantananensis/magicus
from the Lesser Sundas/Philippines/Mollucas; collari/
manadensis/beccarii from Sangihe/Sulawesi/Biak and
enganensis/alius/umbra from islands off Sumatra). Since
the monograph of Marks et al. [22], one further Otus spe-
cies, O. thilohoffmani has been described from Sri Lanka
[61]. This species is only known in museum collections by
the type specimen, deposited in the National Museum
Colombo (Sri Lanka), and based on morphology, has
been suggested to be related to either O. rufescens or O. spi-
locephalus [61]. As a consequence, we did not have access
to a tissue sample of this newly described species. With
the exception of O. magicus, considered by some authors
to include O. insularis because of similarities in vocaliza-
tions [24], none of the species we were unable to sample
have been considered closely related to the Indian Ocean
taxa [22]. We included two individuals per color morph
for O. rutilus (sensu [23]). Representatives of the Strigidae
genera Aegolius, Athenae and Glaucomys (Surniinae), Asia
(Asoniinae), and Bubo, Strix, New World ‘Otus’
(Megascops) and the African White-faced Owl (‘Otus’ Pti-
loopsis leucotos) (Striginae) were included as proximate out-
groups. We rooted our trees using sequences from a
representative of the Tytonidae (Barn Owl Tyto alba),
which has been recovered as the sister-group of the Strigi-
dae in molecular and morphological analyses [62,63].

Laboratory procedure

Total DNA was extracted from frozen, EDTA or alcohol
preserved tissues (liver, blood, feathers, muscle) using a
CTAB-based protocol [64] with an overnight Proteinase K
(0.1 mg.ml–1) digestion. ND2, ATP6 and ND3 were
amplified and sequenced using primer pairs L5219/
H6313 [65], L9245/H9947 [66] and L10755/H11151
[67], respectively. A 900 bp portion of cytochrome-b was
amplified with the primer pairs L14967-H15487 and
L15424-H15916 [23]). Myoglobin intron-2 and TGFβ2
intron-5 were amplified with primers Myo2/Myo3F
[68,69] and tgf5/tgf6 [70], respectively. The amplification
and sequencing protocol were standard [23].

Phylogenetic analyses

Molecular phylogenies were estimated using parsimony
(P) and model-based approaches (maximum likelihood
[ML], and Bayesian inferences [BI]), as implemented in
PhyML v2.4 [71] and MrBayes 3.1 [72-74]. Parsimony
analyses were conducted with PAUP v4.0b10 [75] using
the heuristic tree bisection and reconnection branch-
 swapping (TBR) algorithm with 100 random addition
replicates. Likelihood models were estimated with
MrModelTest 2.0 [76] using the Akaike Information Cri-
terion [77]. The selected models are listed in Table 4.
Clade support in the individual gene trees for the ML and
Table 3: List of samples used and GenBank accession numbers for the six loci analysed.

| Species | Voucher/Tissue number | Geographic | Myoglobin | TGFB2 | Cytochrome-b | ND2 | ATP6 | ND3 |
|---------|-----------------------|------------|-----------|-------|--------------|-----|------|-----|
| Aegolius acadicus | MVZ 118707 (T) | USA | EU601093 | EU600970 | U89172 | EU601051 | EU601160 | EU601013 |
| Asio otus | MVZ 180184 (T) | USA | EU601097 | EU600975 | AF082067 | EU601055 | EU601165 | EU601018 |
| Athene noctua | MNHN 1995–99 (T) | France | EU601089 | EU600966 | A003948 | No sequence | EU601156 | EU601009 |
| Bubo bubo | MNHN 24–55 (T) | France | EU601069 | EU600949 | A003969 | EU601029 | EU601137 | EU601092 |
| Bubo virginianus | MNHN 179340 (T) | USA | EU601092 | EU600969 | AF168106 | EU601050 | EU601159 | EU601012 |
| Glaucidium cuculoides | MNHN 33–9C (JI50, B) | Laos | EU601088 | EU600982 | No sequence | EU601047 | EU601155 | No sequence |
| Glaucidium gnomas | MVZ 179345 (T) | USA | EU601094 | EU600972 | A003994 | No sequence | EU601162 | EU601015 |
| Otus asio | MNHN 30–10J (T) | Captive | EU601074 | EU600954 | No sequence | EU601134 | EU601141 | EU601097 |
| Otus capnodes | MNHN 32–04G (M. Virani C30577) (B) | Anjouan | EU601078 | EU600957 | No sequence | EU601138 | EU601145 | EU601000 |
| Otus hartlaubi | MNHN 32–06J (B) | Sino Tonè | EU601072 | EU600952 | No sequence | EU601139 | EU601099 |
| Otus hoyi | 2MUC 114834 (B) | Bolivia | EU601061 | EU600942 | No sequence | EU601130 | EU601095 |
| Otus insularis | D. Currie SH21863 (F) | Mahè, Seychelles | EU601059 | EU600940 | No sequence | EU601122 | EU601128 | EU601093 |
| Otus insularis | D. Currie SH21866 (F) | Mahè, Seychelles | EU601060 | EU600941 | No sequence | EU601123 | EU601129 | EU601094 |
| Otus ireneae | MNHN 32–06J (M. Virani C30577) (B) | Kenya | EU601077 | EU600956 | No sequence | EU601137 | EU601144 | EU601099 |
| Otus kenicottii | MVZ 182896 (T) | USA | EU601095 | EU600973 | DQ190850 | EU601053 | EU601163 | EU601016 |
| Otus koepckei | 2MUC 115283 (B) | Peru | EU601062 | EU600943 | No sequence | EU601120 | EU601131 | EU600986 |
| Otus 'lempiji' | UWBM 73860 (T) | Captive | EU601076 | EU600981 | No sequence | EU601136 | EU601143 | No sequence |
| Otus lettia letitia | MNHN 33–4C (F142, B) | Laos | EU601073 | EU600953 | No sequence | EU601130 | EU601136 | EU600996 |
| Otus lettia ussuriensis | UWBM 75379 (T) | Russia | EU601075 | EU600955 | No sequence | EU601135 | EU601142 | EU600998 |
| Otus leucotis | FMNH 429716 (T) | Congo RD | EU601085 | EU600963 | No sequence | EU601130 | EU601152 | EU601006 |
| Otus longicornis | FMNH 433020 (T) | Luzon, Philippines | EU601084 | EU600962 | No sequence | EU601119 | EU601151 | EU601005 |
| Otus longicornis | 2MUC 114206 (B) | Luzon, Philippines | EU601063 | EU600966 | No sequence | EU601126 | EU601132 | EU600987 |
| Otus mayottensis | MNHN R22 (F) | Mayotte | EU601087 | EU600965 | No sequence | EU601122 | EU601146 | EU601008 |
| Otus megalotis | FMNH 433019 (T) | Luzon, Philippines | EU601083 | EU600961 | No sequence | EU601118 | EU601150 | EU601004 |
| Otus megalotis | ZMUC 114208 (B) | Luzon, Philippines | EU601064 | EU600944 | No sequence | EU601105 | EU601133 | EU600988 |
| Otus mirus | FMNH 357429 (T) | Mindanao, Philippines | EU601099 | EU600978 | No sequence | EU601126 | EU601057 | EU601020 |
| Otus melanotis | MNHN E–135 (F) | Philippines | EU601086 | EU600964 | No sequence | EU601121 | EU601145 | EU601007 |
| Otus pauliani | MNHN R24 (F) | Grande Comore | EU601100 | EU600997 | No sequence | EU601125 | EU601058 | EU601021 |
| Otus pembaensis | MNHN uncatalogued (B) | Pemba Island | EU601090 | EU600967 | No sequence | EU601133 | EU601157 | EU601010 |
MP analyses was assessed by 1000 non-parametric bootstrap replicates [78]. The six gene regions sequenced differ considerably in their properties and substitution dynamics, as inferred from the parameters of the models (Table 4). Consequently, analyses of concatenated data set were only performed using a mixed-model strategy. The relevance of partitioning the data set by gene and/or codon position was assessed with the Bayes Factor (BF) [79,80]. Fourteen partitions (myoglobin intron-2, TGFB2 intron-5, first, second and third codon position of each of the four mitochondrial genes) were considered for the Bayesian concatenated analyses according to the functional properties of the markers. Bayesian analyses for the concatenated data set were performed allowing base frequencies, rate matrix, shape parameter and proportion of invariable sites to vary between the partitions (using the unlink and prset commands). Between four and six incrementally heated Metropolis-coupled MCMC chains were run for 15 million generations with trees sampled every 100 generations. The first 2 * 10^6 generations (20000 trees) were discarded (‘burn-in’ period) and the posterior probabilities were estimated from the remaining sampled generations. The default temperature for chain heating (T = 0.2) resulted in not satisfactorily mixing among chains for the concatenated data set; we therefore lowered the temperature to T = 0.05, which resulted in swap frequencies between chains within the 20–70% interval. Two independent Bayesian runs initiated from random starting trees were performed for each data set, and the log-likelihood values and posterior probabilities were checked to ascertain that the chains had reached convergence. We also checked that the Potential Scale Reduction Factor (PSRF) approached 1.0 for all parameters and that the average standard deviation of split frequencies converged towards zero. We detected significant incongruence between the individual gene trees by comparing the topologies and nodal support obtained under different analytical methods (ML, BI). Criteria for incongruence were set at 70% for the bootstrap values [81], and at 0.95 for posterior probabilities [72].

**Molecular dating analyses**

Owls have a very rich fossil record [22,82], yet, the taxonomic history of most of these taxa remains controversial and, to our knowledge, no cladistic analyses including fossil and modern taxa has been conducted to date. This hampers the use of most of these fossils as calibration points in our analyses. The family Strigidae is usually divided into three subfamilies or tribes: Striginae, Surniinae and Asioninae [21]. These three subfamilies are defined by a combination of shared characters [[82] fide [83]]. The least inclusive of these three subfamilies, the Asioninae, consists of three genera and nine species (two of the genera, Pseudoscops and Nesasio are endemic to Jamaica and the Solomon Islands, respectively, whereas Asio is widely distributed). The Asioninae differ osteologi-
cally from other owls by having: 1) the anterior rim of the internal trochlea not protruding more anteriorly than the anterior rim of the external trochea, 2) the external calcaneal ridge bent posteriorly, 3) bony loop broad and 4) tubercle for Musculus tibialis antiquus displaced externally [83]. The most ancient fossil having this combination of characters Intulula tinnipara, has been dated from the Early Miocene (23.7–16.4 mya) [84]. We therefore used this date as a minimum age for the split between Asio otus (the member of the Asioninae we sampled) and its closest relative. As a second calibration point, we used the point (emergence of Grande Comore), we used a normal distribution with the mean and standard deviation set to 0.5 mya and 0.1 mya, respectively. We assumed a Yule distribution for the molecular clock model Speciation Process for the tree prior and an Uncorrelated Lognormal distribution for the molecular clock model [86]. We used default prior distributions for all other parameters and ran MCMC chains for 75 million generations, as the effective sample size for some parameter estimates was not large enough using the default length (10 million generations).

**Authors' contributions**

JF did part of the laboratory work, edited and aligned the sequences, performed the phylogenetic and dating analyses and wrote the first draft of the manuscript. JMP did part of the laboratory work and helped to draft the manuscript. SMG provided tissue samples for the study, wrote several sections of the manuscript and commented on multiple versions. VB collected tissue samples in the field and commented on the manuscript. MM assisted with sample collection and with the draft of the manuscript.

Table 4: Model selected and parameters values with their 95% credibility intervals when applicable (obtained with MrBayes).

| Model | Myoglobin | TGFB2 | ND2 | ATP6 | ND3 | Cytochrome-b |
|-------|-----------|-------|-----|------|-----|-------------|
|       | K80 + I   | HKY + I | GTR + I + I | GTR + I + I | GTR + I + I | GTR + I + I |
| 1st position | NA       | NA    | GTR + I + I | GTR + I + I | SYM + I    | GTR + I + I |
| 2nd position | NA       | NA    | GTR + I + I | GTR + I + I | GTR + I    | GTR + I + I |
| 3rd position | NA       | NA    | GTR + I + I | GTR + I + I | HKY + I    | GTR + I    |
| Freq A | 0.25     | 0.25  | (0.22–0.28) | 0.37 (0.34–0.39) | 0.33 (0.30–0.36) | 0.32 (0.28–0.36) |
| Freq C | 0.25     | 0.21  | (0.18–0.24) | 0.41 (0.39–0.43) | 0.44 (0.41–0.46) | 0.41 (0.37–0.45) |
| Freq G | 0.25     | 0.24  | (0.21–0.27) | 0.06 (0.05–0.07) | 0.07 (0.06–0.08) | 0.09 (0.07–0.11) |
| Freq T | 0.25     | 0.30  | (0.27–0.33) | 0.16 (0.15–0.18) | 0.16 (0.14–0.18) | 0.18 (0.16–0.21) |
| A-C    | NA       | NA    | 0.016 (0.012–0.020) | 0.020 (0.013–0.027) | 0.022 (0.013–0.032) | 0.014 (0.009–0.020) |
| A-G    | NA       | NA    | 0.663 (0.607–0.718) | 0.564 (0.484–0.642) | 0.535 (0.429–0.637) | 0.549 (0.474–0.621) |
| A-T    | NA       | NA    | 0.021 (0.014–0.029) | 0.035 (0.022–0.050) | 0.015 (0.005–0.029) | 0.032 (0.019–0.047) |
| C-G    | NA       | NA    | 0.005 (0.0002–0.014) | 0.024 (0.012–0.039) | 0.007 (0.0002–0.002) | 0.010 (0.0002–0.002) |
| C-T    | NA       | NA    | 0.232 (0.188–0.281) | 0.323 (0.252–0.399) | 0.327 (0.240–0.425) | 0.321 (0.259–0.390) |
| G-T    | NA       | NA    | 0.062 (0.035–0.094) | 0.035 (0.010–0.069) | 0.094 (0.051–0.146) | 0.074 (0.041–0.114) |
| I      | 0.10 (0.09–12) | 0.586 (0.382–0.936) | 0.997 (0.826–1.195) | 0.901 (0.676–1.158) | 0.910 (0.604–1.295) | 0.869 (0.702–1.069) |
| Ts/Tv  | 4.24 (3.08–5.75) | 5.90 (4.32–7.795) | NA | NA | NA | NA |
| -ln (ML) | 2198.81  | 2131.71 | 10556.45 | 6550.30 | 3266.29 | 8623.79 |
| -ln (BI) | 2442.98  | 2179.36 | 10582.97 | 6585.41 | 3305.87 | 8658.36 |
| -ln (BI partitioned) | NA | NA | 10161.23 | 6232.87 | 3146.47 | 8128.06 |

NA means not applicable. Reported likelihood scores for the BI refer to the arithmetic mean.
RCKB helped with tissue sample acquisition, provided funding and commented on multiple versions of the manuscript. DC acquired field samples. RS acquired field samples, advised on taxon sampling in relation to morphology and vocalizations, and helped to prepare and finalize the manuscript. MZV collected blood samples and commented on the manuscript. ST helped with collecting samples. AH helped with capturing Otus penbaensis. CC did part of the laboratory work. EP collected tissue samples in the field and commented on the manuscript. All authors have read and approved the final version of the manuscript.

Additional material

Additional File 1
Fifty percent majority-rule consensus tree (arithmetic mean \(-ln = 2442.98\)) obtained from the Bayesian Inference analyses of myoglobin intron-2 (749 bp). Values next to branches represent MP/ML bootstrap percentages (below) and BI posterior probabilities (above). Gray blocks represent the Indian Ocean taxa. Species between quotes indicate samples for which geographic origin is unknown (captive individuals). The phylogram represents the relationships among owls as inferred from myoglobin intron-2 sequence data.

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Additional File 2
Fifty percent majority-rule consensus tree (arithmetic mean \(-ln = 3427.03\)) obtained from the Bayesian Inference analyses TGFB2 intron-5 (602 bp). Values next to branches represent MP/ML bootstrap percentages (below) and BI posterior probabilities (above). Gray blocks represent the Indian Ocean taxa. Species between quotes indicate samples for which geographic origin is unknown (captive individuals). The phylogram represents the relationships among owls as inferred from TGFB2 intron-5 sequence data.

Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2148-8-197-S2.pdf]

Additional File 3
Fifty percent majority-rule consensus tree (arithmetic mean \(-ln = 10161.23\)) obtained from the Bayesian Inference analyses of the mitochondrial ND2 gene (1041 bp) under a mixed-model strategy (partitioned by codon position). Values next to branches represent MP/ML bootstrap percentages (below) and BI posterior probabilities (above). Gray blocks represent the Indian Ocean taxa. Species between quotes indicate samples for which geographic origin is unknown (captive individuals). The phylogram represents the relationships among owls as inferred from ND2 sequence data.

Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2148-8-197-S3.pdf]

Additional File 4
Fifty percent majority-rule consensus tree (arithmetic mean \(-ln = 6232.87\)) obtained from the Bayesian Inference analyses of the mitochondrial ATP6 gene (684 bp) under a mixed-model strategy (partitioned by codon position). Values next to branches represent MP/ML bootstrap percentages (below) and BI posterior probabilities (above). Gray blocks represent the Indian Ocean taxa. Species between quotes indicate samples for which geographic origin is unknown (captive individuals). The phylogram represents the relationships among owls as inferred from ATP6 sequence data.

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Additional File 5
Fifty percent majority-rule consensus tree (arithmetic mean \(-ln = 3146.47\)) obtained from the Bayesian Inference analyses of the mitochondrial ND3 gene (351 bp) under a mixed-model strategy (partitioned by codon position). Values next to branches represent MP/ML bootstrap percentages (below) and BI posterior probabilities (above). Gray blocks represent the Indian Ocean taxa. Species between quotes indicate samples for which geographic origin is unknown (captive individuals). The phylogram represents the relationships among owls as inferred from ND3 sequence data.

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Additional File 6
Fifty percent majority-rule consensus tree (arithmetic mean \(-ln = 8128.06\)) obtained from the Bayesian Inference analyses of the mitochondrial Cytochrome-b gene (1041 bp) under a mixed-model strategy (partitioned by codon position). Values next to branches represent MP/ML bootstrap percentages (below) and BI posterior probabilities (above). Gray blocks represent the Indian Ocean taxa. Species between quotes indicate samples for which geographic origin is unknown (captive individuals). The phylogram represents the relationships among owls as inferred from Cytochrome-b sequence data.

Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2148-8-197-S6.pdf]

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