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On the generic status of "*Nyctimystes rueppelli*" (Anura: Hylidae), a tree frog of Halmahera Island, Indonesia

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***Nyctimystes* is currently diagnosed by a combination of two characters, namely vertical pupil and a palpebral venation. In a newly collected series of "*Nyctimystes rueppelli*" this combination of characters is found wanting and the species is therefore removed from *Nyctimystes* and transferred to *Litoria*. Removal of this species from *Nyctimystes* now allows that genus to have a third diagnostic character, unpigmented ova, and confines it to New Guinea and satellite islands.**

INTRODUCTION

The tree frog *Hyla rueppelli*, currently known as *Nyctimystes rueppelli* (Menzies, 2006; Tyler & Davies, 1978; Zweifel, 1958), was described by Boettger in 1895 from a series of 48 specimens collected on Halmahera Island by Dr. W. Kükenthal and deposited in the Senckenberg Museum, Frankfurt. No holotype was designated and his description gave measurements only for five specimens but many of the others were subsequently distributed to various museums in Europe and America, including Basel (adult male, labelled paratype); Vienna, four males, one subsequently transferred to Adelaide; New York (two syntypes) and London, four specimens, labelled "types". The description was repeated by Boettger in 1900 with some additional information on material held in Frankfurt, noting eight adult males, seven females and four young, still with tails, all from Soah Konorah, and two males, one female from Galela, and 28 adults from Kau. There are now only two adult males, two adult females and five young in the Senckenberg Museum. There is no mention, in the description, of pupil shape nor presence of a pattern on the lower eyelid, and Boettger's illustration (1900, plate 16, figure 12) shows an expanded, more or less circular, pupil. One specimen - a gravid female with pigmented ova - had previously been collected by Bernstein in 1866 on either Gebe or Gag Island. Gebe is approximately 60 km from the eastern arm of Halmahera while Gag is 120 km to the south-east. All three localities are part of the same island arc system. Bernstein had spent some time collecting on Halmahera before visiting Gebe and Gag (Wichman, 1906). Brongersma (1948) reported on two males collected on Morotai Island, to the north of Halmahera, by H.J. Lam, and these are now in the Carnegie Museum in Philadelphia. We are not aware of any other specimens of *Nyctimystes rueppelli* in any museum that have not come from Kükental's, Bernstein's or Lam's collections, other than recent collections on Halmahera made by Riyanto in 2010. Fig. 1 illustrates all locations from which the species has been recorded. No other *Nyctimystes* species have been recorded on Halmahera or Morotai Islands.

As a result of lack of recent material, and poor condition of many of Kükenthal's specimens, *Nyctimystes rueppelli* has remained somewhat of a mystery. The two primary diagnostic features of *Nyctimystes* are the presence of a pattern of lines and/or dots on the transparent upper part of the lower eyelid and a vertical pupil, but all the Papuan *Nyctimystes* species now known, except *N. rueppelli*, produce relatively large unpigmented ova from which torrent-adjusted tadpoles emerge, though very few tadpoles have actually been described. By contrast,

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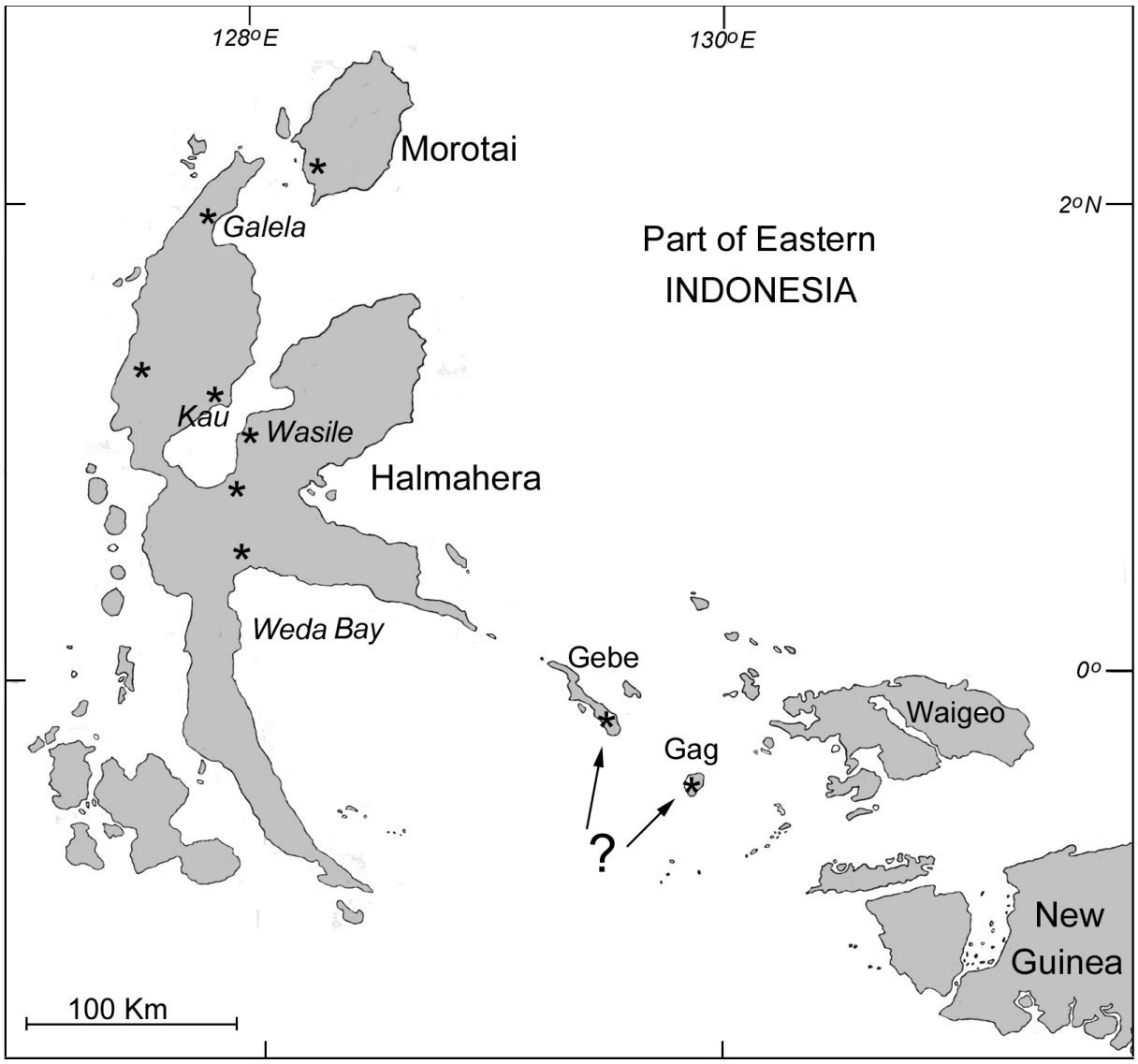

Figure 1. Known localities (*) for *Litoria rueppelli*.

the ova of *Nyctimystes rueppelli* are pigmented brown on the animal pole. This does not necessitate removal of the species from *Nyctimystes*, as currently defined by Zweifel (1958), but does invite further investigation. Apart from the works of van Kampen (1923) and Gorham (1963), which do not add any new information, *Hyla rueppelli* received no further attention until 1958 when Zweifel transferred it to *Nyctimystes*. Tyler (1968) did not include it in his revision of the Papuan *Hyla* (now *Litoria*) because it had already been transferred to *Nyctimystes*.

The genus *Nytimystes* Stejneger

Stejneger (1916) erected *Nyctimystes* to accommodate two species of Papuan tree frogs which, up to that time, had been included, on account of their vertical pupils, in the South American genus *Nyctimantis*. Zweifel (1958) published a revision of the genus *Nyctimystes* and commented that the sole character of a vertical pupil was "a tenuous one for defining a genus" and therefore added the "presence of a vein-like network, the palpebral reticulum", on the lower eyelid. Neither of these characters, individually, is present in any of the Papuan *Litoria* species, the only other hylid genus occurring in the region, and so could be regarded as synapomorphies of the genus *Nyctimystes*. Zweifel (1958) noted that *Hyla rueppelli* had a palpebral reticulum and so transferred it to *Nyctimystes* but was unable to determine the shape of the pupil, which was fully expanded in specimens that he saw. He assumed, because all the other Papuan hylid frogs with palpebral reticula also had vertical pupils, that *Hyla rueppelli* also did so and therefore felt justified in transferring it to *Nyctimystes*. Tyler & Davies (1979) attempted to redefine the genus and made a detailed examination of the skulls of 17 species, comparing them with

Australo-Papuan *Litoria*. They defined *Nyctimystes* by a suite of 39 characters but nearly all these characters were equivocal such as, "well developed or reduced quadrate-jugal". In the montane *Litoria*, to which *Nyctimystes* species were apparently related by their unpigmented large ova and torrent-type tadpoles, this element was always reduced but unreduced in those *Litoria* with pigmented ova. Despite Tyler & Davies' detailed analysis, the genus is left with only two defining characters, contracted pupil shape and eyelid venation. Beyond suggesting that *Nyctimystes rueppelli* may have had an independent origin from other *Nyctimystes* species, they did not question its inclusion in the genus.

Frost *et al*. (2006) returned all *Nyctimystes* species to *Litoria* on the basis of a molecular analysis that included the Australian species, *N. dayi* and the Papuan *N. pulcher*. Wiens *et al.* (2010) also showed *Nyctimystes* to be a paraphyletic genus with seven Papuan species forming a sister group to *Litoria infrafrenata* and *N. dayi* distant from that group. However, Kraus (2013) saw that *Nyctimytes dayi* did not have a vertical pupil, was incorrectly placed in that genus, and therefore the conclusions of Frost *et al*. and Wiens *et al*. were invalid. *Nyctimystes* continues to be recognised as a valid genus (e.g. Kraus, 2013; Menzies, 2014a, b, c). *Nyctimystes rueppelli* was not included in Wiens' analysis and its molecular relationships remain unknown. Kraus (2013) also speculated on the possible incorrect generic allocation of *Nyctimystes rueppelli*, noting, because pupil shape could not be determined in any of the material then available, that "future study of the Halmaheran *N. rueppelli* may show that species to be another mismatch".

Recent collection of material on Halmahera by Riyanto, where the species is common, confirms these suspicions and allows re-assessment of the generic status of *Nyctimystes rueppelli*.

MATERIALS AND METHODS

This published work have been registered in ZooBank. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser. The LSID for this publication is: urn:lsid:zoobank.org:pub:F2B8791E-EDB9-4B94-8F46-D8223D827ACB

For this investigation, we confined ourselves to three characters, the shape of the pupil, the form of the palpebral reticulum and the colour and size of the ova. Approximately 20 recently collected specimens of *Nyctimystes rueppelli* were available, several of which had been photographed in life. Specimens from Kükenthal's original collection had already been examined in Europe and Appendix 1 lists all this material.

Relative eye size was estimated as the horizontal diameter of the eye/length of the body measured from the tip of the snout to the end of the urostyle. Egg diameter was measured in a sample of approximately 20 ova taken from each of the gravid females listed in Appendix 1.

RESULTS

Pupil shape

The eyes are large, mean horizontal eye diameter/body length = 0.15, the iris is dark brown. Fig. 2.A, B, C shows enlargements of eyes of three of specimens of *Nyctimystes rueppelli*, photographed in life. The pupil is rhomboid or diamond-shaped and in fig. 2.A and 2.B the horizontal dimension is clearly greater than the vertical. In all the preserved specimens recently re-examined by Menzies, pupil shape was obscure. Fig. 2.D is the eye of *Litoria amboinensis*, a species that bears some morphological resemblance to *Nyctimystes rueppelli*, photographed in life. This pupil is also diamond or rhomboid in shape and is characteristic of that group of species that includes,

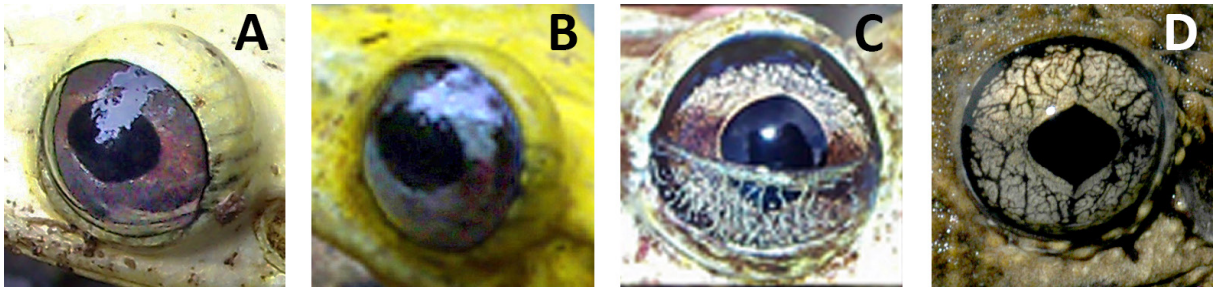

Figure 2. Eyes of **A-C.** *Litoria rueppelli*, **D.** *Litoria amboinensis*. Photo D. by Nick Baker.

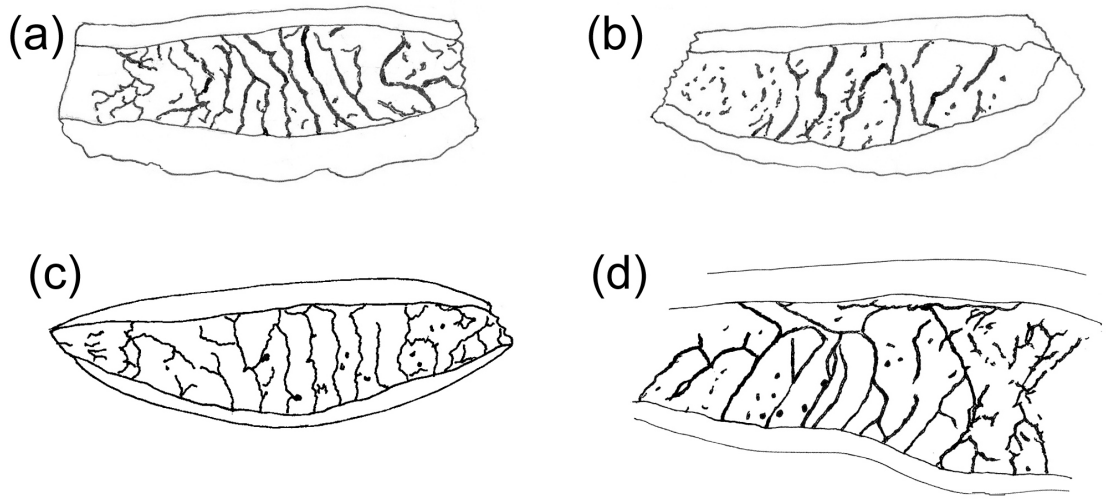

Figure 3. Lower left and right eyelids of *Litoria rueppelli*, **A, B.** SAM R68247 and **C, D.** SAM R 68246; D. is drawn at a higher magnification.

in New Guinea, *Litoria amboinensis*, *L. darlingtoni* and *L. rothi*. Pupil shape in *Litoria amboinensis* confused Brongersma (1953) who transferred the species, on that account, to *Nyctimystes* where it remained until Zweifel (1958) returned it to *Hyla* (now *Litoria*).

Palpebral reticulum

Fig. 3.A and B show the left and right lower eyelids of one specimen, C and D are left and right eyelids of another. The palpebral reticulum consists, in all cases, of thin, meandering, anastomosing, gold lines, supplemented by numerous, isolated pigment spots and covers the entire eyelid. Other specimens examined showed similar patterns but with much variation in the relative number of lines or dots. The pattern, with its wavering lines and dots, is unlike that of any *Nyctimystes* species known (see Menzies 2006, figure 17 for ten different examples) and there is no "typical" eyelid pattern for the genus. The eyes of a number of *Litoria amboinensis* were carefully examined but no trace of a reticulum could be seen in any.

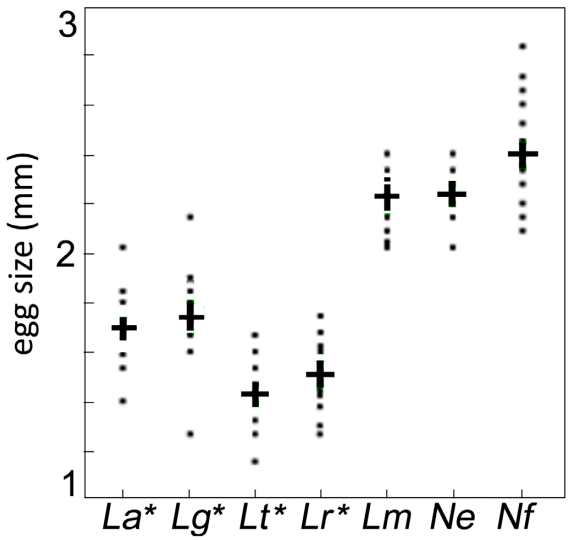

Figure 4. Comparative egg sizes in some New Guinean hylid frogs. *La**, *Litoria amboinensis*; *Lg**, *Litoria genimaculata*; *Lt**, *Litoria thesaurensis*; *Lr**, *Litoria rueppelli; Lm*, *Litoria micromembrana*; *Ne*, *Nyctimystes eucavatus*; *Nf*, *Nyctimystes foricula*. Horizontal bars are the means and vertical bars are their standard deviations. * denotes species with pigmented ova.

Ova

The pigmented ova of *Nyctimystes rueppelli*, taken from two gravid females, have a mean diameter of 1.52 mm (n = 17). For comparison, a sample of ova was taken from gravid females of six other *Litoria* and *Nyctimystes* species (fig. 4). The ova of *Nyctimystes rueppelli* are smaller, on average, than those of the three species that have unpigmented ova and more in line with the pigmented ova of *Litoria amboinensis*, *L. genimaculata* and *L. thesaurensis*. The measurements are probably an underestimate, due to shrinkage and distortion in preservative, but remain useful for comparison.

The larvae of *Nyctimystes rueppelli* are still unknown and the five shrivelled, young specimens in Frankfurt show no larval characters except reducing tails.

DISCUSSION

Results described above show that *Hyla rueppelli* should never have been assigned to *Nyctimystes*, but the limited material available, and with little likelihood of getting more from remote Halmahera Island, left Zweifel (1958) with little choice but to place it there, as no known Papuan *Litoria* species displayed a palpebral venation. Although it does have a palpebral reticulum, *Hyla rueppelli* does not have a vertical pupil, characters which, in combination, are the two diagnostic features of the genus (Zweifel 1958). *Hyla rueppelli* fails to show the synapomorphies of *Nyctimystes* and is therefore removed from that genus and transferred to *Litoria* as *Litoria rueppelli* (Boettger). Other points of difference from *Nyctimystes* species are the pigmented ova and the occurrence, on Halmahera Island.

With the removal of *rueppelli*, the genus *Nyctimystes* is now confined to New Guinea and satellite islands, and all known species have unpigmented ova.

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APPENDIX 1

List of specimens examined

Litoria rueppelli

(AMNH, American Museum of Natural History, New York; BMNH, Natural History Museum, London; CM, Carnegie Museum, Philadelphia; MZB, Museum Zoologicum Bogoriense, Cibinong, Indonesia; NHMB, Naturhistorisches Museum, Basel, Switzerland; NMW, Naturhistorisches Museum, Vienna, Austria; RMNH, Naturalis Museum, Leiden, Netherlands; SAM, South Australian Museum, Adelaide, Australia); SMF, Senckenberg Museum, Frankfurt, Germany.

AMNH 23759-60 (data from Zweifel 1958). Kau, Halmahera

BMNH 95.10.26.19-22; North Halmahera

CM 25554-55 (data from Zweifel 1958); Morotai Island

MZB16517; 16519-16524; 16527-16528; 16531-16532-16534; 16536-16540; 16542-16543; 16546; 16548; I. Desa Iga, Kecamatan Wasile Utara, Kabupaten Halmahera Timur. 1.39N; E 128.26; 165 m asl; and II. Tjetje, Halmahera, Weda Bay 1.39N; E 128.26; 161 m asl

NHMB 2383, North Halmahera

NMV 5899-5901, North Halmahera

RMNH 12368; Gebe (or Gag) Island

SAM R13800; R68246-47; central Halmahera.

SMF 2614-2617; North Halmahera.

Other species

Litoria amboinensis SAM R4890

Litoria genimaculata SAM R10747

Litoria micromembrana SAM R8924A, 13269

Litoria thesaurensis SAM R60645; 64785

Nyctimystes eucavatus SAM R5210B; 5210C; 5210Ae

Nyctimystes foricula SAM R5209A, 5209C