Illustration. Davina Falcão (*Discoglossus galganoi*)
A new brown frog of the genus *Rana* from Japan (Anura: Ranidae) revealed by cytological and bioacoustic studies

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The Japanese brown frog from Nagano Prefecture, previously reported as *Rana tagoi* with 2n = 28 chromosomes, is described as a new species. The new species differs only slightly in morphology from topotypic *R. tagoi tagoi*. It has a greater snout-nostril length, smaller fourth finger length, smaller fourth and fifth toe lengths (all relative to snout-vent length) and a narrower web, but is practically indistinguishable from a neighboring *R. tagoi tagoi* population. However, distinct acoustic differences in their advertisement calls clearly separate the two taxa, and may serve as an effective pre-mating isolation mechanism. Karyotypic difference between the new species and *R. tagoi tagoi* results in nearly complete hybrid sterility. Because the new species is nested within a clade comprising neighboring *R. tagoi* populations, the new species must have originated rather recently by chromosome reconstruction, and subsequent acoustic divergence would have facilitated conspecific mating. This species is an example of an anuran sibling species that is unrelated to molecular phylogeny.

INTRODUCTION

Tago’s brown frog *Rana tagoi* Okada, 1928 is endemic to Japan in the main islands of Honshu, Shikoku and Kyushu, and in neighboring small islands (Maeda & Matsui, 1999). Three subspecies are currently recognized, *R. tagoi tagoi* distributed in Honshu, Shikoku and Kyushu, *R. tagoi yakushimensis* Nakatani & Okada, 1966, in Yaku Island, and *R. tagoi okiensis* Daito, 1969, in Oki Islands. A closely related species, *R. sakuraii*, was described by Matsui & Matsui (1990) from Tokyo Prefecture and was subsequently found in mountain streams of central and western Honshu (Maeda & Matsui, 1999). In contrast to *R. tagoi*, which breeds in the underground waters or seepages of small mountain streams, *R. sakuraii* breeds in deep waters in relatively large mountain streams. However, the phylogenetic relationships among these taxa are complicated and do not fit the taxonomic implications.

Nishioka *et al.* (1987) analyzed by electrophoresis 14 enzymes and two serum proteins in seven populations of *R. tagoi*. Their dendrogram based on genetic distance indicated that three populations of *R. tagoi tagoi* from western Honshu and Shikoku formed a cluster, three populations from Kyushu (*R. tagoi tagoi*) and Oki (*R. tagoi okiensis*) formed another cluster, and *R. tagoi yakushimensis* formed a lineage outside these two clusters. Tanaka *et al.* (1994, 1996) compared the nucleotide sequences of the mitochondrial cytochrome b gene in Japanese brown frogs, including subspecies of *R. tagoi* and *R. sakuraii*. Their analysis indicated a close relationship between *R. t. tagoi* and *R. t. yakushimensis*. *Rana sakuraii* was separated from the cluster of these two subspecies, while *R. t. okiensis* was most remotely related to all three taxa. Eto *et al.* (2012) extensively analyzed mitochondrial 16S rRNA and ND1 (NADH dehydrogenase subunit 1) genes and recognized two clades in this group. One clade
included nine clusters in which the western R. sakuraii populations and R. t. yakushimensis constituted two separate clusters, whereas the eastern R. sakuraii populations constituted a cluster together with the R. t. tagoi populations from eastern Honshu. The other six clusters were composed of R. t. tagoi from north to central Honshu, Shikoku and Kyushu. The other clade included a cluster composed of R. t. okiensis and two other clusters of R. t. tagoi from western Honshu. These observations contradict each other and are inconsistent with the taxonomic implications, especially regarding the species status of R. sakuraii. Eto et al. (2012), therefore, suggested the existence of many cryptic species. Eto et al. (2013) clarified the discrepancy between molecular phylogeny based on mitochondrial and nuclear DNA sequencing.

Nishioka et al. (1987) performed hybridization experiments using females of R. t. tagoi and males of R. t. okiensis and R. t. yakushimensis, and demonstrated weak hybrid inviability in these matings. Artificial hybridization between R. sakuraii females and R. t. tagoi males, and a back-cross experiment using R. sakuraii females and the hybrid males, indicated that the two species are reproductively isolated by incomplete hybrid inviability and hybrid sterility (Daito et al., 1998b). In contrast, all hybrids of R. t. tagoi females and R. t. okiensis males became males and were completely sterile (Daito et al., 1998a). Rana t. okiensis was nearly completely isolated from R. sakuraii by hybrid sterility (Daito, 1999). Based on the biological species concept, R. t. okiensis and R. sakuraii are valid species irrespective of their conflicting molecular relationships. Because of their mountain habitats, it is highly probable that each population of the Rana tagoi complex has long been isolated from neighboring populations and consequently they have diverged considerably. This suggests the existence of many cryptic species.

Distinct divergences in call structure have been reported among populations of R. t. tagoi. Daito & Kawakami (1992) compared the call structures of six populations from Honshu, Shikoku and Kyushu. The advertisement calls of R. tagoi are composed of 1-8 notes (pulse groups), which are classified into two types, one with rapidly repeating pulses and the other with slowly repeating pulses. The proportions of the two kinds of notes, and the total number of notes, differed among populations. Matsuo et al. (2011) reported that the calls of the Goto population in Nagasaki Prefecture consisted of many short notes, in contrast with a few long notes in the other populations of Kyushu. Ryuzaki et al. (unpublished) clarified the call structure of R. t. tagoi from its type locality area in Kamitakara, Gifu Prefecture and showed that the Hakuba population in Nagano Prefecture and the Kurama population in Kyoto Prefecture had quite different call structures, suggesting the existence of cryptic species. In fact, the acoustic differences among different populations of R. t. tagoi were much greater than the differences between subspecies of R. tagoi.

Daito (1967) first reported the chromosome number of R. t. tagoi as 2 n = 26. *Rana t. yakushimensis, R. t. okiensis* and R. sakuraii also have 2 n = 26 karyotypes (Daito, 1981; Maeda & Matsui, 1999). Ryuzaki et al. (1999) described the karyotype of R. t. tagoi more precisely as consisting of five large and eight small chromosome pairs with XX/XY sex determination. Although a total of 81 populations distributed throughout Japan possessed the 2 n = 26 karyotype, a population in Mt. Chausu, Nagano Prefecture was found to have a karyotype of 2 n = 28 (Ryuzaki et al., 2006). Two telocentric pairs specific to the 2 n = 28 karyotype were believed to be derived from central fission of one of the large chromosome pairs (pair no. 1) of the 2 n = 26 karyotype.

This strongly suggests that the Mt. Chausu population is a new species distinct from R. tagoi. In the present study, we compare the morphological and acoustic features of the Mt. Chausu population with those of the neighboring populations, and recognize the former population as a new species.

**MATERIALS AND METHODS**

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Specimens of the putative new species were collected at Mt. Chausu, Neba-mura, Nagano Prefecture at about 1200 m above sea level (asl) on 15 and 20 October 2013 and on 10 May 2014, and specimens of R. t. tagoi were collected at Kamitakara, Takayama, Gifu Prefecture, about 900 m asl on 16 and 17 November 2013, and at Mt. Ena, Achi-mura, Nagano Prefecture, at about 1500 m asl on 19 October 2013 (fig. 1). Kamitakara is the type locality of R. t. tagoi (Shibata, 1988).

Twenty seven body measurements were made using digital calipers, to the nearest 0.1 mm: snout to vent length (SVL); head length (HL); head width (HW); snout to nostril distance (S-N); inter-nostril distance (N-N); nostril to eye distance (N-E); horizontal eye diameter (ED); inter-orbital distance between inner borders of the upper eyelids (E-E); eyelid width (ELW); horizontal tympanum diameter (TD); forearm and hand length (FHL); hand length (HAL); lengths of the four fingers (F1-F4); hindlimb length (HLL); femur length (FEL); tibia length...
(TIL); tarsus and foot length (TFL); foot length (FOL); lengths of the five toes (T1-T5); and inner metatarsal tubercle length (IMT). Examined specimens were deposited in the Institute for Amphibian Biology, Hiroshima University (IABHU). Statistic analyses were performed using SPSS (15.0J) software (SPSS Japan Inc.). The Mann-Whitney U test was used to test for significant differences in these measurements among the populations.

Advertisement calls of the new species were recorded at Mt. Chausu on 13 May 2004 and those of *R. t. tagoi* at Mt. Ena on 1 June 2006 using a cassette tape recorder (Panasonic RX-M40). Sound spectrograms were depicted using Avisoft-SASLab Light software (Avisoft Bioacoustics) with a Hamming window.

**SYSTEMATICS**

*Rana neba* sp. nov.

**Synonymy.** *Rana tagoi* (2 n = 28): Ryuzaki et al. (2006).

**Holotype.** IABHU F2553 (fig. 2). Adult male collected in Mt. Chausu, Neba-mura, Nagano Prefecture at about 1200 m asl on 20 October 2013 by M. Kumagai & Y. Hasegawa. SVL 45.1 mm.

**Paratyptes.** IABHU F2539, F2540 (adult females collected in Mt. Chausu by M. Kumagai on 15 October 2013), F2549-F2552, F2554 (adult males collected in Mt. Chausu by M. Kumagai & Y. Hasegawa on 20 October 2013), F2569-F2575 (adult males collected in Mt. Chausu by M. Kumagai on 10 May 2014), F2576, F2577 (adult females collected in Mt. Chausu by M. Kumagai on 10 May 2014).

**Diagnosis.** Medium-sized brown frog with SVL 37.7-48.3 mm. A sibling species of *R. tagoi*; the morphological differences between the two species are very slight. Differs from totopotypic *R. t. tagoi* in larger S-N, smaller F4, smaller T4 and T5 (all relative to SVL), and narrower web. Chromosome number 2 n = 28, which is unusual for a member of the genus *Rana*; two telocentric pairs probably derived from central fission of a bi-armed pair. Advertisement calls containing high-frequency bands easily distinguished from low-pitched calls of *R. t. tagoi*.
**Description of holotype (measurements in mm).** Vomerine teeth in two oblique lines between choanae; tongue wide, tip bifurcated.

Head wider than long (HL 12.7, HW 16.7); snout moderately pointed; nostril nearer to eye than to tip of snout (S-N 3.9, N-E 3.6); loreal region concave; canthus rostralis blunt; internarial distance larger than inter-orbital distance and eye diameter (N-N 5.7, E-E 3.7, ED 5.1); tympanum distinct, about 2/3 eye diameter (TD 3.4).

Finger free, tip slightly rounded; finger length F2 < F1 < F4 < F3 (F1 4.4, F2 4.0, F3 7.5, F4 4.9); subarticular tubercles well developed; two palmar tubercles distinct; inner side of arm extremely thickened; two nuptial pads, one on inner side of first finger base and the other on outer to upper side of middle part of first finger; first finger bent inward at middle.

Hind limb about 1.6 times SVL (HLL 71.6); tibia slightly larger than femur (FEL 21.0, TIL 22.9); tibio-tarsal articulations overlap slightly when legs folded at right angle to the body axis, and reach the eye when hind limb is stretched forward along the body axis; toe tip small, rounded; toe lengths T1 < T2 < T5 < T3 < T4 (T1 3.4, T2 7.4, T3 10.7, T4 14.3, T5 10.0); subarticular tubercles moderate; web not wide; inner metatarsal tubercle moderate; no outer metatarsal tubercle.

Dorsum smooth; lateral and ventral surface and posterior side of thigh finely granulated; supra-tympanic fold thin; a pair of thin curved dorsolateral ridges from behind tympanum to groin.

**Colour in life.** Reddish brown on upper side with inverted black triangular lines between the eyelids; inverted black V mark behind the triangle; black transverse bands on outer surface of forearm and on upper surfaces of thigh, tibia and foot; ventral side white with many small grayish speckles on throat to venter; lateral side of canthus rostralis and tympanic region black; upper edge of upper jaw white; nuptial pad whitish.

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**Figure 2.** Holotype of *Rana neba* sp. nov. (LABHU F2553): Dorsal view (A) and ventral view (B). SVL 45.1 mm.

**Figure 3.** Scatter plots of individual scores of principal components PC1 and PC2 (left) and those of PC2 and PC3 (right). Circle: *Rana neba*. Square: Kamitakara *R. t. tagoi*. Triangle: Mt. Ena *R. t. tagoi*. Hollow and dotted marks indicate males and females, respectively.
Variation. Measurements are summarized in tab. 1. Sexual dimorphism in size was not obvious, except for the secondary sex characters in males, i.e., nuptial pad and thickness of forearm. Palmar tubercles were more developed in males than in females. All males had an inverse V-shaped black mark on the back just behind head, and an inverse triangular shaped black mark between eyelids, as in the holotype. Dorsal surfaces of the two females were uniformly reddish brown without distinct black markings, and the cross bars on the upper surfaces of thigh, tibia and tarsus were far less conspicuous than in males. Dorsal surfaces of the other two females were finely mottled with reddish and yellowish brown. Mottling pattern on the ventral surface was variable in both males and females, from very faint fine mottling to rather bold black markings.

Comparisons with other species. *Rana tagoi tagoi*: in a principal component analysis using 27 body part measurements on *R. neba* and two *R. t. tagoi* populations (fig. 3), *R. neba* and *R. t. tagoi* from Mt. Ena overlapped extensively, whereas topotypic *R. t. tagoi* from Kamitakara was almost completely separated from the other two. Body ratios relative to SVL and eight other ratios between selected measurements (tab. 2) were similar between *R. neba* and Mt. Ena population of *R. t. tagoi*, but topotypic *R. t. tagoi* differed from the former two in more than six ratios. The ranges in S-N/SVL, F4/SVL, T4/SVL and T5/SVL in *R. neba* overlapped only slightly with those in topotypic *R. t. tagoi*. Principal component analysis using body ratios gave similar results to those in fig. 3.

The hand of *R. neba* male was similar to that of *R. t. tagoi* male (fig. 4. A-C). Probably because of the development of the nuptial pad on the dorsal surface of the first finger, the finger tip of males was bent toward the

**Table 1. Measurements of *Rana neba* specimens (mm).**

<table>
<thead>
<tr>
<th></th>
<th>Male (n = 14)</th>
<th>Female (n = 4)</th>
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<tr>
<td></td>
<td>min-max</td>
<td>median</td>
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<tr>
<td>SVL</td>
<td>37.7-48.3</td>
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<tr>
<td>HL</td>
<td>10.1-15.1</td>
<td>12.6</td>
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<tr>
<td>HW</td>
<td>13.4-17.2</td>
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<tr>
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<tr>
<td>N-E</td>
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<td>3.05</td>
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<tr>
<td>F1</td>
<td>3.5-5.7</td>
<td>4.7</td>
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<tr>
<td>F2</td>
<td>2.7-4.5</td>
<td>3.95</td>
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<tr>
<td>F3</td>
<td>5.5-7.6</td>
<td>6.4</td>
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<tr>
<td>F4</td>
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<tr>
<td>FEL</td>
<td>18.3-24.0</td>
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<td>TIL</td>
<td>19.3-25.4</td>
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<tr>
<td>TFL</td>
<td>28.2-34.6</td>
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<td>FOL</td>
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<td>T1</td>
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<td>T3</td>
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In conclusion, *R. neba* differs from topotypic *R. t. tagoi* in some morphometric characters but is nearly identical to neighboring Mt. Ena population of *R. t. tagoi*.
The advertisement call of *Rana neba* was composed of a series of notes (pulse groups). In the two-note calls (fig. 5.A, tab. 3), the first note was short and involved low frequency bands, whereas the second note was longer, consisting of many pulses that involved a high frequency band at about 5 kHz and less conspicuous band at about 8 kHz. The three-note calls (fig. 5.B, tab. 3) were most frequently heard and thus were considered representative calls of *R. neba*. As in the two-note call, the second note was long and involved high frequency bands. Two distinct call types were observed in the four-note calls, one with a short note and the other with an extremely long lasting note (fig. 5.C, tab. 3). In the latter, the last note had a distinctly slow pulse repetition rate and was composed of only a low frequency band. The second note had high frequency bands as in the two-note and three-note calls. In addition to the above calls, there were calls with 5 to 11 successive notes repeating at 3.3 notes/s. In these, every note was essentially identical to the second note of the above mentioned calls, in length and in the presence of high frequency bands. The dominant frequency bands were at about 1.7 kHz and 1.3 kHz, and a distinct high frequency band was present at about 5 kHz.

Call structures of *R. t. tagoi* were studied by Kuramoto (1980), Daito & Kawakami (1992) and Ryuzaki et al. (unpublished). The calls of topotypic *R. t. tagoi* (Ryuzaki et al., unpublished) were composed of 1-5 notes and, in multi-note calls, the last note was longer, pulse repetition rate was slower and frequency composition was lower than in the other note(s). The notes other than the last note in multi-note calls were similar to each other. The call structure of *R. t. tagoi* from Mt. Ena agreed well with that of topotypic *R. t. tagoi* (fig. 5.D). The fundamental and most dominant frequency bands of Mt. Ena population of *R. t. tagoi*, as well as those of topotypic *R. t. tagoi*, were at 0.4-0.5 kHz in contrast to 1.7 kHz and 1.3 kHz of *R. neba*. Thus, the two species can be easily discriminated by their calls, which are low-pitched in *R. t. tagoi* and high-pitched in *R. neba*. Because intermediate calls have not been recorded where the two species coexist, the differences in call structure may serve as a pre-mating isolating mechanism.

**Figure 4.** Hand and foot of male *Rana neba* (A and D: holotype), *R. t. tagoi* from Kamitakara (B and E: LABHU F2562) and *R. t. tagoi* from Mt. Ena (C and F: LABHU F2546). Not to scale.
**Chromosomes.** *Rana neba* is karyologically distinct in having $2n = 28$ chromosomes in contrast to the $2n = 26$ karyotype of *R. t. tagoi* (Ryuzaki *et al*., 2006). The XX/XY type of sex determination was confirmed in *R. neba* (Ryuzaki *et al*., 2006), whereas such a system was not observed in topotypic *R. t. tagoi* which had $2n = 26$ chromosomes (Ryuzaki, unpublished).

Between male *Rana neba* and female *R. t. tagoi*, viable hybrids with $2n = 27$ chromosomes were produced (Ryuzaki *et al*., 2006). From 500 hybrid larvae, we obtained 8 females and 112 males. Spermatogenesis in the hybrid males became abnormal because of the difficulty in formation of normal bivalent chromosomes at meiosis (Ryuzaki, unpublished); thus the post-isolating mechanism is nearly complete.

In the extensive hybridization experiments using brown frogs with $2n = 26$ and those with $2n = 24$ chromosomes, Kawamura *et al.* (1981) observed a remarkable male dominance in the sex ratio of hybrids and abnormal spermatogenesis in the hybrid males. The same was true for the hybrids in the *R. tagoi* complex (Daito *et al*., 1998a, b; Daito, 1999).

**Phylogenetic relationships.** According to Eto *et al.* (2012), the Neba population is nested in a group (A-6) together with populations from adjacent Prefectures (Yamanashi, Shizuoka, Aichi and Mie) and the Topotypic Takayama (= Kamitakara) population belonged to a different group (A-1a) comprising populations from a wide range of locations from northern Honshu to the northern part of central Honshu. We confirmed by mitochondrial cytochrome b gene sequencing that the mean nucleotide differences were 4.5% between *R. neba* and Mt. Ena.}

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**Figure 5.** Sound spectrograms of advertisement calls of *Rana neba* (A. two-note call; B. three-note call; C. four-note call with long last note) and *R. t. tagoi* from Mt. Ena (D).
Ena. R. t. tagoi, 5.0 % between R. neba and topotypic R. t. tagoi, and 3.9 % between Mt. Ena and topotypic R. t. tagoi (Hasan et al., unpublished). Thus, the molecular relationships do not agree with morphological comparisons. The 2n = 28 karyotype and the high-pitched advertisement call have not been recorded in the Mt. Ena population despite careful examination, indicating that these characteristics are unique to R. neba.

Rana neba is morphologically very similar to R. t. tagoi but nearly completely isolated from the latter by the karyotypic and acoustic differences, thus it is a sibling species as defined by Mayr (1963). Structural changes of chromosomes which result in abnormal gametogenesis can occur without changes in nucleotide sequences, thus the species like R. neba cannot be detected by molecular phylogenetic means.

**Distribution.** Rana neba is currently known to occur in the southern part of Nagano Prefecture and the adjacent parts of Aichi and Shizuoka Prefectures, as follows. Nagano Prefecture: Neba-mura, Urugi-mura, Tenryu-mura, Anan-cho, Hiraya-mura, Achi-mura, Shimojo-mura, Takagi-mura, and Oshima-mura. Aichi Prefecture: Toyone-mura, Shitara-cho, and Asuke-cho in Toyota. Shizuoka Prefecture: Inasa-cho in Hamamatsu. All of these localities are within 40 km from Mt. Chausu.

**Etymology.** The specific name is derived from the type locality, Neba-mura, Nagano Prefecture. 


**ACKNOWLEDGEMENTS**

We thank M. Kumagai and S. Kobayashi for their kind assistance in the field.

**LITERATURE CITED**


Notes on tree frogs, *Nyctimystes* species (Anura: Hylidae) of New Guinea; the *Nyctimystes papua* species group

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The definition of the *Nyctimystes papua* Species Group, as created by Zweifel (1983), is further elaborated and the differences between *N. papua* and *N. disruptus*, including morphology and geography, are discussed. The possibility that *Nyctimystes disruptus*, as currently recognised, includes more than one species, with either green or brown eyes, is also investigated. The diagnostics of *Nyctimystes oktediensis* are reviewed leading to the conclusion that it is not distinct from *N. disruptus*.

INTRODUCTION

Two genera of tree frogs (Hylidae) occur in the Papuan Region, *Litoria* and *Nyctimystes*. *Nyctimystes* species are distinguished from *Litoria* by a vertical (as opposed to horizontal) pupil and a pattern of lines on the clear part of the lower eyelid. The monophyly of *Nyctimystes* is currently in doubt and recent authors (Frost et al., 2006; Rosauer et al., 2009; Wiens et al., 2010), on molecular grounds, have placed the species of *Nyctimystes* within *Litoria* as a sister group to *Litoria infrafrenata*. However, not all authors have accepted this significant reclassification (Kraus, 2012, 2013; Menzies, 2014a, 2014b) and have retained *Nyctimystes* as a valid genus.

If the somewhat aberrant *Nyctimystes rueppelli* from Halmahera is excluded, *Nyctimystes* species are restricted to the island of New Guinea and the d’Entrecasteaux and Louisiade islands to the south-east. Some species have been placed into groups sharing similar morphology such as the *Nyctimystes cheesmanae* species group (Menzies, 1976), the *Nyctimystes papua* species group (Zweifel, 1983) and the *Nyctimystes narinosus* species group (Menzies, 2014b), but the monophyly of *Nyctimystes* has not been conclusively demonstrated.

The *Nyctimystes papua* group of species is distinguished from other *Nyctimystes* species by having an eyelid reticulum that leaves the posterior portion of the eyelid clear (Zweifel, 1958, figure 19a; Menzies, 2006, figure 17e) or with a vestigial venation. Prior to 1963, all *Nyctimystes* with an incomplete palpebral venation had been identified as *N. papua*, giving it a very wide distribution through the central and south-eastern mountains of New Guinea (e.g. Zweifel, 1958, figure 13). Tyler (1963) then described *Nyctimystes disruptus* from the Schrader Mountains of central Papua New Guinea and distinguished it from *N. papua* by “more extensive webbing between the fingers and a palpebral venation which, although disrupted, is far more pronounced than in that species”. His illustration (Tyler, 1963 figure 1) shows an eyelid pattern of short, oblique, broken lines more or less absent from the posterior quarter of the eyelid. There is no mention of eye colour in the original description nor has any author referred to comparative body size in *N. papua* and *N. disruptus*. By 1983, it had been realised that frogs previously identified as *N. papua* from the central highlands of Papua New Guinea were, in fact, *N. disruptus* and that “the only specimens that can be referred with confidence [to *N. papua*] were those of the syntype series” (Zweifel, 1983). In that paper, Zweifel designated a lectotype for *N. papua*, formally recognised a ‘*Nyctimystes papua* species group’ and described two new species of that group, *N. trachydermis* and *N. tyleri*. The only other
relevant discovery has been the description of the new species, *Nyctimystes oktediensis* (Richards & Johnson, 1993) distinguished from *N. disrupta* by, inter alia, iris colour.

The precise type locality for *Nyctimystes papua* is not known. The collector, A.S. Anthony, collecting for Lord Rothschild, had “his final camp on Mt. Victoria and Mt. Knutsford” between April and June 1896 (Wichmann, 1912). Mt. Knutsford is about 16 km north of Mt. Victoria. Mt. Victoria is the highest point in the Owen Stanley Mountain Range which forms the backbone of the south-eastern peninsula of New Guinea. To my knowledge, no one has attempted to trace Anthony’s route and no new material has been collected in the immediate region. However, frogs that agree well with the lectotype of *N. papua* have been collected in several locations in the Owen Stanley Mountains, including Mt. Albert Edward, about 50 km to the north of Mt. Victoria, Woitape 70 km further west and the vicinity of Mt. Dayman, 200 km to the south-east (listed in the species account). It is now possible to give a more information on *Nyctimystes papua* and its allies and their distribution.

**MATERIALS AND METHODS**

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Standard measurements were taken as follows: body length, male or female (HBm, HBF) is taken from the tip of the snout to the ‘bump’ caused by the distal end of the urostyle. This gives a slightly shorter measurement than the more common snout-vent length (SVL) and consequently, a slightly longer tibial/body length ratio. If SVL measurements are taken from the literature for comparative purposes, they are converted to HB by a factor of 0.952 which I derived by comparing both measurements in a batch of *Nyctimystes disruptus* from a single locality. Tibial

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**Figure 1.** Distribution of *Nyctimystes papua* (spots), *N. disruptus* (stars) and *N. oktediensis* (cross). Not all known localities for *N. disruptus* are shown. 1 Mistkamp, Idenburgh River; 2 Star Mountains localities; 3 Western Highlands localities; 4 Southern Highlands localities; 5 Schrader Mountains; 6 Eastern Highlands and Simbu localities; 7 Huon Mountains localities; 8 Kratke Mts. (Wonenara); 9 Mt. Albert Edward and Woitape; 10 Mt. Victoria, Efogi and Myola; 11 Mt. Dayman (Agaun and Bonenau).
length (TL) is the external measurement with the joints held at right angles. Head length is taken from the tip of the snout to the angle of the jaws and head width (HW) is measured at mid-tympanic level. Eye-naris distance (EN) is from anterior rim of the orbit to mid-point of the nostril and inter-narial distance is between mid-points of the nostrils. Eye diameter (EY) and tympanic diameter (TY) are horizontal measurements. F3d and T4d are horizontal widths of the 3rd finger and 4th toe disks.

The statistical analyses used were taken from the JMP statistics package (SAS Inc. Version 4.0.3) for t-Tests and the SPSS package, version 20 (IBM SPSS Corporation, 2014) for multivariate analyses. The use of “significant” in the results indicates a probability of 0.05 or better. Details of the analyses are placed in appendix one.

Museum specimens referred to have the following abbreviations: AMNH for the American Museum of Natural History, New York, AMS for the Australian Museum Sydney, BMNH for the Natural History Museum, London, MZB for the Indonesian Zoology Museum, Cibinong, QMJ for the Queensland Museum, Brisbane, NML for Naturalis, Leiden, SAMA for the South Australian Museum, Adelaide and UPNG for the University of Papua New Guinea, Port Moresby.

All specimen localities are in Papua New Guinea or the Indonesian Province of Papua and provincial abbreviations are as follows: CenP, Central Province; EHP, Eastern Highlands Province; EngP, Enga Province; MadP, Madang Province; MBP, Milne Bay Province; MorP, Morobe Province; NorP, Northern Province.; PapP, Papua (Indonesia); SanP, Sadaun (West Sepik) Province; SimP, Simbu Province; WesP, Western (Fly River) Province; WHP, Western Highlands Province. Provinical boundaries have changed over the years and those given here are current, not always as in the original description. Fig. 1 indicates the localities of all places from where material originated and a list, with geographical coordinates, is included in Appendix 2.

RESULTS

The Nyctimystes papua species group

As diagnosed by Zweifel (1983) this group of species is distinguished by a “palpebral venation sparse or at least much reduced compared with other Nyctimystes, males lacking vocal sac; snout relatively high, short and rounded; EN/IN ratio 1.20 or less; outer fingers one half or less webbed; size moderate to large (males 50 to 80 mm SV).” To this, I can add the coloration, which, on the dorsum, consists of a blotchy mottle of dark on a lighter background which is brown or often with a purplish or greenish tinge. Although males in this group appear to
have no vocal sacs this does not necessarily mean that they have no voice as one specimen, in captivity, made a clucking sound, rather like a chicken.

Zweifel did not suggest monophyly for the *Nyctimystes papua* species group as there was no evidence to show that the shared characters were attributable to common ancestry. The current content of this group is five species, *N. papua* (Boulenger, 1897), *N. disruptus* Tyler, 1963, *N. trachydermis* Zweifel, 1983, *N. tyleri* Zweifel, 1983, *N. oktediensis* Richards & Johnston, 1993 and the distribution of *Nyctimystes disruptus* and *N. papua* in New Guinea is shown in fig. 1. *Nyctimystes trachydermis* and *N. tyleri* are much larger than the other species (fig. 2) and are not included in subsequent statistical analyses.

*Nyctimystes papua* (Boulenger)

*Nyctimantis papua*, Boulenger 1897, 12.
*Nyctimystes papua*, Stejneger 1916, 85.
*Hyla papua* Noble, 1931, 513.
*Litoria papua*, Frost et al., 2006, 362.

**Lectotype.** BMNH 1896.10.31.50 collected by A.S. Anthony on Mt. Victoria, Central Province of Papua New Guinea, between April and June 1896, designated as lectotype by Zweifel (1983). The exact altitude is unknown but as Anthony spent several weeks in the area it is likely that he established camps at different altitudes between the main peak of Mt. Victoria and Mt. Knutsford. Birds collected on this trip were “mostly taken at elevations of from 5000 to 7000 feet (=1500-2100 m).” (Rothschild and Hartert 1896).

The original description did not specify a holotype and merely noted “several specimens.” Of the four remaining in the Natural History Museum one (BMNH 1896.10.31.51), recognised by both Tyler (1963) and Zweifel (1983) as different, has been reallocated to *Nyctimystes ocreptus* (Menzies, 2014b). A fifth specimen, not examined, is now in the Museum of Comparative Zoology at Harvard (MCZ 12838).

**Material examined.** The lectotype, BMNH 1896.10.31.50, and paralectotypes 1896.10.52-53, Mt. Victoria, CenP, 1500-2150 m; UPNG 2876-2879, Avios, Mt. Albert Edward, CenP, 2600 m; UPNG 4049, 4062-4069, 4081-4083 and AMR 23449-23451, Woitape, CenP, 1570 m; UPNG 4735-4736, Agaun, MBP, 1385 m; UPNG 5037, Bonenau, MBP, 1550 m; UPNG 5505-5506, 8853, Efogi, CenP, 1250 m.

**Diagnosis.** The original description listed, inter alia, a short snout with strong canthus rostralis and concave
lores, tympanum distinct, fingers webbed at the base, disks larger than the tympanum, but made no mention of the eyelid venation. In the revision of 1958, Zweifel noted *Nyctimystes papua* to be “A moderate sized species with much reduced and indistinct palpebral venation, scantily webbed fingers and no vocal sac or vocal sac openings in the male.” The original description included “Male with an external subgular vocal sac.” (Boulenger, 1897). This contradiction has not been investigated other than to confirm that there are no openings from the floor of the buccal cavity into a vocal sac. “Moderate size”, with the addition of more material shows that the maximum male HB is 51 mm and HBf is 63 mm (fig. 2). *Nyctimystes papua* is the smallest member of the *N. papua* species group.

**Morphology.** Characteristic features of the external morphology are listed in the diagnosis above and fig. 3 illustrates the head of a typical *Nyctimystes papua*. Zweifel (1983) describes the hand web as ‘scant’ while Parker (1936, figure 2) illustrates a hand with the web not reaching the penultimate tubercle on digit 4. Two specimens from Woitape (fig. 4.A-C) show slightly more webbing so, collectively, the hand web can be described as basal to one third. None of the males from Woitape shows a pigmented nuptial pad but there is an unpigmented, swollen area on the first digit. As these specimens were taken from an (apparently) breeding population, lack of pigmented pads is surprising. Commenting on a large sample from Mondo, about 16 km from Woitape, Parker (1936) notes a “small, round nuptial pad on the first finger of the males but does not say if the pad was pigmented.

The syntype series is now so faded that the palpebral venation is hardly visible but it appears to have been very sparse, perhaps just a few broken lines and dots. In the specimens from Woitape the venation varies from very sparse (fig. 5.A) to extending over the anterior two thirds of the eyelid (fig. 5.D) but, in all cases, the lines are thin and broken. In the two specimens from above Agaun, on the slopes of Mt. Dayman, the venation is just a few vague lines on the lower edge and anterior end of the eyelid. Contrarily, the female from Bonenau, a short distance from Agaun (fig. 5.E) shows a more or less complete venation, but distinctly sparse on the posterior third of the eyelid and the lines are, again, very thin. In summary the venation is usually sparse all over and always reduced on the
posterior part of the eyelid.

Colo(u)rati(ation) of the syntypes (Boulenger 1897) is “Grey, olive or reddish brown above, uniform or marbled with darker or with large insuliform spots; a light line may run along the canthus rostralis and superciliary edge”.

**Figure 6.** *Nyctimystes disruptus* comparison of male and female proportions. Horizontal lines are the means and thick vertical lines their standard deviations. Probabilities for the differences between means are TL/HB $p > 0.7646$; HW/HB $p > 0.0700$; TY/HB $p > 0.6515$; EN/IN $p > 0.1542$; EY/HB $p > 0.0085$; HL/HW $p > 0.6954$.

**Figure 7.** Comparison of TL/HB and EN/IN ratios in *Nyctimystes disruptus* and *N. papua*. Horizontal bars are the means and thick vertical bars their 95% confidence limits. Probabilities for the difference between means are 0.006 (TL/HB) and 0.0001 (EN/IN).
and Parker’s 1936 description of the large series from Mondo is similar. In general, this description would apply to all the material here studied. The colour fades on the flanks but the mottling may persist; ventrum is usually plain white or grey. In specimens from Avios on Mt. Albert Edward the dorsum is brownish without distinct marks, paler on the flanks; on the anterior sides of the thighs and lips are bright yellowish spots and the canthus rostral is and supraciliary margin are outlined in the same bright colour; ventrum dark with white blotches. After many years in spirit the colours duller but otherwise unchanged. The Agraun and Bonenau specimens have the dorsum yellowish brown mottled all over with irregular inter-linking blackish patches which form irregular bars across the limbs; the flanks paler but still with bold blackish markings; the concealed surfaces of the thighs black with white mottle. One specimen is illustrated in life, in Menzies 2006, plate 95. Some Woitape specimens have the dorsum tan with irregular purple-red-brown mottle, vaguely barred on the limbs, others have the dorsum dark slate-coloured with darker mottle, ventrum always white but with darker mottle on throat of females. One specimen from the Kokoda area displays a rather uniform tan coloration with a greenish wash over the dorsum while another has dark green and brown blotches on the hind limbs (fig. 10. A-B).

**Distribution.** The south-eastern peninsula of New Guinea, from 1200-2600 m, the known limits being Mount Albert-Edward in the west to Mt. Dayman in the south-east (fig. 1). The single male specimen (BMNH 1980.650) from Lake Trist, about 100 km north-west of Mt. Albert Edward, may represent this species but is 2 mm longer than maximum HB and has a higher EN/IN ratio. Identification is not confirmed.

**Nyctimystes disruptus**

*Nyctimystes disruptus* Tyler, 1963, 118

*Litoria disrupta* Frost et al., 2006, 362.

**Holotype.** AMR 15923 Kaironk Valley, Schrader Mts, MadP, about 1850 m, collected by R.N.H Bulmer in February 1960.

**Material examined.** 181 specimens from localities in the central highlands of New Guinea as follows: AMR 124127 Nokopo, MadP; NML 12103-04, 47925 Ok Minam PapP; SAMR 5601-a-n Goroka, EHP; SAMR 6155a Rintibe, EHP; SAMR 9373a-c, Lafoyufa, EHP; SAMR 9374 Andandara, EHP; SAMR 8281, 9388a, 8671, 8672 Bundi, MadP; SAMR 9123a-b Dumun, EHP; SAMR 9388a-b Kaironk, MadP; SAMR 5212a-bm, 5066a-h Okapa, EHP; SAMR 933a-c Menyamya, MorP; SAMR 9367 Ialibu, SHP; SAMR 6845-47 Tulum, SHP; SAMR 6824 Tumia, SHP; SAMR 5424a-k, 5417a-b Telefomin, SanP; SAMR 6515a-c, 6455a-b Busilmin, SanP; SAMR 6508a-c Moiyokabip, WesP; SAMR 4928, 9290 Wapenamunda, EngP; SAMR 6156 Ighib, WHP; SAMR 5551a-c Watabung, EHP; SAMR 5616 Baiyer River, WHP; SAMR 5716a-c Koko (not located); SAMR 8655, 8659 New Bonome (not located); SAMR 7156a-c no data. UPNG 3210, 3211, 3461 Chuave, SimP; UP 1226-28 Wonenara, EHP; UP 9223, 9239 Mt. Gulno, MadP; UPNG 3509-10, 5151 Kompia, WHP; UPNG 3258, 3259, 3261, 3262, 3264-3269 Kaironk, MadP.

The sample of *Nyctimystes disruptus* from Okapa (n = 40 females, 33 males) was large enough to test for differences between males and females and showed that females were approximately 20% longer than males but revealed no significant differences in the ratios TL/HB, HL/HW, and EN/IN (fig. 6). There was a significant difference ($p = 0.02$) in eye size, males had larger eyes than females and males appeared to have wider heads but the difference was not significant ($p = 0.07$). Males and females were therefore combined in analyses using all variables except HB.

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1. *Nyctimystes* Stejneger (1916), derived from the Latin mystes, a priest, was formerly regarded as a feminine noun, but was determined by Duellman (1993) to be masculine, hence *disruptus*, rather than *disrupa*. 
Diagnosis. Tyler (1963), in the diagnosis, described *Nyctimystes disruptus* “a species with close affinity to *Nyctimystes papua*” but distinguished from that species by “more extensive webbing between the fingers and a palpebral venation which, although disrupted, is far more pronounced than in that species”. His illustration (Tyler, 1963, figure 1) shows an eyelid pattern of short, oblique, broken lines more or less absent from the posterior quarter. He further distinguished it from *N. papua* by difference in EN/IN ratio (no overlap) and partial overlap in TL/SVL ratios. With the larger samples now available, I find significant differences in the mean ratios but the overlap is so great that the characters have little utility in distinguishing between the species (fig. 7). There is no mention of eye colour in the original description and size is not discussed although the female holotype, at 70 mm, indicates a larger species than *Nyctimystes papua* where the largest female in the current sample measured 63 mm HB.

Zweifel (1983), establishing a ‘*Nyctimystes papua* Species Group’, further diagnosed *N. disruptus* by
“moderately large size” (male SVL < 74 mm, female SVL < 80), outer fingers “about one-half webbed”, tympanum visible and “iris green in life”. The iris colour was not mentioned in Tyler’s original description but a green colour was noted in some other specimens that Zweifel had seen. These included specimens from the Western Highlands Province, “iris green with a greyish tint”, from the Eastern Highlands Province, “iris greenish tan” and one from the Huon Peninsula “iris green” (Zweifel 1983).

Woodruff (1972) noted that a single specimen that he had collected in the Simbai Valley (close to the Kaironk type locality) was intermediate in three diagnostic characters (palpebral venation, finger webbing and EN/IN ratio) between *N. disruptus* and *N. papua* and therefore “*N. disrupta* must be regarded as a species of doubtful validity.” He did not mention body size.

Tyler’s third diagnostic feature concerns the amount of webbing between the fingers. Finger webbing in *N. papua* has been illustrated by Parker (1936, figure 2), Zweifel (1983, figure 1) and here in fig. 4. Zweifel showed hand webbing, in *N. disruptus*, to be somewhat variable. I have examined a large number of specimens and the
Nyctimystes papua is a very common frog in the central highlands of Papua New Guinea and I have collected many of them, but never one with a green iris. A specimen collected at the type locality was described in my field notebook as having “iris dark grey-brown” and one with a brown iris from the same locality is illustrated in colour in Menzies (2006, figure 93). A specimen from the Kratke Mts (Menzies, 2006, figure 92) clearly shows the Eastern Highlands, all show unequivocally, brown, greyish-brown or golden irises. After preservation the iris may appear dark or mid brown but the colour is usually milky and obscure. It is possible that two similar species, with green or brown irises, may exist syntopically but, unfortunately, the majority of museum specimens bear no information on iris colour in life and there is nothing else to separate them. Iris colour obviously needs more study and, presently, cannot be used as a diagnostic feature for Nyctimystes disruptus.

Distribution. Nyctimystes disruptus, as presently understood, is common throughout the central highlands of Papua New Guinea from Wonenara in the east to the international border at 141°E and also in the Huon Peninsula Mountains, approximately 1500-2000 m asl. The distribution map (fig. 1) shows a gap of approximately 120 km between the Star Mountains and 144°E. This is probably due to lack of surveys in that region but may also be due to the predominance of karst formations with scarcity of surface water, so unsuitable for water-breeding frogs. Richards and Venema (1962) describe a specimen from the Ok Minam in the Star Mountains thus “(Nyctimystes papua) was brown with large white-edged black blotches on the after part of its back…”. Iris colour was not mentioned.

Tyler’s diagnostic statement about palpebral venation is true in general but there is far more variation in both species than suggested and this feature does not distinguish Nyctimystes disruptus from Nyctimystes papua. Fig. 9 displays the condition in eight examples of Nyctimystes disruptus covering 470 km through the central highlands of Papua New Guinea. The venation ranges from very sparse (fig. 9.A) to half the eyelid (fig. 9.E-F) to a complete cover (fig. 9.H) but, as with Nyctimystes papua, the lines on the posterior part of the eyelid, when present, are distinctly more broken than those anterior. Specimen NML 12103 is interesting in that the patterns on left and right are different. On the left is a dense network of thin lines and dashes with horizontal lines towards the upper margin and the posterior third clear, on the right, oblique lines fill the anterior half leaving the rest clear.

In colouration, Nyctimystes disruptus is highly variable. I described one specimen in life from the Schrader Mountains as “lead colour, obscurely mottled with purple blotches, flanks paler but with purple blotches, barred purplish on the thighs, reddish purple below but mottled with grey on throat”. Another specimen (Menzies, 2006, plate 93) was similar but more greenish, yet another was spotted with white. Photographs (fig. 10), in life, of four specimens from various eastern highlands localities differ in ground colour from pale to very dark but all display the often inter-linked dark blotches tending to form bars across the limbs. Brongersma and Venema (1962) describe a specimen from the Ok Minam in the Star Mountains thus “(Nyctimystes papua) was brown with large white-edged black blotches on the after part of its back…’’. Iris colour was not mentioned.

Nyctimystes disruptus is a very common frog in the central highlands of Papua New Guinea and I have collected many of them, but never one with a green iris. A specimen collected at the type locality was described in my field notebook as having “iris dark grey-brown” and one with a brown iris from the same locality is illustrated in colour in Menzies (2006, figure 93). A specimen from the Kratke Mts (Menzies, 2006, figure 92) clearly shows a brown iris. Photographs (fig. 10) taken by Fred Parker include eight different examples of Nyctimystes disruptus from the Eastern Highlands, all show unequivocally, brown, greyish-brown or golden irises. After preservation the iris may appear dark or mid brown but the colour is usually milky and obscure. It is possible that two similar species, with green or brown irises, may exist syntopically but, unfortunately, the majority of museum specimens bear no information on iris colour in life and there is nothing else to separate them. Iris colour obviously needs more study and, presently, cannot be used as a diagnostic feature for Nyctimystes disruptus.

Confirmed records from western New Guinea include three specimens in the Naturalis Museum, Leiden (NML 12103-104, 47925, labelled N. papua), from the Ok Minam in the Star Mountains. Ok Minam is quite close to the international border. There are also two specimens in the American Museum of Natural History (AMNH 49671, 49674) from Mistkamp, a site at 1800 m in the valley of the Idenburg River. Zweifel made comment on these, allocating them to the Nyctimystes papua species group and so implying that they had an incomplete palpebral venation though he did not specifically say so. Zweifel did not include these within Nyctimystes disruptus because of their “unusually low EN distance” (EN/IN 0.85). This value is within the range of my very large series of Nyctimystes disruptus, though close to the lower limit, and I would not exclude them from Nyctimystes disruptus because of that character alone. There is also an example (MZB 15010) from the Jayawijaya Mountains, not examined, in the Indonesian Museum that is labelled Nyctimystes papua but, with a high EN/IN ratio, the identification is suspect. It seems likely that
the distribution of *Nyctimystes disruptus* extends right through the central mountains of New Guinea and the mountains of the Huon Peninsula. There are no records for any of the other north coast mountain ranges.

There are no known areas of sympatry for *N. disruptus* and *N. papua*, *N. trachydermis* or *N. tyleri*. The closest approach would be Wonenara (*N. disruptus*) and Mt. Albert-Edward (*N. papua*), about 200 km distant.

**Nyctimystes oktediensis** Richards & Johnston

*Nyctimystes oktediensis* Richards & Johnston, 1993, 73.

*Litoria oktediensis* Frost et al., 2006, 362.


**Material examined**. paratype SAMR 4077 (locality as holotype).

**Diagnosis**: *Nyctimystes oktediensis* was distinguished from *N. papua* by (inter alia) much larger size and more extensive palpebral venation; from *N. trachydermis* by smaller size, smooth skin and a tympanum that is visible, not concealed; from *N. tyleri* by smaller size and by brown, not yellow iris and from *N. disruptus* by similar body size but brown, not green, iris and more extensive palpebral venation.

**Comment**. The question of iris colour has already been discussed and ruled out as a diagnostic feature for *N. disruptus*, therefore one distinction from that species disappears. Other diagnostic features are the “more extensive webbing between the fingers and palpebral venation of very fine disrupted lines” in *N. disruptus*. The holotype eyelid (Richards & Johnston, 1993, figure 1) shows a pattern of thin, forking lines more or less covering the entire eyelid, though rather sparser posteriorly. Comparison of that illustration with fig. 9 here shows some eyelids with more veins. Zweifel (1983, figure 8) has pointed the considerable variation in finger webbing so that character is also unreliable in distinguishing *N. disruptus* from *N. oktediensis*. The excellent photo of the *Nyctimystes oktediensis* holotype, in life, (Richards & Johnston, 1993, figure 3) shows a coloration typical of the *Nyctimystes papua* group and similar to what I have described for *N. disruptus*, above.

My conclusion is that the species *Nyctimystes oktediensis* is of doubtful validity and it is here regarded as a junior synonym of *N. disruptus*.

**Distribution**. *Nyctimystes oktediensis* is only known from two localities in the Star Mountains, 1625-2200 m where *N. disruptus* has also been collected.

**Nyctimystes trachydermis** Zweifel

*Nyctimystes trachydermis* Zweifel, 1983, 12.


**Holotype**. AMNH 82866 collected by R.G. Zweifel by Gapaia Creek, nr. Garaina, MorP, 1280 m, on 1 September 1969.

**Diagnosis**. “tympanum completely concealed; palpebral reticulum relatively sparse; large size-males to 88 mm SVL; maximum size of females unknown; dorsal skin roughened; iris brown in life; males without a vocal sac.” (Zweifel 1983).

**Material examined**. SAMR 24079 from the type locality.

*Nyctimystes trachydermis* is larger than all other species in this group and, in fact, larger than all other *Nyctimystes* species except *granti*, *humeralis*, *pulcher* and *zweifeli*, which all have vocal sacs and complete palpebral reticula. *Nyctimystes trachydermis* is not known to overlap in distribution with *N. disruptus* but the rough, rather than warty, dorsal skin and larger size would distinguish it. In the single specimen that I examined, the tympanum is quite distinct but there may have been some shrinkage after years in alcohol. Measurements given in Menzies (2006) are incorrect, the species is ‘large’ not ‘moderate’ and the illustration (plate 96) is not *N. trachydermis* but the recently described species *N. ocreptus* (Menzies, 2014b).

**Distribution**. Several localities in the south-eastern peninsula of New Guinea from Mt. Kaindi to Garaina (type locality) east to Mt. Simpson (Kraus and Allison 2004), 1280-2480 m. It is known to be sympatric with *Nyctimystes tyleri* and all localities are within the geographic range of *Nyctimystes papua*.

**Nyctimystes tyleri** Zweifel

*Nyctimystes tyleri* Zweifel 1983, 16.

*Litoria tyleri* Frost et al., 2006, 362.
Holotype. AMNH 82878, collected by R.G. Zweifel by Gapaia Creek, nr. Garaina, (MorP) 1280 m, on 1 September 1969. The holotype is the only known specimen.

Material examined, none

Diagnosis. (Zweifel, 1983) “large size, absence of a vocal sac, weak palpebral pigmentation and golden iris.”

Large size (SVL 77.6 mm) rules out confusion with *N. papua* and the yellow iris with *N. disruptus* and *N. trachydermis*, as well as the warty, rather than rugose dorsal skin. The question of iris colour in *N. disruptus* has already been discussed.

Distribution. *Nyctimystes tyleri* is only known from the holotype, and the type locality, near Garaina, is about 180 km distant from any locality for *N. disruptus*. It is sympatric with *N. trachydermis*. Several species of the *Nyctimystes cheesmanae* group (Menzies, 2014a) are known from the Garaina area and it is conceivable that *N. papua* could occur in the mountains above Garaina.

DISCUSSION

In 1983, Zweifel wrote “There remains however, a substantial residue of specimens that do not fit comfortably into any of the taxa that I now recognize but yet are not sufficiently well characterized to be described… As preserved specimens most of these unassigned frogs cannot readily be distinguished from *Nyctimystes disrupta*.” These included some from the Southern Highlands Province with a brown iris and others from the Wau area and as far east as Mt. Dayman, also with brown irises. He suggested that as many as four species were represented. If one disregards the problem of iris colour, and accepts that there is some variation in the extent of hand webbing and palpebral venation, there is no problem in calling all these frogs, including *N. oktediensis*, variants of *Nyctimystes disruptus*, excepting those from the south-east, from Wau to Mt. Dayman, which, by body size alone, can be allied with *N. papua*. If there is, as Zweifel has suggested, more than one species currently included in *N. disruptus*, I have not found any means to separate them. A multi variate (discriminant) analysis dividing specimens into geographical groups selected HL, HW, and IN as the only variables with sufficient power to discriminate between them but the percentage of a priori classifications deemed to be correct was very low, between 7 and 52% except 100% for the south-eastern group. Fig. 11 shows all the geographic groups (1-7) from the central highlands forming a tight cluster, distinct from the south-eastern group (8). In this analysis, the first two axes accounted for 99.9% of the between-groups variance and in axis 1 most weight was carried by head width (HW). A second analysis leaving out the south-eastern group selected tibial length (TL) as the only discriminating variable and the level of ‘correct’ a priori classifications was only 20%. It would be unwise to consider any of these geographical groups, other than the south-eastern group, as more than geographical variants of *Nyctimystes disruptus*.

An alternative way to reveal the presence of sympatric cryptic species is to check for normality in the distribution of different variables. Only in group 6, Star Mountains (*n* = 21), was any possible deviation found, where the HL/HW and HW/HB ratios deviated significantly from normal. In all other groups, where the sample size was large enough to be valid, all variables appeared to be normally distributed. Unfortunately, *Nyctimystes oktediensis* could not be fitted into this figure as the number of specimens for which I had data (*n* = 2) was much too small.

So there may be more than one species masquerading as *Nyctimystes disruptus* but, at present, there is no supporting evidence for this, and the apparent lack of voice these male frogs is a distinct handicap in separating species. Interestingly, the Kalam people of the Schrader Mountains recognise two kinds of *Nyctimystes disruptus* by colour and altitude (Bulmer and Tyler 1968). A light variety (Kwyos) is “Normally found in water but sometimes in pandanus and other vegetation.” The dark variety (Gepgep) is “seldom found in the lower altitude cultivation zone, overlapping with Kwyos in bush fallow”. Both are said to make a whistling call but they also have a very pungent odour, which may be species specific.

With the addition of more specimens of *Nyctimystes papua*, the difference between that species and *N. disruptus* has become clearer though sample sizes (for *N. papua*) are still small. Currently, there is no evidence to support *Nyctimystes oktediensis* or for more than one species in what is known as *N. disruptus*. Elucidation may also have to wait for more data and possibly molecular investigation.

The two very large species, *Nyctimystes trachydermis* and *N. tyleri* appear to be quite distinct but it would certainly be good to have more examples of *N. tyleri*. 
ACKNOWLEDGEMENTS

The Universities of Papua New Guinea and Adelaide and the Australia Pacific Biological Foundation have supported my research; Fred Parker and Allen Allison allowed me to use their photographs and Michael Tyler made many useful comments on the original draft of this paper. Curators of various museums have facilitated the examination of specimens in their care. I sincerely thank them all.

LITERATURE CITED


Rothschild, W., Hartert, E. (1896). Contribution to the ornithology of the Papua Islands. VI On some skins collected from April to June on Mount Victoria, Owen Stanley Mountains, mostly at elevations of from 5000 to 7000 feet. Novitates Zoologicae, 3: 530-533.


APPENDIX 1

Statistics

Table A1. Complementary statistics of figure 2: t-test (assuming equal variances)

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th></th>
<th>Males</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference</td>
<td>t</td>
<td>df</td>
<td>Prob. &gt; [t]</td>
</tr>
<tr>
<td>Estimate</td>
<td>13.8234</td>
<td>10.397</td>
<td>77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SE</td>
<td>1.3295</td>
<td></td>
<td>1.032</td>
<td></td>
</tr>
<tr>
<td>lr. 95%</td>
<td>11.1760</td>
<td></td>
<td>8.8787</td>
<td></td>
</tr>
<tr>
<td>up. 95%</td>
<td>16.4707</td>
<td></td>
<td>13.1304</td>
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</tr>
</tbody>
</table>

Table A2. Complementary statistics of figure 2: means for one-way ANOVA (SE uses a poled estimate of variance).

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th></th>
<th>Males</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>SE</td>
<td>lr. 95%</td>
</tr>
<tr>
<td>N. disruptus</td>
<td>69</td>
<td>68.7854</td>
<td>0.4730</td>
<td>67.843</td>
</tr>
<tr>
<td>N. papua</td>
<td>10</td>
<td>54.9620</td>
<td>1.2425</td>
<td>52.488</td>
</tr>
</tbody>
</table>
**Table A3.** Complementary statistics of figure 7: *t*-test (assuming equal variances)

<table>
<thead>
<tr>
<th></th>
<th>TL/HB</th>
<th>EN/IN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference</td>
<td>Estimate</td>
<td>0.0157</td>
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<tr>
<td></td>
<td>SE</td>
<td>0.0056</td>
</tr>
<tr>
<td></td>
<td>lr. 95%</td>
<td>0.0046</td>
</tr>
<tr>
<td></td>
<td>up. 95%</td>
<td>0.0268</td>
</tr>
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</table>

**Table A4.** Complementary statistics of figure 8: *t*-test (assuming equal variances)

<table>
<thead>
<tr>
<th></th>
<th>TL/HB</th>
<th>HW/HB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference</td>
<td>Estimate</td>
<td>-0.0034</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.0114</td>
</tr>
<tr>
<td></td>
<td>lr. 95%</td>
<td>-0.0265</td>
</tr>
<tr>
<td></td>
<td>up. 95%</td>
<td>0.0197</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>TY/HB</th>
<th>EN/IN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference</td>
<td>Estimate</td>
<td>-0.0133</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.0024</td>
</tr>
<tr>
<td></td>
<td>lr. 95%</td>
<td>-0.0060</td>
</tr>
<tr>
<td></td>
<td>up. 95%</td>
<td>0.0039</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>EY/HB</th>
<th>HL/HW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference</td>
<td>Estimate</td>
<td>-0.0170</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.0061</td>
</tr>
<tr>
<td></td>
<td>lr. 95%</td>
<td>-0.0293</td>
</tr>
<tr>
<td></td>
<td>up. 95%</td>
<td>-0.0046</td>
</tr>
</tbody>
</table>

**Table A5.** Complementary statistics of figure 11 (eight groups): variables in the analysis

<table>
<thead>
<tr>
<th>step</th>
<th>var.</th>
<th>Tolerance</th>
<th>F to remove</th>
<th>Wilk’s lambda</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HW</td>
<td>1.000</td>
<td>233.618</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>HW</td>
<td>0.213</td>
<td>543.539</td>
<td>0.608</td>
</tr>
<tr>
<td></td>
<td>HL</td>
<td>0.213</td>
<td>70.627</td>
<td>0.104</td>
</tr>
<tr>
<td>3</td>
<td>HW</td>
<td>0.186</td>
<td>515.399</td>
<td>0.441</td>
</tr>
<tr>
<td></td>
<td>HL</td>
<td>0.206</td>
<td>24.021</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>IN</td>
<td>0.406</td>
<td>83.530</td>
<td>0.029</td>
</tr>
</tbody>
</table>
Table A6. Complementary statistics of figure 11 (eight groups): Wilk’s lambda.

<table>
<thead>
<tr>
<th>step</th>
<th>No. of vars</th>
<th>Lambda</th>
<th>$d/1$</th>
<th>$d/2$</th>
<th>$d/3$</th>
<th>$\text{Exact } F$ statistic</th>
<th>$d/f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.104</td>
<td>1</td>
<td>7</td>
<td>190</td>
<td>233.618</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.29</td>
<td>2</td>
<td>7</td>
<td>190</td>
<td>132.133</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0.022</td>
<td>3</td>
<td>7</td>
<td>190</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A7. Complementary statistics of figure 11 (eight groups): Pairwise comparisons ($F$-value followed by $p$-value).

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.930; 0.004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.168; 0.955</td>
<td>2.173; 0.074</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7.330; 0.000</td>
<td>1.881; 0.115</td>
<td>1.626; 0.169</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.109; 0.979</td>
<td>1.514; 0.200</td>
<td>0.233; 0.919</td>
<td>1.969; 0.111</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.858; 0.120</td>
<td>7.145; 0.000</td>
<td>0.584; 0.674</td>
<td>9.596; 0.000</td>
<td>0.826; 0.510</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.420; 0.794</td>
<td>3.114; 0.016</td>
<td>0.467; 0.760</td>
<td>5.226; 0.001</td>
<td>0.113; 0.978</td>
<td>2.593; 0.036</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1066.315; 0.000</td>
<td>675.211; 0.000</td>
<td>429.826; 0.000</td>
<td>1421.424; 0.000</td>
<td>320.849; 0.000</td>
<td>879.116; 0.000</td>
<td>701.732; 0.000</td>
</tr>
</tbody>
</table>

Table A8. Complementary statistics of figure 11 (eight groups): Eigenvalues and Wilks’ lambda.

<table>
<thead>
<tr>
<th>Functions</th>
<th>Eigenvalue</th>
<th>% variance</th>
<th>Cumulative % variance</th>
<th>Canonical correlation</th>
<th>Test of Functions</th>
<th>Wilk’s Lambda</th>
<th>Chi-square</th>
<th>df</th>
<th>sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.457*</td>
<td>99.1</td>
<td>99.1</td>
<td>0.985</td>
<td>1-3</td>
<td>0.022</td>
<td>732.223</td>
<td>21</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>0.269*</td>
<td>0.8</td>
<td>99.9</td>
<td>0.460</td>
<td>2-3</td>
<td>0.753</td>
<td>54.368</td>
<td>12</td>
<td>0.000</td>
</tr>
<tr>
<td>3</td>
<td>0.047*</td>
<td>0.1</td>
<td>100.0</td>
<td>2.11</td>
<td>3</td>
<td>0.955</td>
<td>8.761</td>
<td>5</td>
<td>0.119</td>
</tr>
</tbody>
</table>

* first three canonical discriminant functions were used in the analysis

Table A9. Complementary statistics of figure 11 (eight groups): classification results (40% of original grouped cases correctly classified).

<table>
<thead>
<tr>
<th>Group</th>
<th>Predicted group membership</th>
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</thead>
<tbody>
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<td></td>
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</tr>
<tr>
<td>Count</td>
<td>1</td>
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<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
### Table A10. Complementary statistics of figure 11 (seven groups): variables entered/removed

<table>
<thead>
<tr>
<th>step</th>
<th>entered</th>
<th>Wilk’s lambda</th>
<th>Exact F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>statistic</td>
<td>df1</td>
</tr>
<tr>
<td>1</td>
<td>TL</td>
<td>0.722</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table A11. Complementary statistics of figure 11 (seven groups): Wilk’s lambda.

<table>
<thead>
<tr>
<th>step</th>
<th>No. of vars</th>
<th>Lambda</th>
<th>df1</th>
<th>df2</th>
<th>df3</th>
<th>Exact F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>statistic</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0.729</td>
<td>1</td>
<td>6</td>
<td>165</td>
<td>10.204</td>
</tr>
</tbody>
</table>

### Table A12. Complementary statistics of figure 11 (seven groups): variables in the analysis

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<tr>
<th>step</th>
<th>var.</th>
<th>Tolerance</th>
<th>F to remove</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TL</td>
<td>1.000</td>
<td>10.887</td>
</tr>
</tbody>
</table>

### Table A13. Complementary statistics of figure 11 (seven groups): Pairwise comparisons (F-value followed by p-value).

<table>
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<th>Group</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>15.390; 0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.058; 0.810; 6.328; 0.013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>29.219; 0.000; 0.586; 0.445; 5.916; 0.016</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.048; 0.826; 6.914; 0.009; 0.120; 0.729; 6.354; 0.013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.973; 0.162; 21.430; 0.000; 1.204; 0.274; 34.307; 0.000; 0.307; 0.580</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.024; 0.878; 11.654; 0.001; 0.100; 0.752; 14.541; 0.000; 0.012; 0.914; 0.927; 0.337</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

### Table A14. Complementary statistics of figure 11 (seven groups): classification results (24.3% of original grouped cases correctly classified).

<table>
<thead>
<tr>
<th>Group</th>
<th>Predicted group membership</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Count</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
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</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>
## APPENDIX 2

Geographical coordinates (°S, °E) of places mentioned in the text.

### Papua, Indonesia
- Ok Minam, 4.87, 140.87
- Mistkamp, 3.58, 139.05

### Papua New Guinea

#### Central and Milne Bay Provinces
- Mt. Victoria, 8.88, 147.53
- Avios, Mt. Albert Edward, 8.31, 147.21
- Woitape, 8.52, 147.35
- Agaun, 9.5, 147.62
- Bonenau, 9.88, 149.4
- Efogi, 9.13, 147.67

#### Eastern Highlands and Simbu Provinces
- Andandara, SE Kainantu
- Chuave, 6.13, 145.13
- Dumun, 6.11, 145.08
- Goroka; 6.07, 145.38
- Igindi, nr. Goroka
- Kainantu, 6.28, 145.87
- Lafoiyufa, WSW Goroka
- Menyamya, 7.23, 146.02
- Okapa, 6.52, 145.65
- Rintibe, nr. Goroka
- Watabung, 6.07, 145.21
- Wonenara, 6.33, 145.47

#### Western Province
- Moiyokabip, 5.2, 141.27
- Mt. Akrik, 5.17, 141.17

#### Madang Province
- Bundi, 5.73, 145.23
- Nokopo, 5.95, 146.60
- Kaironk, 5.30, 144.48

#### Morobe Province
- Gapaia Creek, nr. Garaina, 7.88, 147.13
- Lake Trist, 7.49, 146.97
- Mt. Kaindi, 7.40, 146.73
- Mt. Simpson, 10.00, 149.50

#### Sandaun (WestSepik) Province
- Telefomin, 5.12, 141.57
- Busilmin, 4.92, 141.10

#### Southern Highlands Province
- Ialibu, 6.28, 143.98
- Tulum, nr. Ialibu
- Tumia, nr. Ialibu

#### Western Highlands, Hela and Enga Provinces
- Baiyer River Sanctuary, 5.57, 144.1
- Wapenamunda, 5.62, 143.85
- Kompia, 5.37, 143.95
Chiromantis simus (Annandale, 1915) is a foam-nesting rhacophorid frog and known only from eastern India. This species was believed to be extinct from India (Daniels, 2005) but was rediscovered from Assam and West Bengal in 1998 and 1999 respectively (Deuti et al., 2000). In Bangladesh, it is reported from Chittagong and Rangamati districts (Asmat et al., 2003). Though the species is poorly known, it is locally abundant in the area of Rajpur, which is a mature flood plain of the dead river basin of the ancient River Ganges. The soil of Rajpur is fertile and there were patches of dense vegetation comprising large old trees and lianas in the area up to the recent past, though these are fast disappearing due to rapid urbanization (Banerjee, 2010).

Chiromantis simus, a foam-nesting rhacophorid frog, previously considered extinct from India, was re-discovered in 1998. Surprisingly it is abundant at the village of Rajpur and its surroundings. This species is a true monsoon breeder and produces foam nests between June and October. Generally, during foam-nesting the female initially lays an egg mass without foam coating (i.e., “uncovered”). Later, she produces a foamy liquid and evenly covers the egg mass with it. I collected an uncovered egg mass before a female went to the water source below to absorb water. After returning, the female waited for 4 hours when she did not find the egg mass near the twig and then, by a process of continuous rubbing of her hind limbs, she secreted a thick jelly-like substance from the cloaca, instead of the foamy substance. Additional observations on the egg laying behaviour showed that uncovered egg masses were always attacked by ants, while those egg masses covered by foam were never attacked. Chiromantis simus foam-nesting is mostly polyandrous but, when a female has to deal with too many males in amplexus, she leaves the egg mass without depositing an additional foam coating, which may be why some clutches can be found uncovered.

Chiromantis simus (Annandale, 1915) is a foam-nesting rhacophorid frog and known only from eastern India. This species was believed to be extinct from India (Daniels, 2005) but was rediscovered from Assam and West Bengal in 1998 and 1999 respectively (Deuti et al., 2000). In Bangladesh, it is reported from Chittagong and Rangamati districts (Asmat et al., 2003). Though the species is poorly known, it is locally abundant in the area of Rajpur, which is a mature flood plain of the dead river basin of the ancient River Ganges. The soil of Rajpur is fertile and there were patches of dense vegetation comprising large old trees and lianas in the area up to the recent past, though these are fast disappearing due to rapid urbanization (Banerjee, 2010).

In amphibians, foam nests are associated with the evolution of terrestriality (Heyer, 1975). Foam-nesting has evolved several times and is reported in eight families, namely Rhacophoridae, Leptodactylidae, Myobatrachidae, Hylidae, Microhylidae, Hyperoliidae, Leiuperidae and Limnodynastidae (Haddad et al., 1990; Wells, 2007). The nature of foam nests varies among families and foam-nesting species are placed among different reproduction modes (Wells, 2007). Though foam-nesting is widely reported in different species, the function of the foam is still poorly understood and differs among the families in which it is known (Wells, 2007). High predation pressure in aquatic environments is generally assumed to be the ultimate cause of leading to the evolution of terrestrial breeding in anurans (Magnusson & Hero, 1991).

The genus Chiromantis has a discontinuous distribution with some species in Africa and most in southern Asia. Li et al. (2013) suggested that inter-continental migration of Chiromantis took place during the Cenozoic as a consequence of the movement of the Indian plate. Deuti (2001) and Banerjee (2010) reported detailed ecology and breeding biology of species at Rajpur. The minimum span of the foam-nesting period of C. simus was 74 days.
in 2009 (01 July 2009 to 12 September 2009) while the maximum period was 115 days in 2013 (14 June 2013 to 06 October 2013). The average length of 22 *C. simus* foam nests was about 6.06 cm (SE ± 0.15), breadth 2.77 cm (SE ± 0.10), thickness 2.27 cm (SE ± 0.08), and clutch size varied between 153 and 234 eggs (Deuti, 2001).

Since 2007, I have been observing the breeding behaviour of *Chiromantis simus* in a small pool in a natural garden (22° 42′36″, 88° 39′23″) in Rajpur, near Kolkata, India. I observed that during construction of the foam nest, the female initially lays a mass of eggs that is irregular in shape and uncovered by foam i.e., some eggs remaining exposed (fig. 1.A). After laying the exposed egg mass on a leaf (or any other substrate over a water body), the female descends to the water source below, soaks water through her abdominal skin and then transports water to the nest when she returns. She then produces a foamy liquid and spreads it evenly over the egg mass with her hind limbs (Banerjee, 2010) (fig. 1.B). Additional layering of foam is reported only in the genus *Chiromantis* from Africa (Jennions *et al.*, 1992).

On 31 August 2012 at 23:00, I observed a female *C. simus* that after laying her egg mass on a leaf went below to soak up water from the pool. After she descended, I collected the leaf on which the egg mass rested ensuring that she could not observe me doing so. When the female returned to the tree branch she could not find the egg mass. She waited there for about four hours with her swollen abdomen containing a large volume of water and rubbed both her hind limbs behind her cloaca intermittently. From 03:00, she started secreting a transparent thick jelly-like substance (not foam) from the cloaca (fig. 2) by a process of continuous rubbing of her hind limbs vigorously, squeezing her swollen abdomen. There were no males on her back to stimulate while doing this. A foam-nesting species secreting jelly (not foam) has not been reported earlier by any previous study. Phylogenetic relationships among rhacophorid frogs have been in a state of flux since Liem (1970). *Chirixalus vittatus* is the only species closest to *Chiromantis simus*, which is known to lay eggs inside a jelly mass instead of a foamy mass. Yu *et al.* (2009) merged *Chirixalus vittatus* and *Chirixalus doriae* with the genus *Chiromantis*. Fei *et al.* (2010) renamed *Chiromantis vittatus* as *Feihyla vittatus*. In-depth study on the foam nesting process may provide some clue on the classification of Rhacophoridae.

My observations on *C. simus* reveal that uncovered egg masses were always attacked by carnivorous ants.

**Figure 1.** Foam nests of *Chiromantis simus*: A. an uncovered egg mass freshly laid and attached to a leaf; it is irregular in shape and with some eggs exposed; B. sectional view of a foam nest after additional layering; thickness of the outer foam cover is 3 to 4 mm ‘red outline’.
Figure 2. Adult female of *Chiromantis simus* rubbing her hind legs vigorously near her cloaca and expelling all jelly-like materials by constricting her flanking muscles. After secretion of jelly from the cloaca, her balloon-like swollen abdomen shrunk to normal size. Constricted flanking muscles are marked with an arrow.

Figure 3. *Oecophylla smaragdinae* ants killed two resting *C. simus* during daytime.
Figure 4. Ant predation on foam nests: A. A covered egg mass, i.e. a complete foam nest (brown) remains untouched by ants while an uncovered egg mass (white) is attacked by ants *Camponotus* sp.; B. A covered egg mass i.e., a complete foam nest (brown) remains untouched by ants while an uncovered egg mass (white) is attacked by ants *Oecophylla smaragdina*; C. Foam nests of *Polypedates leucomystax* were often attacked by ants (*Tetraponera* sp.).
Over the past six years, I have observed approximately 700 covered foam nests of *Chiromantis simus* and none was attacked by ants. Foam nest predation by arthropods, frogs, snakes, birds, and mammals has been widely reported (Wells, 2007) but nobody has reported any mechanism that protects foam nest from predation. Some tree ant species are opportunistic carnivores and common in sub-tropical forests. *Oecophylla smaragdina* can be ferocious and I have observed them attacking resting *C. simus* during the day (fig. 3). I have occasionally noticed uncovered egg masses hanging from leaves and invariably those egg masses were attacked by carnivorous ants such as *Camponotus* sp. (fig. 4.A) and *Oecophylla smaragdina* (fig. 4.B). *Polypedates leucomystax* also makes

Figure 5. Nineteen males of *Chiromantis simus* (15 are in picture frame and 4 just out side) surrounding a polyandrous foam nest, waiting for the female to join in amplexus. One old foam nest is also visible on the left side.
foam nests regularly over the same water pool. I have not observed *P. leucomystax* to make additional external foam sheaths after laying eggs like *C. simus*, and several times I have found that the foam nests of *P. leucomystax* were attacked by *Tetraponera* ants (fig. 4.C).

Making uncovered foam nests is not a regular phenomenon. Over 6 years, the frequency of uncovered foam nests was less than 5%. I have observed an uncovered foam nest only once, but the reason for not covering the nest remains unclear. Foam nesting in *C. simus* mostly involves multiple males. However, when a female is courted by too many males simultaneously (fig. 5; see also figure 1 in Banerjee, 2010), I have observed the female to abandon the foam nest without covering it with an additional foam layer. I recorded this behaviour with photographs only once (on 20 July 2010 at 00:30 night) when a female was surrounded by 19 males (fig. 5; 15 males are in frame and 4 just outside) waiting to join in amplexus. This may not be the reason for all the cases.

Whether the jelly substance is an altered foam that was retained for some hours has the same properties (of protection and hydration), and to which extent the usually secreted foam cover has any particular property that repels the carnivorous tree ants, are subjects that could be further investigated.

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A short note on the use of humeral spines in combat in *Espadarana prosoblepon* (Anura: Centrolenidae)

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There are currently about 150 species of glass frogs in the family Centrolenidae, which range throughout tropical rain forests in Central and South America (Frost, 2014). Some species of glass frog are known to engage male-male combat where males dangle by their toes and grapple venter to venter for extended periods of time (Jacobson, 1985; Bolivar et al., 1999; Hutter et al., 2013). Humeral spines, present in the males of some glass frog species, are thought to be involved in this ritualized combat behavior. Here we present photographic evidence that the spines are indeed used in this combat behavior in one species, *Espadarana prosoblepon* (Guyasamin et al., 2009).

The night after a large rain on 25 November 2012 at 22:31, we observed two male *Espadarana prosoblepon* hanging venter-to-venter from their toe pads. The frogs were suspended from a branch approximately 1.5 m above

![Figure 1. Two male Espadarana prosoblepon as found grappling from the overhanging branch. Male A is on top, and male B is towards the bottom of the image. The white arrow indicates the blue humeral spine of male A.](image-url)
a fast-moving rocky stream in the cloud forests of Omar Torrijos National Park, El Copé Province, Panama. Each male had his humeral spines interlocked in the other’s axial region (fig. 1). At 22:35, the two frogs separated and perched on the branch from which they were both hanging (fig. 2). Immediately after separation, male A (the frog on top in fig. 1 and 2) began calling with the typical three “peeps” (Jacobson, 1985) while male B subsequently jumped to lower hanging vegetation.

Male-male combat behaviour has been described elsewhere in *E. prosoblepon* (Jacobson, 1986) and in other glass frogs with humeral spines (Bolivar et al., 1999; Guayasamin and Barrio-Amorós, 2005), but none of these reports directly commented on the use of the humeral spines. However, Kubicki (2007) provides hypotheses and anecdotal evidence of the humeral spines of this species being used in male-male combat. Our photographs corroborate these findings and provide additional evidence of the use of humeral spines during combat in *E. prosoblepon*. Bolivar et al. (1999) and Hutter et al. (2013) observed males of *Centrolene buckleyi* and *Nymphargus grandisonae*, respectively, using humeral spines in possibly injurious combat. It is possible that the spines are used to pry off combatting males, or to injure the frogs by repeatedly driving them into the opposing male as described by Hutter et al. (2013). Because injuries were not observed, we could not determine whether spines caused injury in this case. It is also possible that the position of the humeral spines in figure 1 is simply an artifact of the grappling behavior. However, given the anecdotal evidence that the spines are used in combat in *E. prosoblepon* (Kubicki 2007), and the fact that these spines are likely used in combat in other species (Bolivar et al., 1999; Hutter et al., 2013), it is likely that the interlocking position of the spines of *E. prosoblepon* observed here is not coincidental.

Combat behaviour in glass frogs is often quite complex and may be phylogenetically informative when the behaviour is fully described (Hutter et al., 2013). Apart from observations that humeral spines may be used in combat, there is little information available regarding the purpose of the spines within the genus *Espadarana*. Detailed observations and photographs may reveal heretofore unnoticed peculiarities of aggressive behaviors and confirm the functional role of humeral spines in combat.

**Figure 2.** Two *Espadarana prosoblepon* immediately after separating from the grappling behavior. Male A is at the top of the image; Male B is at the bottom. The arrow indicates the humeral spine of Male A.
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