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## A new *Ninox* owl from Sumba, Indonesia

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**Abstract.** Since the late 1980s ornithologists have reported an unknown *Otus* owl from the island of Sumba, Indonesia. From a specimen of this unknown owl we analysed the mitochondrial cytochrome *b* gene in feathers and compared its sequence with those in our data library, which includes most owl genera. A phylogenetic analysis unequivocally places the unknown owl into the *Ninox* clade. It differs from *N. novaeseelandiae* and *N. scutulata* by 8.2 and 9.1% nucleotide substitutions, but our *Ninox* data set contains few representatives so we cannot define to which other *Ninox* species it is most closely related. There was no overlap in measurements of body length or mass for this specimen and those of other *Ninox* known from this region. The call of this new owl was a monosyllabic hoot repeated about every three seconds and quite unlike the repeated ‘cluck-cluck-cluck’ made by the endemic *N. rudolfi* or the disyllabic notes made by most *Ninox*, including others in the region. For this new species we assign the common name Little Sumba Hawk-Owl. The conservation status of this owl has yet to be determined, but the species might be threatened and we propose it as Data Deficient.

### Introduction

The island of Sumba is located at 10°S and 120°E in the Lesser Sunda chain in south-eastern Indonesia. The island is 210 km long with a surface area of about 12000 km<sup>2</sup>; at 1225 m, the highest point is Gunung Wangameti. The island experiences a winter dry and summer wet season, and annual rainfall is between 500 mm on the south coast and 2000 mm on the inland hills. Closed canopy forest, mostly deciduous with some evergreen, now covers less than 11% of the island and is confined to small and fragmented pockets due to clearing and the repeated burning of vegetation to provide land for grazing and cultivation (Jones *et al.* 1995).

Since the late 1980s ornithologists have reported an unknown owl on Sumba (Coates and Bishop 1997; Linsley *et al.* 1999; del Hoyo *et al.* 1999; King and Yong 2001). Debus (2002) said that this owl should be described and its status determined. Most writers considered the undescribed owl to be an *Otus*, and del Hoyo *et al.* (1999) noted that, somewhat surprisingly, some sources dismissed the reports as misidentification of the relatively large, endemic Sumba Boobook, *Ninox rudolfi*. King and Yong (2001) stated the bird was ‘undoubtedly a scops owl, at the smaller end of the *Otus* range’, much smaller than the Sumba Boobook, with fine wavy barring on its underparts rather than the broader regular barring of *N. rudolfi* and likely to be the Flores Scops Owl, *Otus alfredi*, Flores being the island 45 km due north of Sumba. However, the song of the unknown Sumba owl, a low

whistled ‘who’ repeated at intervals of 3–4 s (King and Yong 2001) was unlike any other known scops owl; the song of *O. alfredi* is unknown, and when King played songs of the unknown Sumba owl on Flores near the location where *O. alfredi* was recently rediscovered, they failed to elicit a response. Furthermore, *O. alfredi* has ear tufts, as are characteristic of *Otus*, but the owls seen on Sumba by King and Yong had no visible ear tufts.

On 20 December 2001 two of us (Olsen and Trost) visited Sumba and, with a resident guide, checked one of the locations where the Sumba owl had been reported: south of the Km 49 marker on the Lewa–Waingapu road west of Waingapu (King and Yong 2001). Most of Sumba is grassland on rolling hills with remnant patches of forest, like small islands, dotting these open areas. At 1830 hours we broadcast a tape recording of the unknown owl, at one of these remnant patches. A pair came to the edge of forest and both called; we located two other pairs in adjacent forest, and audio-recorded, video-recorded and photographed the three pairs.

On 30 December 2001 a specimen of this owl, shot by a local and said to have come from degraded forest near Km 45, some 4 km east of the forest where we had been observing, was shown to Olsen. As all three pairs we observed were subsequently present and some distance from where the specimen was said to be taken, the specimen was apparently not one of these owls, but it was clearly of the same species.

The analysis of nuclear or mitochondrial marker genes has become a widely applied tool during the last 15 years in all fields of zoology, including ornithology, to reconstruct phylogenies and phylogeographic relationships (overviews in Avise 1994; Mindell 1999). Molecular data have the great advantage that convergence does not impair an analysis to the same degree as morphological data do. Molecular data allow both a phylogenetic and phylogeographic analysis of the unknown past of a particular group of organisms. DNA sequences have therefore become an important tool for taxonomy and evolutionary studies including owls (Heidrich and Wink 1998; Wink and Heidrich 1999; Wink 1998). For this communication we have analysed the mitochondrial cytochrome *b* gene of the unknown owl and compare its sequence with those in our data library, which includes most owl genera.

## Materials and Methods

We photographed and recorded a description of the specimen and compared morphological data for its wing, tail, length and mass with those of other *Ninox* taxa in the region. Total DNA was extracted from feather samples by an overnight incubation at 37°C in lysis buffer (10 mM Tris [pH 7.5], 25 mM EDTA, 75 mM NaCl, 1% SDS) including 1 mg of Proteinase K (Merck, Darmstadt), followed by a standard phenol/chloroform protein extraction. DNA was precipitated from the supernatant with 0.8 volume of cold isopropanol, centrifuged, washed, dried and resuspended in TE buffer.

### PCR and DNA sequencing

The mitochondrial cytochrome *b* gene was amplified from total DNA under the following conditions: 50 µL reaction buffer contained 1.5 mM MgCl<sub>2</sub>, 10 mM Tris, 50 mM KCl, 100 µM dNTPs, 0.8 units Taq polymerase (Pharmacia Biotech, Freiburg), 200 ng DNA and 5 pmoles PCR primer mt-A1 5'-GCCCTACCAACATCTCAGCATGATGAAACTTCG-3' and mt-FR 5'-CTAAGAAGGGTGGAGTGTTCAGTTTTGGTTTACAAGA-3'

PCR: initially 94°C for 4 min; 31 cycles with 94°C for 45 s, 52°C for 60 s, 72°C for 120 s; finally 72°C for 5 min. PCR products were stored at 4°C. PCR products (1 volume) were precipitated in 4M NH<sub>4</sub>Ac (1 volume) and 6 volumes ethanol. After centrifugation for 15 min at 13000 rpm, DNA pellets were washed in 70% ethanol and taken up in 15 µL distilled water.

A cycle-sequencing reaction (final volume 10 µL) was carried out next. The reaction buffer consisted of 2 µL reaction mix with BigDye terminators (according to the BigDye Terminator Protocol: ABI Applied Biosystems), 10 pmole primer (smtA-5'-CAACATCTCAGCATGATGAAACTTCG-3' and mt C-5'-TAYGTCCTACCATGAGGACAAATATCATTCTGAGG-3'). The cycle sequencing was carried out in 25 cycles at 96°C for 10 s, 52°C for 5 s and 60°C for 4 min.

Sequencing products were purified by precipitation: 1 vol reaction mix, 1/10 3M NaAcetate (pH 4.6), 2.5 vol ethanol. After centrifugation for 15 min at 13000 rpm, DNA pellets were washed in 70% ethanol and taken up in 20 µL distilled water. The purified sample was diluted 1:5 in water and applied to a 16-column automatic capillary sequencer (ABI 3100) using 50-cm capillaries and POP6 as a polymer.

Sequences of the other owl taxa shown in Fig. 1 and Table 1 were obtained earlier using radioactive sequencing or ALFexpress II, as described previously (Heidrich and Wink 1998; Wink and Heidrich 1999, 2000).

Sequences of >1000 nt were obtained directly from the sequencer and aligned. Deletions, insertions or inversions were not encountered. A 900-bp section of the cytochrome *b* gene from the unknown owl and those of *Ninox novaeseelandiae* of Australasia and *N. scutulata* of south-east Asia are given in Fig 4.

### Phylogenetic and statistical analysis

Maximum Parsimony and Maximum Likelihood searches were conducted with the heuristic search approach of PAUP\* 4.0 (Swofford 2001) using the 'tree-bisection-and-reconnection' swapping algorithm. For Maximum Parsimony the default settings were applied. For Maximum Likelihood (ML) the following parameters were specified: number of substitution types = 6, settings correspond to the GTR model. ML has proven to be powerful and is now widely applied (Swofford *et al.* 1996; Huelsenbeck and Crandall 1997).

### Analysis of vocalisations

Following the Biological Species Concept, König *et al.* (1999) regard as full species the members of a reproductive community that have evolved different patterns from members of another reproductive community. Often with owls these patterns are most easily perceptible in their vocalisations. Owls' vocalisations are inherited and, unlike those of some passerines, have little geographic variation in dialect, so they are diagnostic of species and critical to the study of owl taxonomy (König *et al.* 1999; Higgins 1999). For a mean 140 minutes per night we observed and video/audio-recorded episodes of calling with a Panasonic NV-MX300 digital video camera: (a) three different calling pairs of the new owl over five nights near Km 49, and (b) four different calling pairs of *N. rudolfi* at four different locations over 13 nights (three pairs near Melolo, and one pair near Km 49 in the same forest,

**Table 1. Origin of owl taxa analysed at Heidelberg University**

Taxon	Accession No	Origin
<i>Aegolius acadicus</i>	IPB-6242	Canada
<i>Aegolius funereus</i>	IPB-6237	Munich, Germany
<i>Asio flammeus</i>	IPB-465	Zoo, Leipzig, Germany
<i>Asio otus</i>	IPB-6945	Raisting, Germany
<i>Athene noctua</i>	IPB-2877	North-east Greece
<i>Bubo bubo</i>	IPB-2609	Museum, Tromsø, Norway
<i>Bubo sumatrana</i>	IPB-6340	Zoo, Villars des Dombes, France
<i>Bubo virginianus</i>	IPB-6096	Zoo, Berlin, Germany
<i>Bubo zeylonensis</i>	IPB-9577	Zoo, Hong Kong
<i>Glaucidium brasilianum</i>	IPB-6050	Salta, Argentina
<i>Glaucidium passerinum</i>	IPB-6067	Zoo, Innsbruck, Austria
<i>Ninox novaeseelandiae</i>	IPB-5681	Western Australia
<i>Ninox novaeseelandiae</i>	IPB-6172	Australia
<i>Ninox scutulata</i>	IPB-6271	Taiwan, China
<i>Nyctea scandiaca</i>	IPB-6131	Zoo, Leipzig, Germany
<i>Otus atricapillus</i>	IPB-6318	Arroyo, Argentina
<i>Otus megalotis</i>	IPB-6290	Philippines
<i>Otus sanctacatarinae</i>	IPB-6129	Misiones, Argentina
<i>Otus scops</i>	IPB-6251	Museum, Chur, Switzerland
<i>Otus usta</i>	IPB-6157	South America
<i>Phodilus badius</i>	IPB-6222	Zoo, Berlin
<i>Scotopelia peli</i>	IPB-6343	Zoo, Pretoria, South Africa
<i>Strix aluco</i>	IPB-6159	Zoo, Leipzig, Germany
<i>Strix uralensis</i>	IPB-6329	Finland
<i>Surnia ulula</i>	IPB-2593	Museum, Tromsø, Norway
<i>Tyto pratincola</i>	IPB-16499	Dominican Republic

calling at the same time as, and within 50 m of, a pair of the new owl). The calls of each owl species were digitised from a Sony DAT Walkman (TCD-D8) with an IBM microcomputer and were sampled at 44.1 kHz. Cool Edit Pro (Ver. 1.2) was used to produce all sonograms and to make all measurements. Three measurements of three call properties were made from each individual: dominant frequency (from the entire call), call duration (plus note durations in the Boobook) and call rate (only

1 measurement per species). Average values  $\pm$  standard deviation are presented for each individual and sonograms generated to compare the territorial songs of the new owl, *N. rudolfi* and (from a commercial audio tape: Buckingham and Jackson 1990) *N. novaeseelandiae*.

## Results and Discussion

### Phylogenetic relationships

A phylogenetic analysis that includes members of the genera *Otus*, *Bubo*, *Asio*, *Aegolius*, *Athene*, *Glaucidium*, *Surnia*, *Nyctea*, *Scotopelia*, *Ketupa*, *Strix*, *Phodilus*, *Tyto* and *Ninox* unequivocally places the unknown owl into the *Ninox* clade (Fig. 1). This placement is obtained in MP, NJ and ML tree reconstructions and supported by a bootstrap value of 100% (NJ). Thus, the new owl belongs to the genus *Ninox*. It differs from *N. novaeseelandiae* and *N. scutulata* by 8.2 and 9.1% nucleotide substitutions. Since our *Ninox* data set contains few representatives, we cannot determine to which other *Ninox* species it is most closely related. The phylogenetic relationships shown in Fig. 1 correspond with those published previously (Wink and Heidrich 1999).

### *Ninox sumbaensis*, sp. nov. (Figs 2–5)

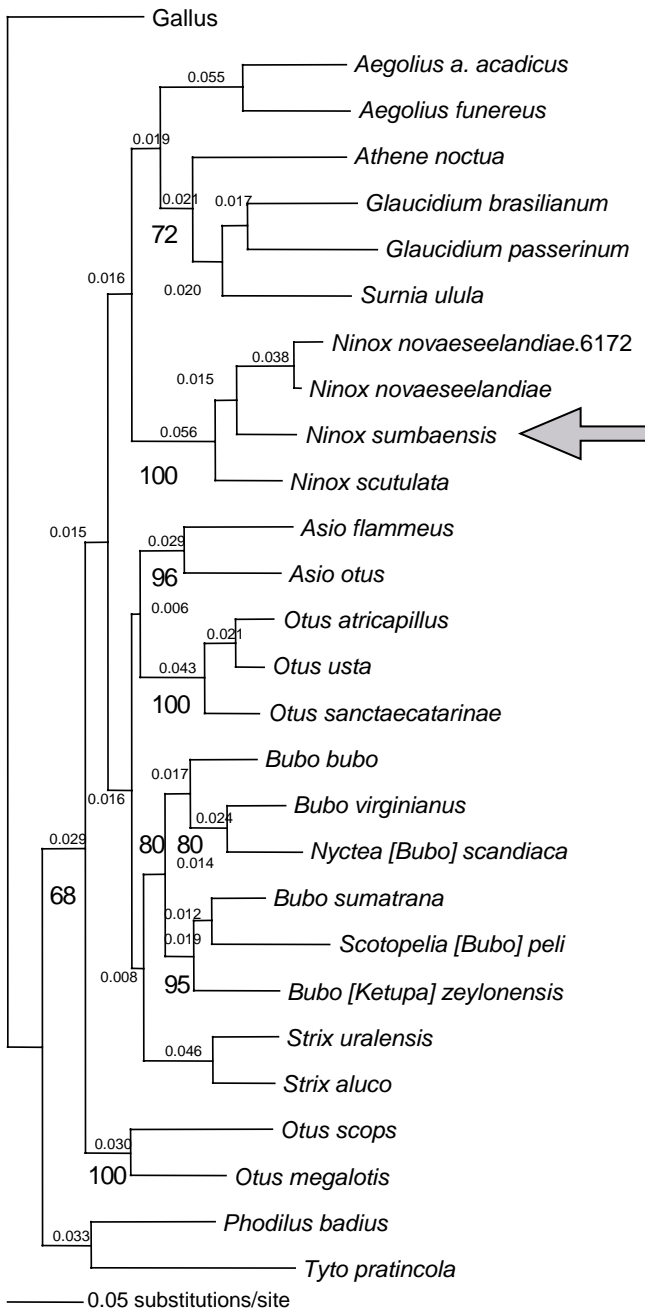
### Holotype

The adult specimen (sex unknown) was collected on the night of 30 December 2001, from degraded forest near Km 45 west of Waingapu, on the Lewa road, Sumba, Indonesia (approximately 9°44'S, 119°57'E) by a local bird hunter. The body was left with villagers on Sumba. Feathers and photographs are lodged at Heidelberg University (Accession No. IPB-20415).

### Description

Generic characters of the new owl that place it within *Ninox* are the indistinct facial disc (Fig. 5), the lack of ear tufts, and the bulbous cere with nostrils located frontally rather than at the sides (Fig. 3) (cf. König *et al.* 1999)

Descriptive notes of specific characters taken in the field for the holotype (see Figs 2, 3, 5) of the new Sumba owl were: crown — greyish with fine, close barring; face — grey, with prominent white eyebrows; face not flat, eyes slightly on sides of head, somewhat like an Australian Owllet-nightjar, *Aegotheles cristatus*; prominent beak, no prominent facial disk; ear tufts not apparent; bill — horn then yellow  $\frac{1}{3}$  to tip; eyes — yellow; underparts — throat rufous with dark vermiculations; lower breast white with fine dark vermiculations (chevrons); upperparts — light brown with fine widely spaced dark brown vermiculations; scapulars white; flight feathers — (underside) barred rufous and cream (upperside) barred rufous and dark brown; wings quite long; tarsi — feathered on front to toes then bristly type feathers on tops of toes; back of tarsi fairly bare; feet — yellow, claws yellow



**Fig. 1.** Phylogenetic relationships of the Little Sumba Hawk-Owl (maximum-likelihood phylogram). Distances (substitutions per site) are given in small letters above each furcation; bootstrap values above 65% (from a NJ analysis) are shown in larger and bold letters below each furcation. A = 0.265; C = 0.357; G = 0.135; T = 0.243; 439 characters are variable, and 363 are parsimony-informative.



**Fig. 2.** The holotype of *Ninox sumbaensis* collected near Km 45 on the Waingapu–Lewa road on 30 December 2001.



**Fig. 3.** The holotype of *Ninox sumbaensis* collected near Km 45 on the Waingapu–Lewa road on 30 December 2001.



**Table 2. Measurements of the type specimen of *Ninox sumbaensis* and of other *Ninox* species from the region**

Comparative data are from del Hoyo *et al.* (1999), König *et al.* (1999) and Johnstone and Darnell (1997). Linear measurements are in millimetres; mass is in grams

Species	Wing length	Tail length	Body length	Total mass
<i>N. sumbaensis</i>	176	99.5	230	90
<i>N. rudolfi</i>	227–243	145	300–360	
<i>N. scutulata</i>	176–245	98–142	280–320	172–227
<i>N. novaeseelandiae</i>	198–261	127–162	300–360	170–360
<i>N. n. rotiensis</i>	188	100	270	146
<i>N. squamipila</i>	190–241	135–157	250–360	210
<i>N. natalis</i>	178–199	117–124	260–290	130–190

with grey-black tips; pads of feet yellow; reversible outer toe; feet proportionately similar to *N. novaeseelandiae*; tail — 12 or 13 dark brown bars on a light rufous-brown background; tail very concave.

The owl had been moulting — on the right wing Primaries 1–3, and on the left wing Primaries 4 and 5 and Secondaries 9 and 10 were new. On the right wing Primary 5 was longest, then 7, then 6. On the left wing Primaries 6 and 7 were equal longest; 1 and 5 were missing. The ends of the primaries were nicked off as if broken or worn and there was some damage to Primary 8 on both wings as the bird hunter had tied these together to carry the bird. As other *Ninox* undergo a post-juvenile moult into adult-like plumage at several months old, but do not moult remiges and retrices until probably the end of their first year (Higgins 1999), our specimen was therefore at least a year old and in adult (or adult-like), not juvenile, plumage. Furthermore, on 24 December 2001 Olsen and Trost sighted a fledged, food-begging juvenile with two calling adults. It was lighter reddish and with no visible wavy barring and no chevrons on its underparts compared with the specimen and the adult pair.

#### Diagnosis

For tail and wing there was little overlap between measurements for this specimen and those for other *Ninox* known for this region; for body length and mass there was no overlap

(Table 2). Additional measurements (in millimetres) for the new Sumba owl are: culmen (from cere) 12.4; mid-toe 21.5; sternum 25.9; head width (measured at the widest point) 30.2; talon span 43.0; tarsi 34.5; girth of body (measured just behind the humerus) 13.7; wingspan 570; radius–ulna 62.4; tibia 60.0; tip of beak to back of head 46.3.

The new owl was much smaller and with proportionately longer wings than the sympatric *N. rudolfi* (Fig. 6), the latter also being distinguished by its white-spotted crown and mantle, white underparts with heavy reddish-brown barring, closely white-barred upperparts including scapulars, plain white throat, and brown, not yellow, irises (del Hoyo *et al.* 1999 incorrectly suggested that *N. rudolfi* has yellow irises) (König *et al.* 1999; Olsen and Trost, unpublished).

The wing:tail ratio of the new owl is 1.8 versus 1.6 for *N. rudolfi* (from Table 3); these proportions are similar, respectively, to mainland Australian *N. novaeseelandiae* (1.8) versus *N. n. novaeseelandiae* (1.6) and *N. natalis* (1.6) (data from Higgins 1999).

#### Analysis of vocalisations

The voice of the new owl was a single note, a monosyllabic whistle or ‘hoot’ repeated about every 2.5 seconds, quite unlike the repeated ‘cluck-cluck-cluck’ uttered about 2.3 times per second by the endemic, much larger, *N. rudolfi* (Fig. 7, Table 3), the only other owl that we saw and heard in the same forest. The pairs of *N. rudolfi* never used a disyllabic call, they only used the ‘cluck-cluck-cluck’ call in reply to playback; that is, this call appeared to be their territorial song and not equivalent to the ‘por’ call of *N. novaeseelandiae*, as suggested by del Hoyo *et al.* (1999). In response to playback of its own call, the new owl exclusively used a monosyllabic ‘hoot’, very different from the call of *N. rudolfi*, and this monosyllabic ‘hoot’ appeared to be the species’ territorial song. As the territorial song of the new owl was monosyllabic, it was unlike the disyllabic notes made by most other *Ninox* species; for example, *N. novaeseelandiae*, which utters its call about every 3 seconds (Fig. 7, Table 3) or those owls listed in Table 3 (Coates and Bishop 1997; König *et al.* 1999; Higgins 1999; King and Yong 2001). The three owls compared in Table 3 have differing dominant frequencies,

**Table 3. Call properties for three owl species**

Values = mean  $\pm$  standard deviation. From audio track of video recorded with a Panasonic NV-MX300 digital video camera of (a) *N. rudolfi*, near Melolo on 28 December 2001, (b) *N. sumbaensis* — one of the six adults we observed in forest south of Km 49 (from Waingapu) on the Lewa road on 2 January 2002 (recorded by J. Olsen and S. Trost after owls responded to playback), and (c) *N. novaeseelandiae* from recordings edited by Buckingham and Jackson (1990)

Species	Dominant frequency (Hz)	Call duration (ms)	Duration of 1st note (ms)	Duration of 2nd note (ms)	Call rate (calls/min)
<i>N. rudolfi</i>	714.8 $\pm$ 23.6	38.6 $\pm$ 2.6	–	–	138.0
<i>N. sumbaensis</i>	871.8 $\pm$ 21.5	383.0 $\pm$ 26.1	–	–	24.0
<i>N. novaeseelandiae</i>	846.8 $\pm$ 35.1	609.7 $\pm$ 49.2	222.0 $\pm$ 21.9	222.0 $\pm$ 21.9	18.0



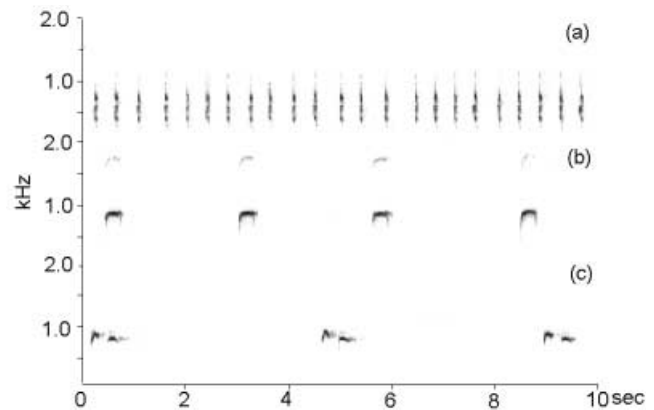


**Fig. 5.** One of the six adult *Ninox sumbaensis* observed in forest south of Km 49 (from Waingapu) on the Lewa road, photographed on 2 January 2002.



**Fig. 6.** *N. rudolfi* photographed near Melolo on 1 January 2002.

call durations, and call rates. There is no similar single-note 'hoot' vocalisation for any known *Ninox*. The single note uttered by the New Britain Hawk-Owl, *N. odiosa*, is rapidly repeated (König *et al.* 1999; del Hoyo *et al.* 1999). Although del Hoyo *et al.* (1999) argue that the Solomon Hawk-Owl, *N. jacquinoti*, has a repeated single 'hoot', they cite no recordings whereas König *et al.* (1999) based their description of a double-note song on tape recordings by D. Bishop. Although del Hoyo *et al.* (1999) describe the call of the Philippine Hawk-Owl, *N. philippensis*, as a repeated single-note 'who', König *et al.* (1999), on the basis of tape recording by



**Fig. 7.** Sonagrams from video taken with a Panasonic NV-MX300 digital video camera of (a) *N. rudolfi*, near Melolo on 28 December 2001, (b) *N. sumbaensis* (one of the six adults observed in forest south of Km 49 (from Waingapu) on the Lewa road on 2 January 2002 (recorded by J. Olsen and S. Trost after owls responded to playback)), and (c) *N. novaeseelandiae* from recordings edited by Buckingham and Jackson (1990).

P. Morris, describe the call as becoming disyllabic and then climaxing as a three- or four-note call, quite unlike the call of the new owl.

#### Etymology

*Ninox sumbaensis*, the Little Sumba Hawk-Owl, is named for the island of Sumba, where it is the smallest known hawk-owl.

#### Distribution

Known only from the island of Sumba near 9°44'S, 119°57'E.

#### Ecology

The species is known only from primary and secondary forest around 600 m on Sumba. Unlike *N. rudolfi* (Olsen and Trost, unpublished), these owls did not perch or hunt in more open areas outside the forest, but appeared to be limited to remnant forest patches on Sumba. Furthermore, we saw *N. rudolfi* in coastal swamps near Melolo but not the new owl, though we broadcast its call there. The conservation status of this owl has yet to be determined, but the species might be threatened and we propose it as Data Deficient.

Though its small size and uncharacteristic voice led earlier observers to classify this owl as an *Otus* species, its phylogenetic analysis unequivocally places it within *Ninox*. There is no similar voice for any known *Ninox*, and no overlap for more than two of the four morphological measurements for *Ninox* in Table 2 (König *et al.* 1999).

Owls in this region, including Flores, are poorly known (see Widodo *et al.* 1999) and it is possible that other bird species remain undescribed. The discovery of a new owl on Sumba continues the trend of recent discovery of new *Ninox*

species or subspecies in Wallacea (Johnstone and Darnell 1997; Rasmussen 1999). It is critical to describe new species, determine the conservation status of new and known species, and conserve the unique yet increasingly degraded forests on these islands.

### Acknowledgments

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