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A new species of masked-owl (Aves: Strigiformes: Tytonidae) from Seram, Indonesia

KNUD ANDREAS JØNSSON[1,4], MICHAEL KØIE POULSEN[2], TRI HARYOKO[3], ANDREW HART REEVE[1] & PIERRE-HENRI FABRE[1]

[1]*Center for Macroecology Evolution and Climate at the Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark*
[2]*Nordic Agency for Development and Ecology (NORDECO), Skindergade 23, DK-1159 Copenhagen K, Denmark*
[3]*Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Jl. Raya Jakarta-Bogor KM. 46, Cibinong 16911, Indonesia*
[4]*Corresponding author. E-mail: kajonsson@snm.ku.dk*

Abstract

We describe a new species of masked-owl from the lower montane forest of Seram, one of the largest islands in the Moluccas of eastern Indonesia, for which we propose the name *Tyto almae* (Seram Masked-Owl), **sp. nov**. Molecular (mitochondrial cyt-*b*) differences show that *Tyto sororcula* of Buru and Tanimbar is closely related to *T. novaehollandiae* of Australia and New Guinea (~1% uncorrected pairwise distance), and that *Tyto almae* of Seram differs by ~3% (uncorrected pairwise distance) from both of them. These differences are further corroborated by morphology and colouration. Although a photograph from Seram published in 1987 had already established the presence of a *Tyto* owl on the island, ours represents the first specimen of this species. The bird was mist-netted in wet, mossy lower montane forest at an elevation of 1,350 m. No further observations of the owl were made during four weeks of fieldwork in Seram.

Key words: Manusela, Moluccas, new taxon, owl, phylogeny

Introduction

Wallacea is the island region that forms the faunal transition between Asia and Australia. Faunal studies in this biologically unique part of the world inspired Alfred R. Wallace to write one of the earliest modern evolutionary syntheses (Wallace 1860, 1869, 1876), yet it remains one of the ornithologically least-studied regions on Earth, and some endemic Wallacean bird species have only been recorded a few times since their discovery (White & Bruce 1986; Coates & Bishop 1997).

One secretive and little known avian group from Wallacea is the masked-owl complex within the genus *Tyto*, which is closely related to the two species of Australo-Papuan sooty-owls (*Tyto tenebricosa* Gould and *T. multipunctata* Mathews; Bruce 1999; Norman *et al.* 2002; Wink *et al.* 2009). Current hypotheses about the evolutionary relationships within the masked-owls are largely based on morphology, and no comprehensive DNA studies have previously been undertaken to determine the systematic relationships within the group. The seven currently recognized species (Bruce 1999) are found (from west to east) on Sulawesi (*T. rosenbergii* Schlegel and *T. inexspectata* Schlegel), Taliabu in the Sula Islands (*T. nigrobrunnea* Neumann), the Moluccan islands of Buru and Tanimbar (*T. sororcula* Sclater), Australia and New Guinea (*T. novaehollandiae* Stephens), the island of Manus (*T. manusi* Rothschild & Hartert) and New Britain (*T. aurantia* Salvadori).

Tyto sororcula, from the south Moluccan islands of Buru (*T. s. cayelii* Hartert) and Tanimbar (*T. s. sororcula*), is sometimes considered a subspecies of the Australo-Papuan *Tyto novaehollandiae*, but differs by being smaller overall (Higgins 1999). *Tyto sororcula* is known from only four museum specimens representing these two insular subspecies. The nominate subspecies was collected on Tanimbar in 1882 and 1923 (Sclater 1883; Stresemann 1934), and *T. s. cayelii* is known only from two specimens from Buru collected in 1898 and in 1921 (Hartert 1900;

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Siebers 1930). Additionally, a bird presumed to be *Tyto sororcula* was photographed during the Operation Raleigh Expedition by Rudi Badil and Sukianto Lusli in Manusela National Park in Seram in 1987 (Voice of Nature Magazine 1987; not seen, cited on p. 358 of Coates & Bishop 1997). However, it remained uncertain whether this individual belonged to one of the two already described subspecies, or whether it represented a hitherto undescribed form (Bruce 1999). Nothing appears to have been published about this first record of a *Tyto* owl from Seram, and it is not mentioned alongside the other bird records from the Operation Raleigh Expedition by Bowler and Taylor (1989, 1993) or elsewhere in the book "*Natural History of Seram: Maluku, Indonesia*" by Edwards *et al.* (1993), which covers the results of the expedition. *T. s. cayelii* of Buru has probably not been sighted since the two specimens were collected, but the vocalization of *T. s. cayelii* was recorded during a trip to Buru in 2009 by George Wagner (http://www.xeno-canto.org). In Tanimbar, surveys in 1985 and 1993 failed to locate nominate *Tyto s. sororcula*, but in 1995 it was rediscovered (Bruce 1999), and recent bird tours to Tanimbar have successfully seen and photographed it on several occasions (e.g, http://ibc.lynxeds.com/photo/lesser-masked-owl-tyto-sororcula/frontal-view-bird).

From 25 January through 18 February 2012, an international team organized by the Natural History Museum of Denmark (SNM) and the Indonesian Institute of Sciences (LIPI) conducted avifaunal surveys of the forests in Manusela National Park in the central parts of the 17.100 km^2 large island of Seram in Maluku Province of Indonesia. On 10 February 2012, a *Tyto* owl was netted at 1,350 m at the edge of a large, natural opening in the forest. The owl, which was vocal, emitting a continuous series of drawn-out shrieks, was immediately taken back to the camp for further assessment.

Methods

Morphometrics. We compared the newly collected *Tyto* specimen housed in the Museum Zoologicum Bogoriense (MZB; measured by Tri Haryoko) with study skins of *Tyto sororcula cayelii* (N=2) from Buru and *Tyto sororcula sororcula* (N=2) from Tanimbar in the collections of the American Museum of Natural History (AMNH; measurements by P. Capainolo and P. Sweet and photos by M. Shanley and T. J. Trombone), Naturalis, Leiden (RMNH; measurements by C. S. Roselaar and S. van der Mije and photos by Eelco Kruidenier), and the Natural History Museum, Tring (BMNH; measurements and photos by Hein van Grouw).

Plumage characters. We compared photos taken of the following museum specimens: an adult female *T. s. cayelii* collected in Buru in 1898 (AMNH 629476, type specimen); an adult female *T. s. sororcula* collected in Tanimbar in 1923 (RMNH.AVES.162520); and another adult female specimen of *T. s. sororcula* (type specimen) (BMNH 1883.5.30.89, type specimen) (Figs. 1–3 and Table 1). These photos demonstrate overall similarities, but also some rather conspicuous differences. Ridgway (1912) was used as a standard for colour terminology.

Molecular analyses. We isolated and purified DNA from the fresh muscle tissue preserved in DMSO of the Seram *Tyto*. We also sampled toe-pads of *Tyto s. sororcula* from Tanimbar (RMNH.AVES.162520 and BMNH 1883.5.30.89, type specimen) and *Tyto sororcula cayelii* from Buru (AMNH 629476, type specimen), as well as *Tyto inexspectata* (AMNH 298887), *Tyto manusi* (AMNH 334763), *Tyto aurantia* (AMNH 777865), *Tyto rosenbergii* (AMNH 298884), and *Tyto novaehollandiae calabyi* (AMNH 425940) using QIAamp Mini Kit (Qiagen Inc., 2003) following the manufacturer's recommendations, and sequenced 877 base pairs (bp) of the mitochondrial gene region cytochrome *b* (cyt-*b*) (only 523 bp for one of the two *T. s. sororcula* specimens from Tanimbar, but the full 877 base pairs for the *T. s. sororcula* type specimen), using the following primers specifically designed for this study: CytbTytoF1: TGCTGGGCATTTGTCTAACCCT; CytbTytoR1: TGTGCAGGTATGAGCCGTAGTA; CytbTytoF2: ATCTGCATCTACCTACACATCG; CytbTytoR2: CGCCTCAGGCCCATTCTACA; CytbTytoF3: CTACCATATGTGGGCCAAACCA; CytbTytoR3: GAGAAGTATGGGTGAAATGGGAT; CytbTytoF4: ACCCACTAGGAATTACATCAAATTG; CytbTytoR4: ATGGAGCGTAAAATAGCGTATGC; CytbTytoF5: CCCCCCTCACATCAAACCAGA; CytbTytoR5: GCTGGCTGCCGATTCAGGT.

TABLE 1. Morphometrics of *Tyto sororcula* specimens compared to the *Tyto* collected in Seram. Morphological measurements in parentheses represent measurements as provided in original publications. Some measurements are given in inches in the original publication. These have been converted to millimeters by a factor 1 inch = 2.54 mm.

Species	Sex	Island	locality	wing (mm)	tail (mm)
Tyto almae **sp. nov.**	female adult	Seram	Manusela	252	116
Tyto s. sororcula	female adult	Yamdena, Tanimbar archipelago	Saumlaki	242 (235)	100 (93)
Tyto s. sororcula (type)	female adult	Larat, Tanimbar archipelago	Larat	225 (227)	87.4 (93.5 converted from inch)
Tyto s. cayelii (type)	female adult	Buru	Cayeli	255.7	129.5 (120)
Tyto s. cayeli	male	Buru	Wai Eno	248 (251)	115

continued.

Species	tarsus (mm)	bill length (mm)	Collecting date	data by
Tyto almae **sp. nov.**	63.85	37.35	10 Feb 2012	Knud Jønsson
Tyto s. sororcula	57.5	35.2	22 April 1923	Kees Roselaar
Tyto s. sororcula (type)	60.5 (58.8 converted from inch)	30.5	24 Sept 1882	Hein van Grouw
Tyto s. cayelii (type)	57.4	32.9	Oct 1898	Peter Capainolo
Tyto s. cayeli	56.8	36.5	7 Sept 1921	Tri Haryoko

Thermocycling conditions included a hot start at 95°C, an initial denaturation step at 95°C for 5 min, followed by 4 cycles at 95°C for 30 s, 59°C for 30 s, and 72°C for 45 s; 4 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, 32 cycles at 95°C for 30 s, 52°C for 30 s, and 72°C for 45 s and completion by a final extension at 72°C for 8 min. One microlitre of polymerase chain reaction (PCR) products was electrophoresed on a 1.5% agarose gel and visualized under UV light with ethidium bromide to check for correct fragment size and to control for the specificity of the amplifications. The PCR products were purified using ExoSap enzymes (exonuclease and shrimp alkaline phosphatase). Purified PCR products were cycle-sequenced using Big Dye terminator chemistry (ABI; Applied Biosystems, Foster City, CA, USA) in both directions with the same primers used for PCR amplifications, and run on an automated ABI 3730 DNA sequencer. Sequences were assembled with Sequencher v4.7, and checked for the presence of stop codons or indels that would have disrupted the reading frame, indicative of having amplified pseudogenes. Furthermore, we blasted each of the five individual DNA fragments in GenBank to confirm the validity of the sequences. These new sequences have been deposited in GenBank (KC492082–KC492090).

We downloaded cytochrome *b* sequences from eight other *Tyto* species in GenBank, including *Tyto n. novaehollandiae* and *Tyto n. castanops* from Australia, which are considered closely related to *Tyto sororcula*. Uncorrected pairwise distances were computed in PAUP* (Swofford 2003) for the individuals for which we had all 877 base pairs, to assess the genetic divergence between Australasian *Tyto* specimens (Table 2).

We used BEAST v1.6 (Drummond & Rambaut 2007) to estimate the divergence dates among members of *Tyto*, by applying the best-fitting model, as estimated by MrModeltest 2.0 (Nylander 2004), following the Akaike information criterion (Posada & Buckley 2004). We assumed a Yule Speciation Process for the tree prior and an uncorrelated lognormal distribution for the molecular clock model (Ho 2007), and we used default prior distributions for all other parameters, and ran MCMC chains for 20 million generations. We repeated the analysis twice to ensure independent convergences of models and used the program Tracer (Rambaut & Drummond 2007) to assess convergence diagnostics. To obtain absolute dates, we used a rate of 0.01 substitutions per site per lineage per million years (Myr) for cyt-*b* (Weir & Schluter 2008).

Vocalization. The captured *Tyto* vocalized almost incessantly and we compared sonograms of these calls to calls of other masked-owls as well as subspecies of *Tyto* (*alba*) *delicatula* from Australasia available from www.xenocanto.org. Because vocalizations of *Tyto* owls are poorly documented and some give a variety of screeches, screams and shrieks, we cannot be certain that the analysed calls are directly comparable (Bruce 1999). Thus, analyses of sonograms for masked-owls remain to be further tested based on additional vocal data.

TABLE 2. Uncorrected pairwise distances in % in the mitochondrial cytochrome-*b* (877 base pairs) between Australasian Masked-Owls.

	Tyto almae **sp. nov.**	*Tyto aurantia*	*Tyto manusi*	*Tyto n. calabyi*	*Tyto n. castanops*	*Tyto n. novaehollandiae*	*Tyto s. cayelii*
Tyto aurantia	3.28						
Tyto manusi	3.08	2.82					
Tyto novaehollandiae calabyi	3.08	2.83	0.46				
Tyto novaehollandiae castanops	3.07	2.96	0.95	0.95			
Tyto n. novaehollandiae	3.07	2.66	0.47	0.47	0.95		
Tyto sororcula cayelii	3.53	2.98	0.68	0.68	1.41	0.94	
Tyto sororcula sororcula	2.85	2.69	0.23	0.23	0.71	0.24	0.68

Results and discussion

Morphometrics. The wing length of *T. almae* (252 mm) falls within the wing length range of *T. s. cayelii* from Buru (248 mm and 255.7 mm), both measurements of which are slightly larger than those of *T. s. sororcula* from Tanimbar (225 and 242 mm). The bill length is similar for *T. s. cayelii* and *T. s. sororcula*, but about 10% bigger for *T. almae*, which could reflect a different specialization for feeding. However, we note that the sample size is very small (N=5) and that five different people took the measurements. Therefore, we cannot say much about the differences without further controlled morphometric data.

Molecular analyses. *Tyto sororcula sororcula* and *T. s. cayelii* are closely related to *Tyto novaehollandiae* and *Tyto manusi* from Australo-Papua, showing molecular differences of only 0.23–1.41% (uncorrected pairwise distances). *Tyto almae*, however, differs by 2.85–3.53% in mtDNA from all of these (Table 2). *Tyto aurantia* represents the deepest lineage of the Australo-Papuan masked-owls, and the two masked-owl species of Sulawesi (*Tyto rosenbergii* and *Tyto inexspectata*) are not closely related to this assemblage (Fig. 4), nor to each other.

Applying the 2% rule (Weir & Schluter 2008) to our dataset produced a dated chronogram (Fig. 4) that suggests that *Tyto almae* diverged from the *Tyto novaehollandiae/sororcula/manusi* complex around 1.7 Million years ago (Mya) and that diversification leading to *Tyto novaehollandiae/sororcula/manusi* started later around 0.7 Mya.

Vocalization. The collected specimen of *T. almae* vocalized almost incessantly while being handled, giving screams quite similar to those of the masked-owls *T. sororcula cayelii*, *T. s. sororcula*, and *T. novaehollandiae*, as well as to the Australian Barn Owl (*T.* [*alba*] *delicatula*). The screams were decidedly lower-pitched than those of *T. alba* (*deroepstorffi* of the Andaman Islands and *stertens* of India) (Fig. 5). Although the pitch is quite similar within *Tyto almae* and *Tyto novaehollandiae*/*sororcula*, it seems the pitch of *Tyto almae* is slightly lower (Figs. 5 and 6), but larger sample sizes are needed to establish the consistency of these differences. We also note that the calls given by *Tyto almae* may be distress calls and thus not entirely representative of its usual voice.

Habitat and biology. The type locality is in mossy montane forest at 1350 m, where the owl was captured in a natural treefall gap with a small landslide, ~50 m wide and ~100 m long. The surrounding forest is about 15 m tall, and characterized by the angiosperm families Fagaceae (dominated by *Castanopsis buruana* and *Lithocarpus* species) and Myrtaceae (dominated by *Syzygium* species), the gymnosperms Podocarpaceae, and also tree ferns, rattans, bamboo, non-woody climbers, epiphytes and bryophytes (Edwards *et al.* 1990, 1993).

Taxonomy

We base our taxonomic assessment on the general lineage-based species concept (de Queiroz 1999), which is an extension of the evolutionary species concept (Simpson 1961; Wiley 1978). This way we employ a lineage-based species concept that recognizes taxa with a unique evolutionary history. In contrast to the biological species concept (Mayr 1963), the lineage-based species concept is better suited for allopatric insular taxa for which it is impossible to assess the level of reproductive isolation. We base our species assessment primarily on genetic divergence and plumage patterns, but also draw on morphometrics and vocalization.

Based on evidence from genetic divergences and plumage patterns, and to a lesser extent morphometrics and vocalization of Moluccan and Australo-Papuan masked-owls, it is apparent that a tight-knit assemblage including *Tyto novaehollandiae*, *T. sororcula* and *T. manusi* may be considered a single species, which should be referred to as *Tyto novaehollandiae*. Our data further shows that the newly collected *Tyto* specimen from Seram falls outside the variation seen in *T. sororcula* of Buru and Tanimbar and *T. novaehollandiae* from Australo-Papua. Consequently, we describe it here as a new species, which we name:

***Tyto almae*, sp. nov.**
Seram Masked Owl

Holotype. Museum Zoologicum Bogoriense (MZB), Cibinong, Indonesia, study skin voucher: MZB 33.231, female (ovary 17 × 5 mm), Mount Binaiya, at Waensela above Kanikeh Village, Manusela National Park, Seram, Maluku Province, Indonesia, elevation 1,350 m, 3°08.668'S, 129°28.434'E, mist-netted on 10 February 2012; prepared by Knud Andreas Jønsson, field catalogue number KAJ1-10.02.12.

Measurements of holotype. Wing length (chord): 252 mm; tarsus: 63.6 mm; tail: 116 mm; bill from base of skull: 37.4 mm; bill height at base: 16.1 mm; bill width: 13.7 mm; weight: 540 g.

Description of holotype. Facial area light pinkish cinnamon, more dusky in the eye-pit, with short feathers that do not cover the auricular troughs (probably as a consequence of heavy moult), with a ruff of orange tawny feathers with dark brown tips bordering the facial area outside the auricular troughs. Crown, nape, mantle and wing coverts ochraceous orange, each feather with a short white stripe embedded within dusky-mottled feather-tips; lower back to upper tail-coverts with more spindle-shaped and pale buffy spots within the dark feather-tips. Greater wing-coverts and remiges ochraceous orange with well-spaced fuscous bars and only a few darker mottles between bars; upper side of rectrices similar, ochraceous tawny with five exposed fuscous bars, each 5–8 mm wide, and with a few dusky mottles or stipples within the broader ochraceous zones. The entire extent of the underparts, including wing linings, yellow-ocher with whitish basal parts of the feathers, and small, roundish subterminal spots on most feathers of the breast and belly; tarsi with yellow-ocher feathers all the way to the base of the toes. Irides dark brown, bill pale horn, feet pinkish drab with pale grey talons.

Diagnosis. Head large and round with a heart-shaped facial area, tail short, and legs rather long as is typical of masked-owls of the genus *Tyto* (Bruce 1999). The general colouration, size and geographical location initially led us to believe that it belonged within *Tyto sororcula*. Generally similar to both forms of *Tyto sororcula*, but differs by having yellow ochre instead of white or whitish underparts. Also shows a unique pattern on crown and upper back, with a short white or buff shaft stripe within the dusky terminal part of each feather, instead of two roundish, or bar- or heart-shaped white spots per feather, as in most *Tyto* species. Additionally, feathers of the mid- and lower back have only a limited amount of light (buffy) mottles within the dusky terminal parts, unlike in *T. s. cayelii* and, notably, *T. s. sororcula*. In the latter subspecies, distal parts of the dorsal feathers are densely mottled with dusky and white, and there is also a broad mottled zone inside each of the dark bars, giving a rather 'peppered' greyish appearance to much of the upperparts. The tail of *T. almae* is extensively golden brown with broad dark bars, showing no pale mottling, and only a trace of dark mottling between the bars. This is unlike the more extensive dark mottling found in both forms of *T. sororcula*, which additionally shows pale mottling. Thus, with more extensively golden brown and distinctly barred wings and tail, *T. almae* is phenotypically quite distinct from the two currently recognized forms of *Tyto sororcula*. Furthermore, *T. almae* has longer and completely feathered tarsi, similar to *T. s. cayelii*, whereas the lower tarsi of *T. s. sororcula* have short bristle-like feathers only.

Etymology. The specific epithet honours Alma Jønsson, daughter of the senior author, acknowledging that she had to be without her father while he was out exploring.

FIGURE 1. Comparisons of *Tyto* owl specimens. From left to right: *Tyto sororcula cayelii* from Buru (AMNH 629476, type specimen), photo by M. Shanley and T. J. Trombone; *Tyto almae* from Seram (MZB 33.231), photo by K. A. Jønsson; *Tyto sororcula sororcula* from Tanimbar (RMNH.AVES.162520), photo by Eelco Kruidenier; and *Tyto sororcula sororcula* from Tanimbar (BMNH 1883.5.30.89, type specimen), photo by Hein van Grouw.

FIGURE 2. Close-up photos of heads of: *Tyto sororcula cayelii* from Buru (AMNH 629476, type specimen), photo by M. Shanley and T. J. Trombone; *Tyto almae* from Seram (MZB 33.231), photo by K. A. Jønsson; and *Tyto sororcula sororcula* from Tanimbar (RMNH.AVES.162520), photo by Eelco Kruidenier.

FIGURE 3. Close-up photos of tails of: *Tyto sororcula cayelii* from Buru (AMNH 629476, type specimen), photo by M. Shanley and T. J. Trombone; *Tyto almae* from Seram (MZB 33.231), photo by K. A. Jønsson; and *Tyto sororcula sororcula* from Tanimbar (RMNH.AVES.162520), photo by Eelco Kruidenier.

Conservation. Both known records of *Tyto* owls from Seram are from within the protected Manusela National Park. Manusela National Park covers about 10% of Seram (17,100 km^2). This is one of the only areas in Seram that is visited with any regularity by ornithologists (Bowler & Taylor 1989, 1993; Marsden *et al*. 1997; Rheindt & Hutchinson 2007a,b) and other individuals who would recognize the scientific significance of a *Tyto* owl on Seram. The natural vegetation of Seram is tropical lowland evergreen and semi-evergreen rain forest, with tropical montane rain forest above ~800 m. Seram is still well-forested, although the low-lying areas where the human population is concentrated along the coast and in the west have been cleared (BirdLife International 2012). It is very problematic to assess the threat status of this species as so little is known about its distribution, status and habitat requirements. The little information available suggests that *T. almae* is not threatened at present, but lives at rather low population densities within montane and perhaps lowland rain forest on Seram. Timber extraction and bird trapping for the wild bird trade are known threats to other forest bird species of Seram (Marsden 1998; Chan *et al*. 2004; BirdLife International 2012). The most likely threat to *Tyto almae* is disappearance of its forest habitat due to anthropogenic activities. The existence of Manusela National Park may be of high importance for the long-term conservation of this species.

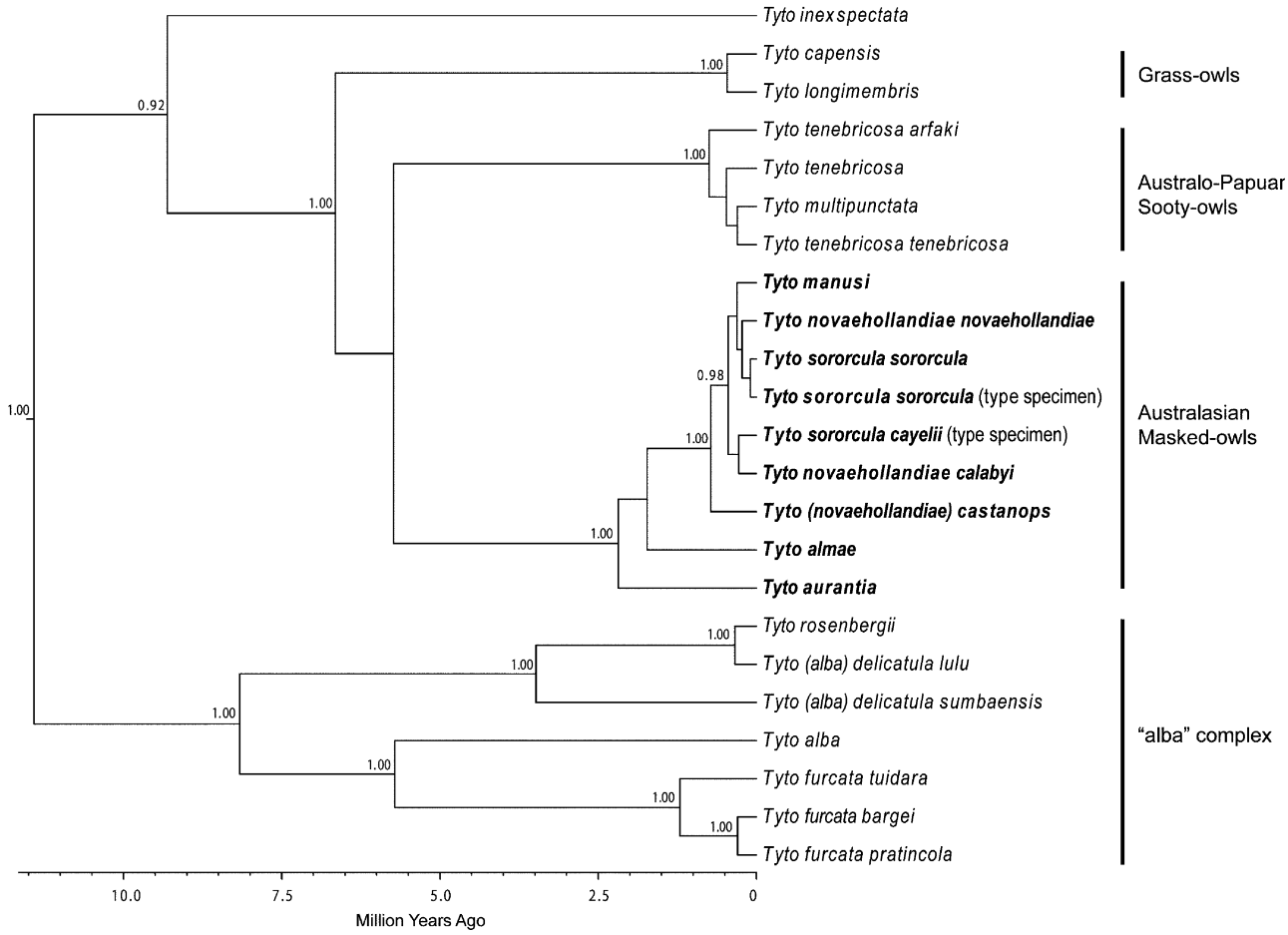

FIGURE 4. Chronogram based on the BEAST analysis of members of *Tyto*. Divergence dates were estimated by using a rate of 0.01 substitutions per site per lineage per million years in Cytochrome-*b* (the 2% rule, Weir & Schluter 2008). Only posterior probabilities ≥ 0.90 are shown on the figure.

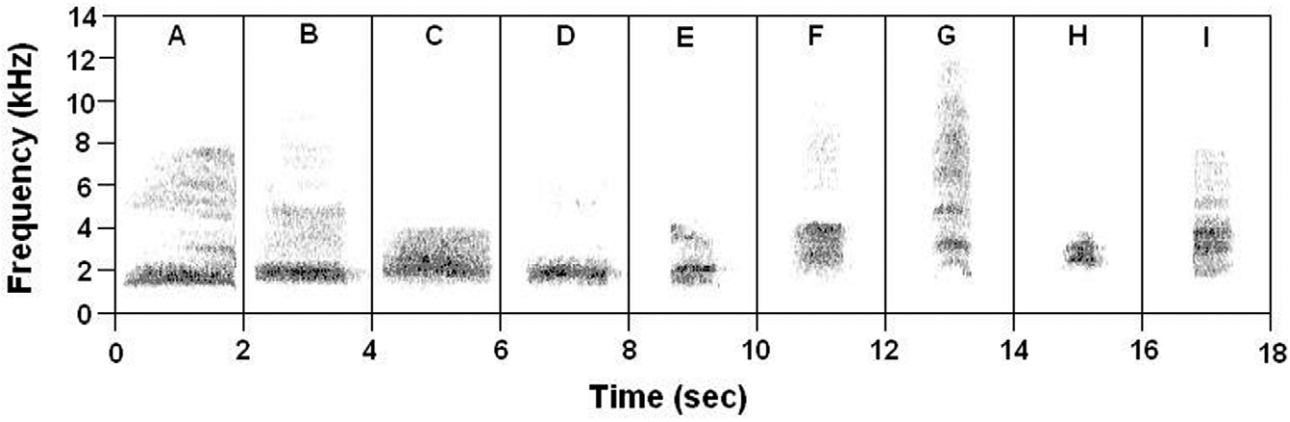

FIGURE 5. Sonograms of **A)** *Tyto almae* (recording by Michael Køie Poulsen) from Seram, **B)** *Tyto sororcula cayelii* (XC42300, recording by George Wagner) from Buru, **C)** *Tyto sororcula sororcula* (http://ibc.lynxeds.com/video/lesser-masked-owl-tyto-sororcula/bird-tree-looking-around-calling-once, recording by Josep del Hoyo) from Tanimbar, **D)** *Tyto n. novaehollandiae* (XC102921, recording by Nigel Jackett) from Australia, **E & F)** *Tyto (alba) delicatula* (XC41851, recording by Vicki Powys) from Australia, **G)** *Tyto rosenbergii* (XC88280, recording by Frank Lampert) from Sulawesi, **H)** *Tyto (alba) deroepstorffi* (XC94701, recording by Pankaj Koparde) from the Andaman islands, **I)** *Tyto (alba) stertens* (XC92296, recording by Sudipto Roy) from India.

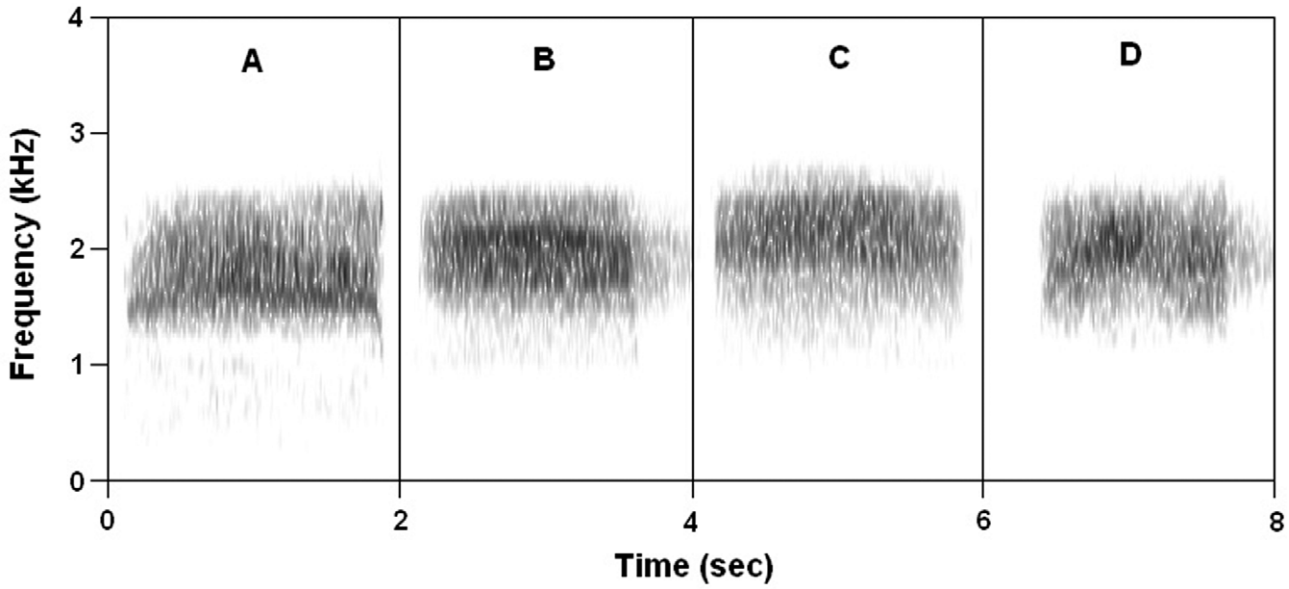

FIGURE 6. Sonograms of **A)** *Tyto almae* (Michael Køie Poulsen) from Seram, **B)** *Tyto sororcula cayelii* (XC42300, recording by George Wagner) from Buru, **C)** *Tyto sororcula sororcula* (http://ibc.lynxeds.com/video/lesser-masked-owl-tyto-sororcula/bird-tree-looking-around-calling-once, recording by Josep del Hoyo) from Tanimbar, **D)** *Tyto n. novaehollandiae* (XC102921, recording by Nigel Jackett) from Australia. Notice how the pitch of *Tyto almae* (A) is slightly lower than the pitch of *Tyto sororcula/novaehollandiae* (B-D), although this result should be interpreted with caution because *Tyto almae* gave incessant alarm calls, which may not be directly comparable to other field recordings.

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