

IDENTIFICATION AND EVOLUTION OF AUSTRALIAN TORRENT TREEFROGS (ANURA: HYLIDAE: *LITORIA NANNOTIS* GROUP)

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All four species of Australian Torrent Treefrogs (*Litoria nannotis* group) have declined since 1990 and are classified as Endangered. I address confusion in identification and reconstruct evolutionary relationships among Torrent Treefrogs. Species differ particularly in size, calls, head shape, webbing, and secondary sexual characteristics such as the form and distribution of nuptial spinules. Torrent Treefrogs are united by spinose nuptial pads, *Litoria rheocola* is basal within the group and the clade (*nyakalensis*, (*lorica*, *nannotis*)) is defined by coarse nuptial spines, enlarged thumbs in males, and robust forearms. The only external characters which separate *nannotis* from *lorica* are size and slight differences in snout shape. Mitochondrial DNA from *nannotis* and *rheocola* suggest that this group arose well before the late Miocene - Pliocene contraction of rainforest in eastern Australia. *Litoria lorica* is known from two published localities on the Thornton uplands. I report two further localities, one of which represents the last sighting of *lorica* in the wild, and a series of museum specimens from Carbine Tableland. Neither *nyakalensis* nor *lorica* have been seen since 1991 and they should be classified as Critically Endangered. Recent discoveries of other declining species and this range extension for *lorica*, suggest a need for intensive surveys of the Carbine and Thornton uplands. □ *Litoria*, decline, phylogeny, distribution, conservation.

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Australian Torrent Treefrogs comprise four species of *Litoria* (Australian Waterfall frog, *nannotis*; Common Mistfrog, *rheocola*, Mountain Mistfrog, *nyakalensis*, and Armoured Mistfrog, *lorica*) which, although modest, is the second-most speciose vertebrate radiation in the Wet Tropics rainforests (after *Cophixalus* frogs, with ten endemic species). Torrent Treefrogs share adaptations to life along torrent streams, such as dorsoventrally flattened, suctorial tadpoles, and adults are rarely observed away from riparian microhabitats (Hodgkison & Hero, 2002). These species differ in appearance, habitat choice and life history. *Litoria nannotis* is usually confined to areas around cascades and waterfalls, with tadpoles generally found under rocks in cascades (Hodgkison & Hero, 2001). *Litoria lorica* closely resembles *nannotis* and appears to be similarly confined to cascades along torrent streams. *Litoria nyakalensis* and *rheocola* resemble each other in appearance and ecology and are more evenly distributed along streams than the preceding two species (Liem, 1974). *Litoria nannotis*, *rheocola* and *nyakalensis* differ markedly in mating calls (Hero & Fickling, 1994) but due to their torrent stream habitat these calls are difficult to record and have not been

compared among populations, within species. The call of *lorica* has not been described.

Distributional records of Torrent Treefrog species are nested across altitudes and latitudes (Fig. 1) but see below for current distribution. All four species occurred in sympatry at several upland sites on the Thornton Massif (145° 20'26"E, 16°03'11"S), and *lorica* is known only from these sites (Covacevich & McDonald, 1993), and from a site on the Carbine Tableland, reported here. *Litoria nyakalensis* occurred with *nannotis* and *rheocola* above 400m, between the Thornton Massif and the Kirrama Range (145°48'E, 18°13'S) (McDonald, 1992). Bioclimatic models predict suitable climates for *nyakalensis* on the Windsor Tableland to the northwest, (144° 58'-145°09'E, 16°10'-18'S), but it has not been recorded in this area (Nix & Switzer, 1991). *Litoria rheocola* has a wide distribution in lowlands and uplands between Amos Bay (145°19'-20'E, 15°41'S) at the northern end of the Wet Tropics rainforest, and Broadwater Ck (145°57'E, 18°23'S) near the Herbert River (Covacevich & McDonald, 1993). *Litoria nannotis* occurs throughout the distribution of the other three species, from foothills to uplands, and is the sole species

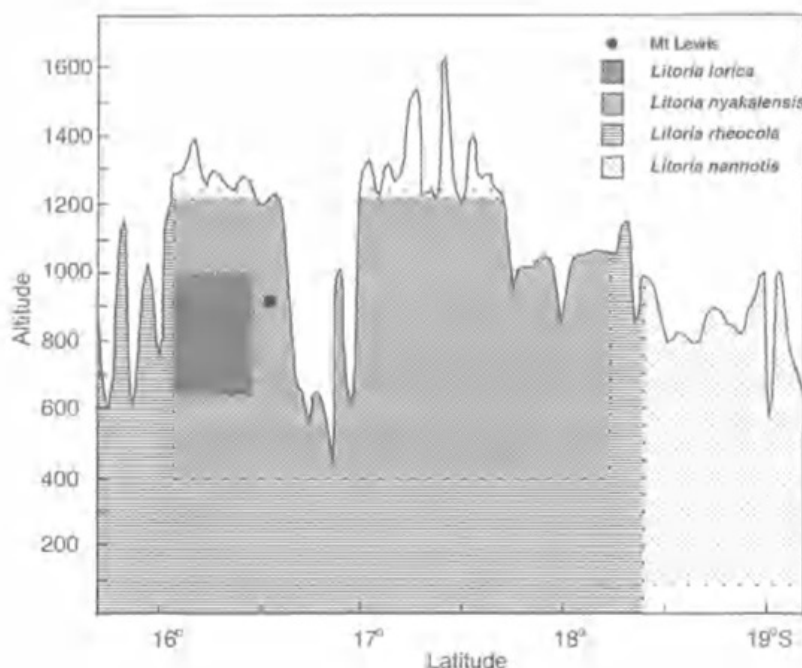


FIG. 1. Schematic profile of the Wet Tropics showing altitudinal and latitudinal range of Torrent Treefrog species and location of Mt Lewis Rd.

present south of the Herbert River to the Blue-water Range (146°27'E, 19°11'S) (Covacevich & McDonald, 1993; Fig. 1). In the last decade, all Torrent Treefrog species have undergone catastrophic declines, particularly upland populations (> 300m), and these frogs are no longer found in many former localities (Richards et al., 1993; McDonald & Alford, 1999). All four species are formally listed as 'Endangered' under Queensland and Commonwealth legislation.

Although Liem (1974) and Davies & McDonald (1979) carefully described differences among Torrent Treefrogs, and provided keys, there is still confusion over the identification of *lorica* and *nyakalensis*. Photos, pictures and descriptions in field guides are inconsistent and may be misleading (Hero 1996a). For example, a photo labelled *nyakalensis* in Nix & Switzer (1991: 94) appears to be *rheocola* and the painting Nix & Switzer (1991: 92) inaccurately depicts *lorica* colouration and micro-habitat; in Tyler (1992: 26, 32), a photo of *nyakalensis* appears to be *rheocola* and vice versa; in Cogger (1992: 144), a photo from Windsor Tableland is labelled *lorica*; this photo is again labelled *lorica* in Barker et al. (1995: 137), but is emended to *rheocola* in Cogger (2000), the photo of *nyakalensis* in Hero & Fickling (1994: 144) may be *rheocola*. It seems that there are few published

photographs of *nyakalensis* (but see Barker et al., 1995:139; Tyler, 1992: 32; Queensland Parks & Wildlife Service, 2000; McDonald, 2000:180), and no photographs of *lorica* are known.

Evolutionary relationships within *Litoria* are poorly resolved but morphological (Tyler, 1982) and immunological (Hutchinson & Maxson, 1987) comparisons suggest that the Torrent Treefrog radiation is independent of other torrent-adapted hylids in Australia and New Guinea, and is therefore an important component of phylogenetic diversity in Hylidae. Within Torrent Treefrogs the ecologically similar species pairs (*nannotis*, *lorica*) and (*rheocola*, *nyakalensis*) share morphological features and are difficult to distinguish from one-another (Davies & McDonald, 1979). Liem (1974), however, regarded *rheocola* as

the 'ancestral stock' and presented a phylogenetic hypothesis with *nyakalensis* sister group to *nannotis*.

The primary aim of this paper is to clarify differences among Torrent Treefrogs and to provide a revised guide for field identification. Secondly, I use these differences to reconstruct a phylogeny of this group. I compare this phylogeny against mitochondrial DNA divergence within and between *nannotis*, *rheocola* and outgroup species. Only formalin fixed museum specimens are available from *nyakalensis* or *lorica*, so it is difficult to address Liem's hypothesis with DNA sequence data, or to determine genetic divergence among sister species. Thirdly, I refer museum specimens from the Carbine Uplands to *lorica* and report the last sighting in the wild of this species.

COMPARISONS AMONG TORRENT TREEFROGS

Existing primary literature reports 44 differences among Torrent Treefrogs (Liem, 1974; Davies & McDonald, 1979; Richards, 1992; Hodgkison & Hero, 2002). These include 12 internal structures, and 14 ecological, behavioural, developmental, colouration or geographical differences (reviewed above) which could not be scored from preserved specimens. Type

collections in the Queensland Museum, including holotypes of *lorica*, *nyakalensis* and *rheocola*, along with additional specimens, were examined for the remaining 21 aspects of external morphology. Specimens were also measured for 10 morphometric traits following (Tyler, 1968) and (Alford et al., 1999): Snout-Vent length, SV; Tibial Length, TL; Forearm Length, FL; Interlimb Length, IL, from axilla to groin; Head Width, HW; Head Length, HL; InterNasal distance, IN; Eye - Nasal distance, EN; Eye Diameter, ED; Tympanum - Nasal distance, TN, from the anterior edge of the tympanum to the posterior edge of the nostril. Specimens examined are given in appendix.

Differences among Torrent Treefrog species (Table 1) include aspects of external morphology, features 1-14, which are grouped as a key and listed, within each group, according to ease of use in the field. Features 1-6 separate the ecological pairs, (*nannotis* + *lorica*) from (*nyakalensis* + *rheocola*). Features 7-12 separate *nyakalensis* from *rheocola*. Features 13 and 14 separate *nannotis* from *lorica*. Morphometric ratios incorporating SV, TL, FL, HW and HL measurements (features 29-31) show strong differences in mean values but overlap in range, and should be used as corroborative evidence in identification. Features 23-28 are colouration, larval and behavioural traits applicable to live specimens or preserved tadpoles (see Richards, 1992 for a tadpole key). Features 15-22 are osteological which generally require cleared-and-stained specimens or skeletal preparations, but may also be visible in X-ray radiographs. Features 7 and 12 include both external appearance and associated osteological features, which are not essential to field identification but add resolution to species discrimination. For example in character 7, thumbs of *nannotis* and *lorica* are similar in external appearance but differ internally in the number of metacarpal flanges, conversely, the enlarged prepollex of *lorica* and the flattened, conical thumb of *nyakalensis* differ externally but are osteologically similar (Davies & McDonald, 1979).

Species differ particularly in head shape, webbing, size and secondary sexual characteristics. *Litoria nannotis* and *lorica* have short, broad heads ($HW/SV > 0.35$; $HL/HW < 1.01$) with a rounded canthus rostralis and concave loreal area. *Litoria lorica* has a truncate snout, when viewed from above or in profile, with the nostrils virtually at the tip. *Litoria nyakalensis* and *rheocola* are more gracile ($HW/SV < 0.36$;

$HL/HW > 0.93$) with *nyakalensis* somewhat intermediate between the relatively angular head and protruding snout of *rheocola* and the rounded head and snout of *nannotis*. This difference is difficult to characterise but may be seen in ventral view where the triangular snout of *rheocola* protrudes beyond the margin of the mandible, whereas the margin of the snout of *nyakalensis* tends to approximate the jaw line. Torrent Treefrogs have sparsely webbed fingers and near fully webbed toes. Although webbing varies within species (Davies & McDonald, 1979) there are also consistent differences between species and these are the most useful characters for separating female *rheocola* from female *nyakalensis*. In most *rheocola* specimens webbing reaches the second-subarticular tubercle at the end of the first phalanx, measured on the outer edge of the third finger (Fig. 2A). In *nannotis*, *lorica* and *nyakalensis* hand-webbing is less extensive and rarely extends more than half-way along the first phalanx (Fig. 2B). One *nyakalensis* specimen (J29287 from Mt Lewis Rd) has extensive finger-webbing, but agrees with *nyakalensis* in other respects. In *nannotis*, *lorica* and *rheocola* webbing along the fifth toe extends to intercalary element or disc (Fig. 2C), whereas *nyakalensis* has slightly reduced toe-webbing, which does not extend $>2/3$ along the first phalanx of the fifth toe (Fig. 2D).

Litoria nannotis is larger than other Torrent Treefrogs, and body-size varies geographically in this species. The Carbine Tableland population comprises very large individuals (male SV 54-60mm, Mt Lewis Rd, Fig. 1) whereas remaining populations range from 40-53mm in male SV (pers. obs., I shall discuss the status of the Carbine population elsewhere). Other species are smaller and similar to each other in size, but there is considerable variation in *nyakalensis*, males 30-31mm SV among type specimens, from the southern Atherton Tableland, and males up to 41mm SV from other areas. Males of *nannotis*, *lorica* and *nyakalensis* have robust, enlarged arms, evident in sexual dimorphism for relative limb length (FL/TL males $>$ females, Table 1). *Litoria rheocola* does not have enlarged arms and differs from *nyakalensis* males on this ratio (male FL/TL, *nyakalensis* $>$ *rheocola*, Table 1). Mature male *lorica* and *nannotis* have accessory spines on the chest, and develop further spines around the jaws and legs during the breeding season. In males of these species the proximal $2/3$ of the thumb, around the prepollex, is enlarged up to three times the base thumb width and the dorsal

TABLE 1. Morphological variation in Torrent Treefrogs.

Feature	<i>Litoria nannotis</i>	<i>Litoria lorica</i>	<i>Litoria nyakalensis</i>	<i>Litoria rheocola</i>
1. Accessory spinules on chest, limbs and face (♂♂)	Present (a)	Present (a)	Absent (b)	Absent (b)
2. Tympanic annulus	Indistinct or obscured (a)	Indistinct or obscured (a)	Distinct (b)	Distinct (b)
3. Expansion of discs on fingers 2, 3, 4, cf. finger-width at base of discs	Large, > 2 × finger-width (a)	Large, > 2 × finger-width (a)	Moderate, < 2 × finger-width (b)	Moderate, < 2 × finger-width (b)
4. Canthus rostralis in cross section, horizontal curvature	Rounded, indistinct, moderately curved (a)	Rounded, moderately curved (a)	Distinct, ± angular, pronounced curvature (b)	Sharp, angular ridge, ± straight or only slightly curved (b)
5. Loreal curvature in cross-section	Distinctly concave (a)	Strongly concave (a)	Slightly concave (b)	Not or only slightly concave (b)
6. Snout length (horizontally from eye to tip-of-snout)	Shorter than eye diameter (a)	Shorter than eye diameter (a)	Longer than eye diameter (b)	Longer than eye diameter (b)
7. Prepollex and thumb, bony flanges on the first metacarpal (♂♂) (ordered: a-b-c)	Greatly enlarged for proximal 2/3, multiple metacarpal flanges (a)	Greatly enlarged for proximal 3/4, single metacarpal flange (b)	Enlarged to terminal disc, conical appearance, single metacarpal flange (b)	Not enlarged, no metacarpal flange (c)
8. Nuptial pad, dorsal surface of thumb and prepollex (♂♂) (ordered: a-b-c)	Large, dense covering of coarse spines (a)	Large, dense or sparse covering of coarse spines (a)	Large (> 1/2 thumb; gap < thumb-width between nuptial pad and disc), coarse spines (a)	Small (< 1/2 thumb; gap > thumb-width to disc), oval pad of fine spines (b)
9. Terminal disc on thumb, cf. width at base of disc	Distinctly wider than thumb (a)	Distinctly wider than thumb (a)	Reduced, ± thumb width (b)	Distinctly wider than thumb (a)
10. Hand webbing along the outer margin of the longest finger (between fingers 3 & 4)	0-3/5 length of proximal phalanx (average 1/5) (a)	1/3-2/3 proximal phalanx (average 1/2) (a)	0-1/1 (complete length of proximal phalanx)* (average 1/3) (a)	1/2-1/1 (complete length of proximal phalanx) (average 4/5) (b)
11. Foot webbing along the inner margin of shortest toe, (between toes 1 & 2)	1/2-1/1 along proximal phalanx to disc (average >4/5) (a)	1/1, complete, reaches base of disc (all specimens 1/1) (a)	0-3/4 along the proximal phalanx (average 1/3) (b)	3/4-1/1, from end of proximal phalanx to disc (average >4/5) (a)
12. Forearm width, bony flanges on humerus (♂♂) (Davies & McDonald 1979, fig. 5)	Robust, pronounced dorsal and ventral humeral flanges (a)	Robust, moderate humeral flanges (a)	Robust, moderate humeral flanges (a)	Slight, no flanges (b)
13. Adult body size	Large, SV > 40mm (average: ♂ 48mm, ♀ 51mm) (a)	Moderate, SV < 40mm (average: ♂ 33mm, ♀ 34mm) (b)	Moderate, SV < 41mm (average: ♂ 35mm, ♀ 36mm) (b)	Moderate, SV < 40mm (average: ♂ 31mm, ♀ 35mm) (b)
14. Snout shape, position of nostrils relative to tip-of-snout and end-of-mouth, in profile view	Bluntly rounded, nostrils slightly posterior to snout, ± level with mouth (a)	Truncate, nostrils ± terminal, slightly anterior to mouth (b)	Bluntly rounded to pointed, nostrils posterior to snout and mouth (a)	Bluntly pointed, nostrils posterior to snout, ± level with mouth (a)
15. Nasals - sphenethmoid	In contact (a)	Separated (b)	Overlapping (a)	Overlapping (a)
16. Frontoparietal fontanelle	Elongate, length > 3 × width (a)	Elongate, length > 3 × width (a)	Oval, length < 3 × width (b)	Oval, length < 3 × width (b)
17. Ossification of medial prootic	Robust, complete (a)	Incomplete (b)	Robust, complete (a)	Robust, complete (a)
18. Quadratojugal	Complete, articulating with squamosal (a)	Complete, articulating with squamosal (a)	Incomplete, does not contact squamosal (b)	Incomplete, does not contact squamosal (b)
19. Preorbital process on pars facialis of maxillary	Present (a)	Absent or indistinct (b)	Absent or indistinct (b)	Absent or indistinct (b)
20. Alary processes of pre-maxillae, in dorsal view	Not projecting beyond jawline (a)	Projecting forward beyond jawline (b)	Projecting forward, beyond jawline (b)	Projecting forward, beyond jawline (b)
21. Dentigerous processes of prevomers	Truncate, 4-5 teeth (a)	Moderate, 6-7 teeth (b)	Short, 4-6 teeth (a)	Short, 4-6 teeth (a)
22. Intercalary elements	Cartilage (a)	Cartilage (a)	Bone (b)	Bone (b)
23. Dorsal head colouration (live)	Similar to dorsum, mottled slate (a)	Similar to dorsum, uniform or mottled (a)	Similar to dorsum, uniform or mottled (a)	Conspicuous lighter triangle from interorbital to snout (b)
24. Ventral colouration (live)	Cream (a)	Cream (a)	Cream, pink flush (b)	Cream (a)
25. Iris colouration (live)	Silver to golden (a)	Silver to golden (a)	Golden or sandy to brown, not reddish (a)	Dark red to russet brown (b)
26. Larval colouration	Diffuse dark pigment on tail and fins, fins yellow tinged with yolk cells	Unknown	Discrete, dark blotches on cream tail and clear fins	Diffuse dark pigment on tail, clear, sparsely pigmented fins
27. Larval oral papillae on anterior upper labium	Two or more complete rows	Unknown	One complete row	One complete row
28. Call (♂)	Deep 'crawk, crawk, crawk' and quiet popping	Unknown	Rasping, slow growl	Harsh, repetitious 'wreck'
29. Head shape (HL/HW). Range; mean ♂, mean ♀	Range: 0.87-1.01 ♂: 0.93, ♀: 0.94	Range: 0.89-0.95 ♂: 0.92, ♀: 0.92	Range: 0.93-1.04 ♂: 1.01, ♀: 1.00	Range: 0.94-1.11 ♂: 1.04, ♀: 1.00
30. Limb ratio (FL/TL). Range; mean ♂, mean ♀	Range: 0.37-0.51 ♂: 0.46, ♀: 0.43	Range: 0.40-0.55 ♂: 0.47, ♀: 0.45	Range: 0.44-0.55 ♂: 0.50, ♀: 0.46	Range: 0.40-0.47 ♂: 0.44, ♀: 0.44
31. Relative head-width (HW/SV). Range; mean ♂, mean ♀	Range: 0.35-0.42 ♂: 0.37, ♀: 0.36	Range: 0.33-0.38 ♂: 0.37, ♀: 0.34	Range: 0.31-0.36 ♂: 0.33, ♀: 0.33	Range: 0.32-0.34 ♂: 0.33, ♀: 0.33

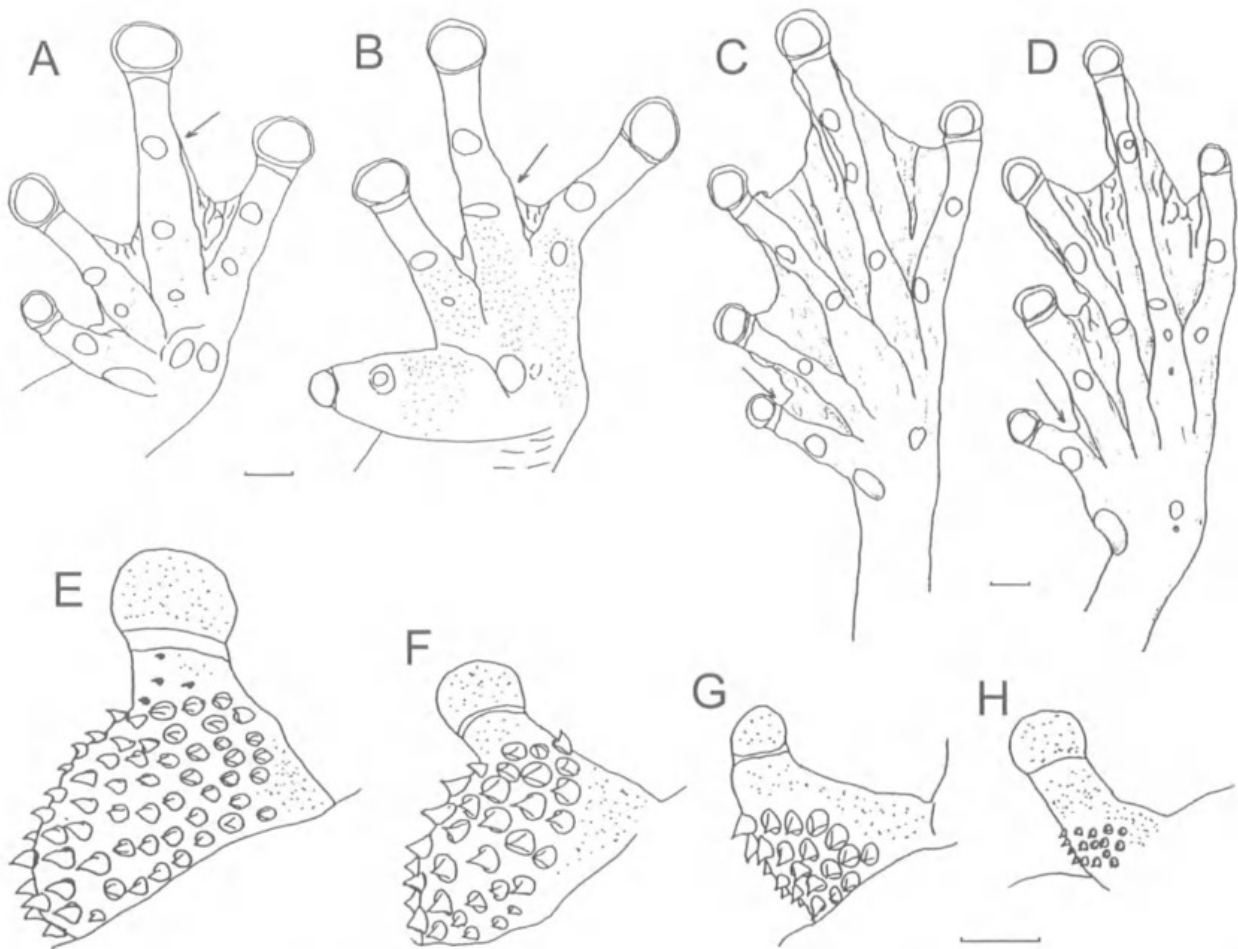


FIG. 2. Differences among Torrent Treefrogs in: Hand-webbing: A, *rheocola* J22631; B, *nyakalensis* J22624. Foot-webbing: C, *rheocola* J22631; D, *nyakalensis* J22624. Thumb shape and nuptial pad: E, *nannotis* J52965; F, *lorica* J36090; G, *nyakalensis* J22624; H, *rheocola* J22631. Arrows show differences in hand and foot-webbing between *rheocola* and *nyakalensis*. Scale bars: 1mm.

surface is covered in coarse nuptial spines. This part of the thumb is also enlarged in females, up to twice the base thumb width, but the distal third of the thumb is not enlarged in either sex (Fig. 2E, F). By contrast, in male *nyakalensis* the thumb is enlarged up to the disc, which is reduced in size, giving the thumb a conical appearance (Fig. 2G). Coarse nuptial spines cover most of the dorsal surface of the male *nyakalensis* thumb and the gap between this nuptial pad and the disc is less than the thumb width at the base of the disc (Fig. 2G). Female *nyakalensis* do not have an enlarged thumb, spines or enlarged arms, and closely resemble female *rheocola*. *Litoria rheocola* does not have enlarged thumbs and males have a small, oval, finely spinose nuptial pad on the prepollex, which is separated from the thumb disc by more than the base thumb width (Fig. 2H).

No other morphometric ratios or measurements gave significant separation among species. Liem (1974) suggested IN/EN as a diagnostic separating *nyakalensis* (> 0.98) from *nannotis* and *rheocola* (< 0.97). These variables are error prone and this ratio is inconsistent (Davies & McDonald, 1979), indeed, Liem's measurements misdiagnose the holotype of *nyakalensis* ($IN/EN = 0.91$). Nevertheless, on the limited samples considered here, IN/EN separated female *nyakalensis* (1.01-1.14) from female *rheocola* (0.93-0.96). The alternative ratio (HL/HW), suggested by Davies & McDonald (1979), gave similar mean values in both species. Liem (1974) noted a single outer metacarpal tubercle in *nannotis* and paired tubercles in *nyakalensis* and *rheocola* but this feature is often indistinct or grades within species from a single tubercle with two confluent lobes to paired tubercles. An inner

tarsal fold is present in all species but this varies from distinct to indistinct or absent within some *lorica* and *nyakalensis* specimens (cf. Liem, 1974, 'absent in *nyakalensis*'). Male *nannotis* do call (cf. Liem, 1974), although more commonly they may use other modes of communication (Torr, 1994). All species have a small tympanum which is covered with skin and varies with specimen preservation (Czechura et al., 1987), but the tympanic annulus is generally less distinct in *nannotis* and *lorica*.

PHYLOGENETIC ANALYSIS

Phylogenetic trees were derived using parsimony and outgroup rooting, with exhaustive searches in PAUP*4.01 (Swofford, 1998). The intent of this analysis is to examine morphological change within the group, not to challenge its monophyly, which was forced as a constraint on analyses. Previous phylogenetic analyses suggest that the *Citropa* group (represented here by *Litoria pearsoniana*) is a sister to the Torrent Treefrogs, and that the *Eucnemis* group (*Litoria serrata*) and *Lesueuri* group (*Litoria lesueuri*) are basal to this clade (Hutchinson & Maxson, 1987). Tyler & Davies (1979) suggest a phylogenetic association between *Nyctimystes dayi*, Torrent Treefrogs and the *Eucnemis* group. Representatives from each of these species groups were examined and scored as outgroups (Appendix). The dataset consists of characters 1-25 in Table 1 and nine additional characters which do not vary among Torrent Treefrogs (Table 2) (character, states a/b: 26, fringes along toes absent/present; 27, vocal sac absent/present; 28, ovarian eggs large, >1.5 mm/small, <1.5 mm diameter; 29, eggs unpigmented/pigmented; 30, larvae suctorial/non-suctorial; 31, larval tooth rows complete/incomplete; 32, larval papillae fully surround oral disk/anterior gap in papillae; 33, larval anal tube medial/dextral; 34, tympanum small, <5% SV/large, >5% SV). Characters seven and eight were ordered, with a single flange on the first metacarpal considered intermediate between no flange and multiple flanges (character 7), and finely spinose nuptial pads intermediate between non-spinose and coarsely spinose pads (character 8). All other characters were unordered. Photographs and field observations were used to corroborate scoring of external features and colouration. The yellow-tinged venter of male *lesueuri* and dark iris of *N. dayi* were considered unique, uninformative states (characters 24, 25; state c). Scoring of osteological characters was

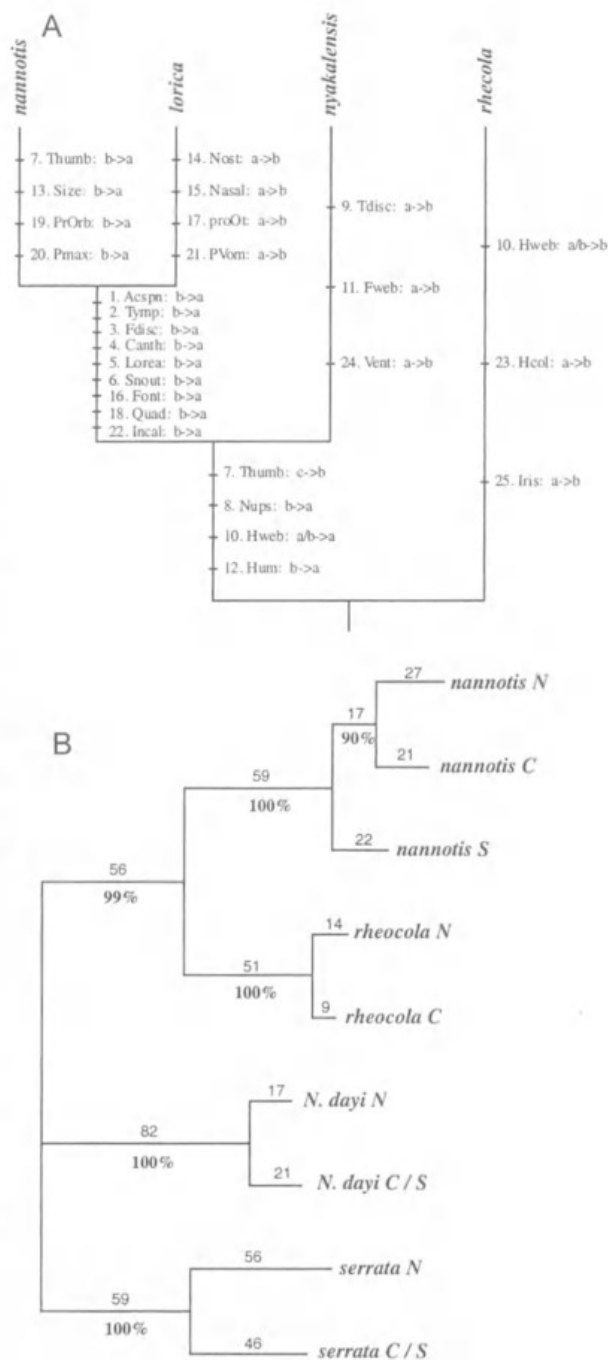


FIG. 3. A, phylogeny of Torrent Treefrogs based on 34 morphological characters showing state-changes along branches. Outgroups *serrata*, *lesueuri*, *pearsoniana* and *N. dayi* are not shown. B, mitochondrial DNA phylogeny among species and intraspecific lineages of *nannotis*, *rheocola*, *serrata* and *N. dayi*, from combined fragments of 16S (500bp) and cytochrome *b* (530bp) genes. Branch lengths are shown along with percentage bootstrap support. N = Northern, C = Central and S = Southern Wet Tropics.

based on published descriptions (Tyler & Davies, 1978; Davies & McDonald, 1979; McDonald & Davies, 1990) and cleared and stained specimens of *serrata*, with corroboration from related species (Eucnemis group: Tyler & Davies, 1979; Tyler et al., 1986). Tadpole characters were taken from the literature (Liem, 1974; Davies, 1989; Davies & Richards, 1990; McDonald & Davies, 1990; Richards, 1992; Anstis et al., 1998) and scored as ambiguous in *lorica*.

The single shortest tree, 60 steps, is identical in topology to Liem's (1974) hypothesis, with *rheocola* basal within Torrent Treefrogs (Fig. 3A). This tree differs from Liem's analysis in character support. Of the nine characters examined by Liem (1974), three are considered uninformative here (inner tarsal fold, outermetacarpal tubercle, male call), for reasons discussed above, and a further three are interpreted differently (the distinct tympanum and angular canthus rostralis are qualitatively similar in *nyakalensis* and *rheocola*, although these differ in degree, and the enlarged prepollex of male *nyakalensis* differs from *rheocola* cf. Liem, 1974). The consistency index for the tree is 0.63 and the retention index 0.58 with no homoplasy in ingroup comparisons. Within the constrained search space of 225 trees there were no 61 step trees and four 62 step trees (2 steps longer), one of which gave an alternate topology with *nyakalensis* and *rheocola* as sister species. The clade (*nyakalensis*, *lorica*, *nannotis*) is uniquely supported by enlarged thumbs with metacarpal flanges, coarsely spinose nuptial pads, and robust arms with humeral flanges (Fig. 3A). No unique character states support the competing hypothesis (*nyakalensis* + *rheocola*). When the constraint of ingroup monophyly was relaxed 10,395 trees were searched and two further 60-step trees were discovered, with *N. dayi* either as sister to *rheocola* or sister to *nannotis* + *lorica*. The close association of *N. dayi* with Torrent Treefrogs is supported by egg, larval and adult characters (large, unpigmented eggs; suctorial tadpoles with a median anal tube; tympani obscured by skin) which may be adaptations to torrent streams. Monophyly of Torrent Treefrogs is supported by a unique morphological trait; spinose nuptial pads.

Although data were not obtained from *nyakalensis* or *lorica*, mitochondrial DNA sequences from *nannotis* and *rheocola* are consistent with current understanding of Torrent Treefrogs (Fig. 3B). 16S and COI sequences were obtained from geographically separated lineages of *nannotis*, *rheocola*, *serrata* and *N. dayi*, in the Northern (N), Central (C) and/or Southern Wet Tropics (S). These samples represent the maximum divergence within each species. Genbank accession numbers are (COI, 16S: *nannotis* N AF304236, AF136325; C AF304243, AF136323; S AF304259, AF136322; *rheocola* N AF304283, AF136327; C AF304293, AF136326; *serrata* N AF304221, AF136299; C/S AF304222, AF136297; *N. dayi* N AF304268, AF136329; C/S AF304266, AF136328). Sequences were aligned by eye with alignment gaps in 16S treated as ambiguous states. The combined sequences (500bp 16S + 530bp COI) were analysed using an exhaustive parsimony search and 1000 bootstrap reanalyses in PAUP*4.01 (Swofford, 1998). The shortest tree, with 557 steps, is shown in Fig. 3B. There is strong support for *nannotis* + *rheocola* (100% bootstrap) and negligible support for other interspecific groupings, in this limited comparison. The shortest tree conflicting with this *nannotis* + *rheocola* clade was 575 steps (18 steps longer). Divergence within *nannotis* and *rheocola* is from one half to one eighth that between these species. Applying rough estimates of divergence rates, around 2% per million years for mitochondrial protein coding genes (Moritz, Dowling & Brown, 1987) and around 1% per million years for the ribosomal 16S gene (Caccone et al., 1997), to mean divergences between *nannotis* and *rheocola* (19.7% at COI and 10.4% at 16S, using HKY 85 distances from

TABLE 2. Phenotypic character state matrix. Character states in *nannotis* are coded 'a', with alternative states 'b' and 'c'. Characters 7 and 8 are ordered (a b c).

Character	1234567891111111111222222222233333 0123456789012345678901234
<i>nannotis</i>	aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
<i>lorica</i>	aaaaaabaaaaabbbababbbbaaaaaa????a
<i>nyakalensis</i>	bbbbbbbabababaababbbabababaaaaaaa
<i>rheocola</i>	bbbbbbcbababbaababbbabababaaaaaaa
<i>Nyctimystes</i>	babbabccababbaababbbababaaacbbaaaaaa
<i>pearsoniana</i>	bbbbbbccaabbbbaababbbababaaabbbbbbba
<i>genimaculata</i>	bbababccabababaaabaaaaaaaababbbbbb
<i>lesueuri</i>	bbbbbbccbabbaabaaaabaacaaabbbbaabb

PAUP*) suggests a mid-Miocene split within Torrent Treefrogs compared with roughly Pliocene splits among lineages within *nannotis* (COI 7.1–11.2%; 16S 2.2–2.4%), and Pleistocene divergence within *rheocola* (COI 3.3%; 16S 1.3%). The discrepancy in ratios of these values indicates that interspecific divergences are underestimates, particularly at COI, due to site saturation and rate variation among nucleotide sites.

GEOGRAPHIC DISTRIBUTION OF *LITORIA LORICA*. Several misclassified individuals were discovered among the specimens examined including one *N. dayi* registered as *nyakalensis*, and two *serrata*, two *nyakalensis* and one *lorica* registered as *nannotis*. In addition, a search of the combined USA herpetological collection register (www.calacademy.org/research/herpetology/Comb_Coll_Index) revealed further specimens registered as *lorica* in the University of Michigan collection (UMMZ). These *lorica* specimens are of particular interest as they extend the known range of this enigmatic species. QMJ42235, is a mature male with densely spinose nuptial pads and accessory spines on the chest, jaws and legs. The size (32.3mm SV) and truncate snout distinguish QMJ42235 from mature *nannotis* and there are no notable differences between this individual and *lorica* type specimens. Thirteen specimens from the University of Michigan were similarly consistent with *lorica*, but not with *nannotis*. At present *lorica* is known from only three sites on the Thornton uplands (Table 3). The maximum straight line distance between sites is approximately 10 km. Two of these are published records, from streams flowing off Thornton Peak into the Bloomfield and Daintree Catchments (Davies & McDonald, 1979; McDonald, 1992). The third is a personal observation at Roaring Meg Creek, below Mt Pieter Botte, from 3–5 December 1991. Numerous *lorica* (>12 individuals) were observed at night clustered in the splash-zone of a major cascade, and also in deep cracks beside this cascade during the day (DM Hillis, M Cunningham, C Moritz, pers.

obs.). Several *nannotis* were present at this cascade and at other points along the stream, and these two species were clearly distinguished by size. This appears to be the last observation of *lorica* in the wild. Other declining species (*rheocola* and *Taudactylus acutirostris*) were observed at the same locality at this time. I saw a single female *nannotis* at the same site on 5–6 April 1993, and none of these species was found during searches in November 1994 and 11–12 June 1995. Trennery (1994) attributed a torrent tadpole found in surveys of Roaring Meg Ck (23–24 August 1993) to *nannotis* but it is also conceivable that this was *lorica*, the tadpole of which is unknown.

Specimen QMJ42235 was collected by G. Montieth, D. Yeates and G. Thompson on 2 November 1983, on an unnamed tributary of the Mossman River which crosses the Mossman Bluff track at around 900m altitude. The UMMZ specimens were collected in the same area, '3.5 miles west of Mossman near vertical rock faces in turbulent stream bed', by J. Bailey on the 19 August 1971. These sites increase the linear range of *lorica* to 46km, with a total extent of 120km². More importantly, these records extend the range of *lorica* to a second upland rainforest area, the Carbine Tableland approximately 34km south of the type locality. Although all four Torrent Treefrogs have undergone sudden declines, the geographically restricted upland species, *lorica* and *nyakalensis*, have not been observed since December 1991 (above) and November 1990 (Hero & Fickling, 1994), respectively, despite ongoing monitoring and searches of historically known sites (Richards, et al., 1993; Trennery, et al., 1994; Hero, 1996b; Williams & Hero, 1998; Hero et al. 1998; McDonald & Alford, 1999). *Litoria lorica* and *nyakalensis* fulfil all criteria for listing as 'Critically Endangered' under the Commonwealth *Environment Protection and Biodiversity Conservation Act (1999)*, these being: severe declines, very restricted distribution, very low estimated population size and high probability of extinction from further declines. Recent

TABLE 3. Records and distribution of *Litoria lorica*. * see text for further details.

Site	Latitude	Longitude	Altitude	Year	Reference
Roaring Meg Ck	145°25'E	16°04'S	680m	1991	Pers. obs.*
Alexandra Ck	145°21'E	16°07'S	640m	1976	Davies & McDonald, 1979
Hilda Ck	145°23'E	16°10'S	1,000m	1990	Covacevich & McDonald, 1993
Mossman Gorge	145°17'E	16°28'S	?	1971	J. Bailey (UMMZ register)*
Mossman Gorge	145°17'E	16°28'S	900m	1983	G. Montieth, pers. comm.*

publications report the rediscovery of two other upland rainforest frogs, *Taudactylus rheophilus* and *Taudactylus acutirostris*, as small, isolated populations in the central and northern Wet Tropics, including Mt Lewis (Marshall, 1998; Hero et al. 1998). Increasing search effort has also resulted in the discovery of upland populations of *nannotis* and *rheocola* which may have been previously overlooked (Hero et al. 1998; McDonald pers. comm.). These discoveries fuel hope that remnant populations of *lorica* and *nyakalensis* remain to be discovered in the Wet Tropics. Rediscoveries of other species on the Carbine Tableland, together with these new records, suggest an urgent need for intensive surveys across the entire Thornton and Carbine uplands.

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APPENDIX

SPECIMENS EXAMINED.

Litoria nannotis - All QM specimens of this species between registration numbers J17017 to J71334. Sample sizes: ♂♂ 60; ♀♀ 44; Juveniles: 22.

Litoria lorica - ♂♂: J36090 (holotype), J36092-93 (QM paratypes), R17349-50 (SAM paratypes), J42235 (QM new record), UMMZ202427-39; ♀♀: J36091 (QM paratype), R17351 (SAM paratype).

Litoria nyakalensis - ♂♂: J22624 (holotype), J22625-30 (paratypes), J29287, J32117, J34415, J43680, J43682-83, J43856, J52942, J54188, J55584, J55598, J55768; ♀♀: J55592, J55595-96, J55770, J55776-77, J55786.

Litoria rheocola - ♂♂: J22631 (holotype), J22632-42 (paratypes); ♀♀: J22643-45 (paratypes).

Nyctimystes dayi - ♂♂: J61844, J66044, J66127-28; ♀♀: J66108.

Litoria genimaculata - ♂♂: J43650, J43653, J53685, J53688, J53707, J53743 (cleared & stained), J56093; ♀♀: J53741, J53747 (cleared & stained), J56092.

Litoria pearsoniana - ♂♂: J70861, J70894, J72661; ♀♀: J68178.

Litoria lesueuri - ♂♂: J35611, J35620-21; ♀♀: J36068.



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