Biological Journal of the Linnean Society, 2007, **91**, 549–563. With 7 figures

Description, biology and conservation of a new species of Australian tree frog (Amphibia: Anura: Hylidae: *Litoria*) and an assessment of the remaining populations of *Litoria genimaculata* Horst, 1883: systematic and conservation implications of an unusual speciation event

CONRAD J. HOSKIN*

School of Integrative Biology, The University of Queensland, St Lucia, Qld 4072, Australia

Received 6 January 2006; accepted for publication 10 August 2006

The Australian populations of the green-eyed tree frog *Litoria genimaculata* consist of a northern and southern genetic lineage that meet in a mosaic contact zone comprising two independent areas of contact: one where the main ranges of the lineages overlap, and the second where a population of the southern lineage is isolated within the range of the northern lineage. A recent study failed to find significant reproductive isolation between the main ranges of the two lineages, despite deep genetic divergence, partial postzygotic isolation, and call differences. The study did, however, demonstrate rapid phenotypic divergence and speciation of the isolated population of the southern lineage from both the parapatric northern lineage and from the allopatric, but genetically similar, main range of the southern lineage. Herein, the isolated population of the southern lineage is described as a distinct species, ***Litoria myola* sp. nov.**, whereas the remainder of the southern lineage and the northern lineage are retained as a single, paraphyletic species, *Litoria genimaculata*. Resolving this unusual systematic situation demonstrates the value of using multiple lines of evidence in delimiting species. ***Litoria myola* sp. nov.** has a very small distribution and population size and warrants a Critically Endangered listing (B1, 2) under IUCN criteria. Threats and management recommendations are outlined, and the conservation of hybrid zones as areas of evolutionary novelty is discussed. *Biological Journal of the Linnean Society*, 2007, **91**, 549–563.

ADDITIONAL KEYWORDS: allopatric speciation – contact zone – delimiting species – hybridization – reinforcement – Wet Tropics.

INTRODUCTION

The green-eyed tree frog, *Litoria genimaculata*, occurs in tropical rainforest in north-east Australia and is also widely distributed in New Guinea (Richards, McDonald & Ingram, 1993). The Australian and New Guinean populations are genetically highly divergent from each other and the species is deeply paraphyletic within the '*Litoria eucnemis*' species group (Moritz *et al*., 1997; Cunningham, 2001). The present study resolves the systematic relationship between the Australian populations, which are restricted to the 'Wet Tropics' region (between Townsville and Cooktown) of north-east Queensland. The Wet Tropics populations of *L. genimaculata* comprise two deeply divergent [13% cytochrome oxidase subunit I (COI) mtDNA] sister lineages: one in the north and one in the south (Schneider, Cunningham & Moritz, 1998; Cunningham, 2001; Hoskin *et al*., 2005). The level of divergence between these lineages is greater than seen between some recognized sister species of the '*L. eucnemis*' group (Moritz *et al*., 1997; Cunningham, 2001). A geographically congruent pattern of divergent northern and southern lineages has been recognized across a broad range of low vagility, rainforest-restricted vertebrates and invertebrates in the Wet

*Current address: School of Botany and Zoology, The Australian National University, Canberra, ACT 0200, Australia.
E-mail: conrad.hoskin@anu.edu.au

Tropics (Schneider *et al*., 1998, 1999; Hugall *et al*., 2002; Moritz *et al*., 2005). This pattern has been attributed to contraction of rainforest to isolated Pliocene and Pleistocene refugia in the northern and southern Wet Tropics (Joseph, Moritz & Hugall, 1995; Schneider *et al*., 1998; Hugall *et al*., 2002).

The lineages of *L. genimaculata* are currently in secondary contact in the central Wet Tropics (Fig. 1; Hoskin *et al*., 2005). This is an area of secondary contact between lineages of multiple species (Schneider *et al*., 1998; Phillips, Baird & Moritz, 2004) and the congruence in the location of these secondary contacts has led to the recognition of the central Wet Tropics as a suture zone (Phillips *et al*., 2004). The current suture zone is believed to have formed approximately 7500–6000 years ago when northern and southern lineages came back into contact with the spread of rainforest from refugia (Hugall *et al*., 2002; Phillips *et al*., 2004). Hoskin *et al*. (2005) used a combination of genetic, morphological and call analyses, experimental crosses, and mate choice trials to characterize the contact zone between the northern and southern lineages of *L. genimaculata* and assess the degree of reproductive isolation between them. The northern and southern lineage of *L. genimaculata* meet in a mosaic contact zone. This consists of a main area of contact between the lineages (Contact A) 13 km to the south of a second area of contact (Contact B), where an isolated population of the southern lineage (termed iS) occurs in the northern lineage area (Hoskin *et al*., 2005: fig. 1). Experimental crosses and genetic data revealed asymmetric postzygotic isolation between the lineages: crosses between southern females (including iS) and northern males fail, whereas the reciprocal crosses are successful (Hoskin *et al*., 2005).

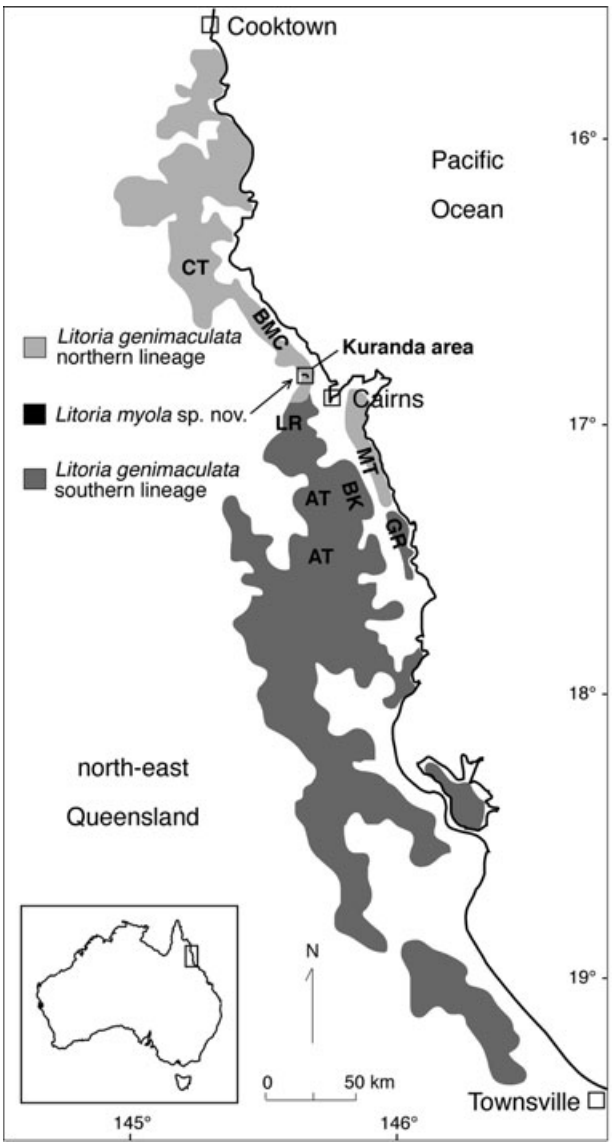

Figure 1. Distribution of ***Litoria myola* sp. nov.** (formerly termed iS) and the northern (N) and southern (S) lineages of *Litoria genimaculata* in the Wet Tropics, north-east Queensland. CT, Carbine Tableland; BMC, Black Mountain Corridor; LR, Lamb Range; BK, Bellenden Ker Range; AT, Atherton Tableland; MT, Malbon Thompson Range; GR, Graham Range.

No morphological or ecological differences were detected where the lineages meet at the main contact (Contact A), but a significant difference in male call is evident (Hoskin *et al*., 2005). However, females from Contact A did not show significant positive assortative mating when these calls were used in laboratory-based mate choice trials (Hoskin *et al*., 2005). At Contact B, the iS population was found to have diverged significantly in male size and call from the co-occurring northern lineage (Hoskin *et al*., 2005). Once again, no ecological divergence was detected. Laboratory-based mate choice trials revealed highly significant premating isolation between the iS population and co-occurring northern lineage. A preliminary genetic analysis of the mosaic contact zone supported these results by concluding that hybridization is significantly lower at Contact B (0–1.4%), where the northern lineage and iS co-occur, than at the main contact between the northern and southern lineage (Contact A, 3.1–6.8% hybrids) (Hoskin *et al*., 2005). The iS population was also found to have diverged significantly in male size and call from the genetically similar allopatric main range of the southern lineage and mate choice trials revealed highly significant premating isolation between iS and the remainder of its southern lineage (Hoskin *et al*., 2005).

Hoskin *et al*. (2005) concluded that the process of reinforcement (driven by natural selection against maladaptive hybridization between the northern and southern lineage) has led to rapid speciation of iS from both the co-occurring northern lineage and also, inci-

dentally, from the main range of the southern lineage. By contrast, they concluded that speciation has not occurred at the main contact (Contact A) between the northern and southern lineages. Speciation was defined by statistically significant reproductive isolation, which is similar to the 'substantial reproductive isolation' interpretation of the Biological Species Concept defined by Coyne & Orr (2004). The present study deals with the systematic and conservation implications of these conclusions. The iS population is herein described as a distinct species (*Litoria myola* sp. nov.) that is diagnosed from *L. genimaculata* by male size and call. The biology, threatened status, and conservation of this new species are outlined, and the importance of preserving the evolutionary potential of contact zones is discussed. The remaining Australian populations of *L. genimaculata*, comprising a deeply divergent northern and southern lineage, are retained as a single, paraphyletic species, pending further investigation of Contact A. The value of the approach used here to delimit species, that combines multiple lines of data with direct and genetic tests of reproductive isolation, is discussed.

MATERIAL AND METHODS

Morphological measurements

Measurements of the type series were taken from alcohol-preserved specimens held in the Queensland Museum. All other measurements were taken from live individuals in the field. All measurements were taken using Mitutoyo vernier callipers to the nearest 0.1 mm. Measurement of the following characters follows Zweifel (1985) and Hoskin (2004): snout–vent length (SVL), tibia length (TL), head width (HW), head length (HL), body width (BW), eye diameter (ED), eye to naris distance (EN), distance between the nares (IN), third finger disc width (3DW), third finger length (3FL), fourth toe disc width (4DW), and fourth toe length (4TL). Only SVL, TL, and HW were measured from live individuals in the field. Additionally, field measurements included weight, measured to the nearest 0.05 g using spring-loaded Pezolas. Only mature individuals were measured, with mature males being determined by the presence of enlarged nuptial pads on the thumbs and the breeding status of females being determined by visual inspection for eggs through the body wall in the groin region. The lineage of individuals in the contact region was determined by genetic analysis of tissue samples (Hoskin *et al*., 2005).

Morphological analysis

A principal component analysis (PCA) was performed to provide a visual multivariate comparison of the difference in male morphology between *L. myola* sp. nov., *L. genimaculata* in the Kuranda area (where it co-occurs with *L. myola* sp. nov.), and all *L. genimaculata* across the Wet Tropics. The analysis was performed on field measurements of SVL, TL, HW, and weight, which were tested for normality within each group. The data for *L. genimaculata* were collected over a broad altitudinal range (30–1160 m) and the confounding effects of altitude on the each of the morphological traits was tested. Given the lack of altitudinal range in the *L. myola* sp. nov. data (20 m), the relationship between traits and altitude could not be tested in this group. Linear regressions were performed on the *L. genimaculata* data to test for, and remove, significant altitudinal effects (all traits showed a significant relationship with altitude) and the unstandardized residuals from this relationship were taken for both the *L. genimaculata* and *L. myola* sp. nov. data. Principal components 1, 2, 3, and 4 account for 87.8%, 5.9%, 3.8%, and 2.5% of the variance, respectively. Principal component 1 (PC1) is loaded equally and positively by all four characters (approximately 0.94 for all) and therefore represents body size, with large PC1 values corresponding to large body size. A PCA was not performed on the females because field measurements were available for too few individuals. The results from this analysis are consistent with those from detailed analyses performed by Hoskin *et al*. (2005) in which *L. myola* sp. nov. was included as the 'iS' population.

Call recording and measurements

All recordings were obtained in the field using a Sony Professional DAT recorder (TCD-D100) and an AKG microphone. Recordings were made at approximately 50 cm from the frog with gain controlled manually. For each individual, air temperature was recorded, morphology was measured, and a tissue sample was taken. The lineage of individuals in the contact region was determined by genetic analysis (Hoskin *et al*., 2005). Calls were sampled at 44 100 Hz on a Macintosh G4 and were analysed using the software Canary, version 1.2.1. Generally, four randomly chosen calls were analysed to give the average call characteristics for each male. Unless otherwise stated, call refers to courtship calls (those used to attract females) and only calls deemed to be courtship calls were used for analyses. The classification of call type was determined by observations of calling behaviour in the field and female response to calls in the laboratory. The courtship call is a regularly repeated call of predictable structure within each individual. Other advertisement calls, including interactions between males, are of longer duration and slower note rate than the courtship calls, and are highly variable in structure and call

spacing within each individual. The definition of call characteristics follow those outlined in detail in Zweifel (1985). Each call of *L. myola* sp. nov. or *L. genimaculata* consists of a series of notes (heard as 'tocs'). Herein the terms note, 'toc' and tap are synonymous. The following call characteristics were measured: call duration (beginning of the first note to the end of the last note of a call), number of notes, number of pulses in each note, note rate (number of notes per second), and dominant frequency (the frequency at which the call is of greatest intensity). Frequency modulation (change in dominant frequency over the duration of a call) was assessed by comparing the dominant frequency of several notes in each call with that of the call as a whole.

Call analysis

A PCA was performed to provide a visual multivariate comparison of the difference in call between *L. myola* sp. nov., *L. genimaculata* in the Kuranda area, and all *L. genimaculata* across the Wet Tropics. Call duration, dominant frequency and note rate were used for the analysis. Normality of each trait within each group was tested and an inverse transformation was performed on call duration to normalize this trait. Partial *F*-tests (Bowerman & O'Connell, 1990) were performed to test for confounding effects of air temperature on each of the call traits, with SVL included as a covariate in the models. Only note rate was found to have a significant relationship with temperature. Temperature effects were removed from the note rate data by performing a linear regression and taking the unstandardized residuals. Principal components 1, 2, and 3 account for 67.2%, 28.4%, and 4.4% of the variance, respectively. PC1 is loaded heavily by inverse call duration (0.95) and note rate (0.93), and moderately by dominant frequency (0.51). The results from this analysis are consistent with those from analyses performed by Hoskin *et al*. (2005) in which *L. myola* sp. nov. was included as the 'iS' population.

SYSTEMATICS

Class amphibia linnaeus, 1758

Order anura rafinesque, 1815

Family hylidae grey, 1825

Subfamily pelodryadinae günther, 1859

Genus *litoria* tschudi, 1838

Litoria myola sp. nov. (Kuranda tree frog) (Fig. 2)

This species is assigned to *Litoria* on the basis of external morphology and mitochondrial DNA sequence data. Species of the genus *Litoria* are easily distinguished from the only other hylid genus in Australia with large finger and toe pads, *Nyctimystes*, by having a horizontal pupil and lacking venation on the lower eyelids (Cogger, 2000).

Holotype: Deposited in the Queensland Museum, QMJ82420, male (calling when captured), Jumrum Ck. (16°49.3′S, 145°38.3′E), north-east Queensland, 330 m elevation, C. J. Hoskin and K. R. McDonald, 30 October 2001; measurements of preserved specimen (mm): SVL 36.1; TL 19.8; HW 12.4; HL 11.4; BW 8.9; ED 3.8; EN 3.0; IN 2.6; 3DW 2.0; 3FL 7.7; 4DW 1.8; 4TL 11.8; proportions: TL/SVL 0.55; HW/SVL 0.34; HL/SVL 0.32; HW/HL 1.09; BW/SVL 0.25; ED/SVL 0.11; EN/IN 1.15; 3DW/4DW 1.11; measurements in life: SVL 37.4 mm; TL 20.2 mm; HW 12.1 mm; 3DW 1.9 mm; weight 2.45 g.

Paratypes: QMJ82422-25, males (calling when captured), collection details as for holotype; QMJ82426, male (calling when captured) location details as for holotype, C. J. Hoskin and K. R. McDonald, 9 March 2001; QMJ82421, female (gravid and in amplexus

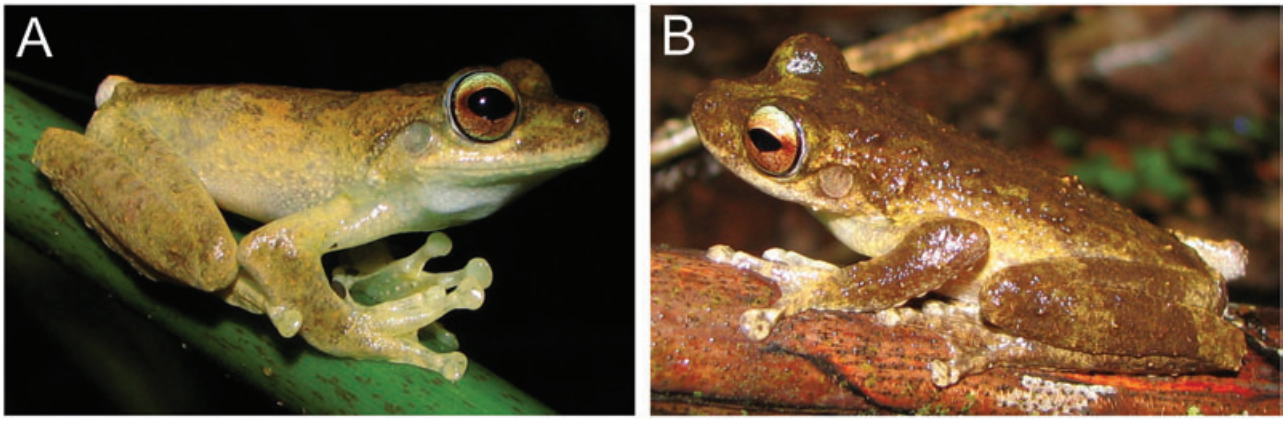

Figure 2. ***Litoria myola*, sp. nov.**, males (A, a pale individual; B, a heavily marked individual), Kuranda, north-east Queensland.

when captured), location details as for holotype, C. J. Hoskin and K. R. McDonald, 6 November 2001; QMJ82427, male (calling when captured), Kowrowa (16°48.2′S, 145°35.4′E), north-east Queensland, 335 m elevation, C. J. Hoskin and K. R. McDonald, 9 March 2001.

DIAGNOSIS

Litoria myola sp. nov. can be distinguished from all Australian congeners by its distinctive short, fast-tapping call (Fig. 3). *Litoria myola* sp. nov. is restricted to the Kuranda area (Fig. 1), where it co-occurs with *L. genimaculata*, the species to which it is most similar. Although there is some overlap in the range of the individual call characters between *L. myola* sp. nov. and *L. genimaculata* (Table 1), multivariate analyses clearly distinguish the two species, particularly in the Kuranda area (Fig. 4; Hoskin *et al*., 2005). In this area, the courtship call of *Litoria myola* sp. nov. is relatively easily diagnosed from that of *L. genimaculata* by its shorter duration (no overlap), faster note rate (no overlap), and generally higher dominant frequency (Table 1). *Litoria myola* sp. nov. can be further distinguished from *L. genimaculata* (from both the Kuranda area and the rest its range) because males of *L. myola* sp. nov. are smaller in all aspects of body size (Table 1; Fig. 5; Hoskin *et al*., 2005). In the Kuranda area, an SVL cut-off of 42 mm, or a weight cut-off of 3.75 g, separates 90% of the males of these two species (weight is the more reliable measure because it is not reliant on measuring technique). Although *L. myola* sp. nov. females are generally smaller than those of *L. genimaculata* (Table 1), there is no significant difference in size between *L. myola* sp. nov. and *L. genimaculata* where the two species co-occur in the

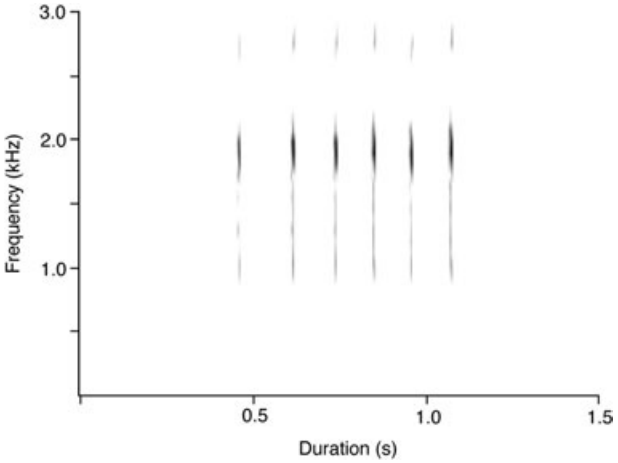

Figure 3. Spectrogram of the courtship call of ***Litoria myola* sp. nov.** (recorded at an air temperature of 25 °C). The spectrogram displays a single courtship call consisting of six notes ('tocs'). The degree of shading displays call intensity.

Table 1. A comparison of call and morphological traits between ***Litoria myola* sp. nov.**, co-occurring populations of *Litoria genimaculata* in the Kuranda area, and populations of *L. genimaculata* across the rest of the Wet Tropics, north-east Queensland

Trait	***Litoria myola* sp. nov.**	*N*	*Litoria genimaculata* Kuranda area	*N*	*Litoria genimaculata* Wet Tropics	N
Courtship call						
Call duration (s)	0.85 (0.57–1.35)	19	2.69 (1.68–4.36)	23	2.06 (0.73–5.38)	89
Note rate (notes s^{-1})	8.8 (6.7–11.4)	19	3.8 (2.5–5.2)	23	5.3 (2.4–9.1)	89
Dominant frequency (kHz)	1.79 (1.53–2.07)	19	1.60 (1.27–1.94)	23	1.56 (1.25–1.95)	89
Morphology						
Males						
Snout–vent length (mm)	40.3 (35.6–45.2)	117	43.8 (39.9–48.2)	169	45.5 (37.3–53.9)	837
Tibia length (mm)	21.2 (18.6–23.4)	117	23.2 (20.3–25.2)	169	23.5 (18.5–27.9)	553
Head width (mm)	13.8 (12.1–15.5)	117	15.4 (13.4–17.0)	169	15.5 (12.4–18.0)	538
Weight (g)	3.2 (1.9–5.0)	121	4.5 (3.2–6.0)	168	4.9 (2.5–8.3)	835
Females						
Snout–vent length (mm)	64.4 (57.2–69.0)	18	67.0 (58.1–74.5)	33	64.9 (57.7–79.6)	81
Tibia length (mm)	34.0 (31.2–37.7)	10	35.5 (32.2–39.2)	27	36.3 (32.2–42.0)	70
Head width (mm)	19.8 (16.7–20.9)	10	21.3 (19.0–23.4)	27	21.6 (18.9–24.0)	70
Weight (g): all females	14.9 (9.3–19.3)	18	17.1 (10.3–23.8)	33	18.5 (8.3–30.0)	79
Weight (g): gravid	15.5 (10.0–19.3)	15	18.6 (13.3–23.8)	22	20.8 (13.3–30.0)	45

Data are presented as mean (range) and sample size (*N*). All morphological measurements were taken on live animals in the field.

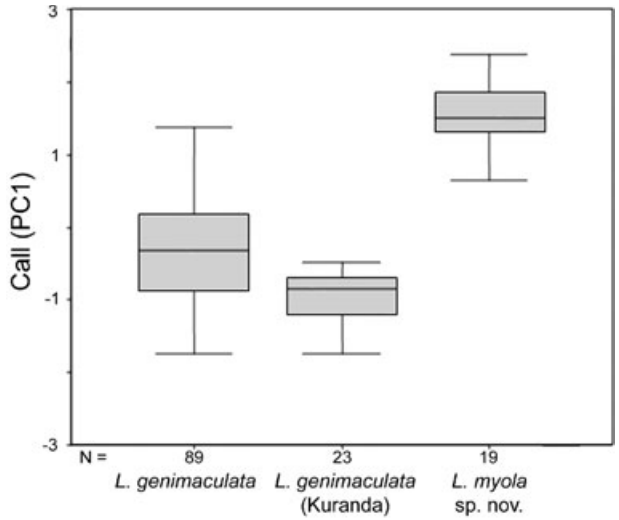

Figure 4. A representation of the difference in call between *Litoria genimaculata* and ***Litoria myola* sp. nov.** The box plots compare calls of *L. genimaculata* from across the Wet Tropics, *L. genimaculata* from the Kuranda area, and ***L. myola* sp. nov.** PC1 accounts for 67.2% of the variation in call (duration, dominant frequency and note rate) across *L. genimaculata* and ***L. myola* sp. nov.** PC1 is loaded heavily by inverse call duration (0.95) and note rate (0.93), and moderately by dominant frequency (0.51). The box plots show the median, 25th and 75th quartiles, and minimum and maximum data of PC1.

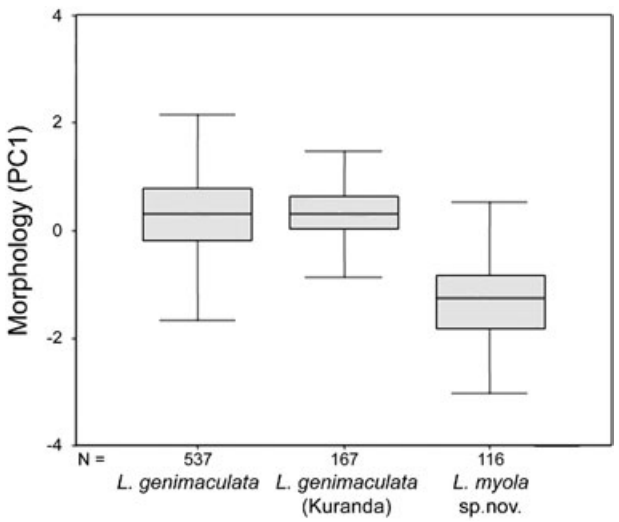

Figure 5. A representation of the difference in morphology between male *Litoria genimaculata* and ***Litoria myola* sp. nov.** The box plots compare morphology of *L. genimaculata* from across the Wet Tropics, *L. genimaculata* from the Kuranda area, and ***L. myola* sp. nov.** PC1 accounts for 87.8% of the variation in morphology (SVL, TL, HW, and weight) across *L. genimaculata* and ***L. myola* sp. nov.** PC1 is loaded equally and positively by all four characters (approximately 0.94 for each) and therefore represents body size. The box plots show the median, 25th and 75th quartiles, and minimum and maximum data of PC1.

Kuranda area (Hoskin *et al*., 2005). *Litoria myola* sp. nov. and *L. genimaculata* cannot be distinguished on the basis of coloration, pattern, body shape, distribution or habitat.

DESCRIPTION

Variation across type series (seven males and one female, all adult): Data are range followed by mean in brackets. *Male measurements in spirit (mm):* SVL 32.8–38.6 (36.3); TL 18.7–21.3 (20.3); HW 11.9–13.8 (12.7); HL 11.4–12.0 (11.7); BW 8.0–10.0 (9.1); ED 3.8–4.4 (4.1); EN 3.0–3.5 (3.3); IN 2.6–3.1 (2.9); 3DW 1.9–2.1 (2.0); 3FL 6.5–7.9 (7.4); 4DW 1.6–1.9 (1.7); 4TL 9.1–11.8 (10.5). *Male proportions:* TL/SVL 0.53–0.58 (0.56); HW/SVL 0.33–0.36 (0.35); HL/SVL 0.31–0.35 (0.32); HW/HL 1.03–1.15 (1.09); BW/SVL 0.24–0.26 (0.25); ED/SVL 0.10–0.12 (0.11); EN/IN 1.06–1.17 (1.13); 3DW/4DW 1.11–1.19 (1.15); *Male measurements in life:* SVL (mm) 36.0–41.1 (38.9); TL (mm) 18.6–21.4 (20.5); HW (mm) 12.1–13.9 (13.1); 3DW (mm) 1.9–2.1 (2.0); weight (g) 2.0–3.3 (2.8); *Female measurements in spirit (mm):* SVL 53.5; TL 30.6; HW 18.4; HL 15.4; BW 15.7; ED 5.5; EN 5.0; IN 4.1; 3DW 2.8; 3FL 9.8; 4DW 2.4; 4TL 12.9; *Female proportions:* TL/SVL 0.57; HW/SVL 0.34; HL/SVL 0.29; HW/HL 1.19; BW/SVL 0.29; ED/SVL 0.10; EN/IN 1.22; 3DW/4DW 1.17; *Female measurements in life:* SVL 59.6 mm; TL 31.2 mm; HW 18.6 mm; 3DW 3.4 mm; weight 10.0 g (8.5 g after laying 1.5 g clutch).

Field measurements: These are presented in Table 1. (N = 117 males and 18 females, all adult).

Sexual dimorphism: Body size dimorphism is pronounced, with females being an average of 1.6-times the SVL and 4.5-times the weight of males. In contrast, there appears to be little sexual dimorphism in external morphological character states, pattern or coloration; thus, the sexes are dealt with together below.

Head: Broad and flattened, slightly wider than body; snout bluntly rounded to truncate in dorsal view and distinctly projecting in profile, canthus rostralis slightly angular, loreal region steep, nostrils much closer to tip of snout than to eye, nostrils directed dorso-laterally; eye very large, diameter greater than eye to naris distance, horizontal pupil; internarial distance less than distance from eye to naris; tympanum large and obvious.

Body: Slender; urostyle moderately prominent to indistinct; cloaca positioned immediately below

urostyle, orientated posteriorly and dorsally, no ornamentation.

Limbs: Hindlimbs long and slender; two pale, distinct pointed tubercles or several indistinct tubercles on the heel; forearms broad, particularly in males; pale serrated ridge along the trailing edge of the forearm (distinct) and along the trailing edge of the foot (moderately distinct); fingers half webbed (webbing formula (Savage & Heyer, 1997): I 2–2$^{1}/_{2}$ II 1$^{1}/_{2}$–2$^{1}/_{2}$ III 2–2 IV); toes nearly fully webbed (webbing formula: I 1$^{1}/_{2}$–2 II 1–2 III 1–2 IV 2–1 V); fingers long, relative length 3 > 4 > 2 > 1, finger discs fleshy and rounded, obviously expanded from penultimate phalanx, first finger short with disc obviously expanded; in males a broad, black or grey nuptial pad covers the base of the first finger; indistinct tubercle present in the centre of the base of the palm; relative length of toes 4 > 3 > 5 > 2 > 1, rounded toe discs expanded from penultimate phalanx, disc on first toe marginally expanded; small yet obvious oval inner metatarsal tubercle; discs on fingers larger than discs on toes.

Skin: Ventral surface coarsely granular; dorsal surface of body, head and limbs smooth or finely granular with scattered low tubercles in some specimens; distinct postorbital skin fold extending along dorsolateral surface to mid-body.

Pattern and colour. In preservative: Dorsal pattern and colour highly variable ranging from blotched brown ($N = 6$), speckled brown ($N = 1$), to evenly grey ($N = 1$). Blotched individuals are generally darker down the centre of the body with paler areas on the shoulders, lower back and forehead. This results in a roughly hourglass pattern. Several individuals are marked with a distinct pale triangle between the eyes and nares. Pigmentation is often darker in the canthal region. Hindlimbs often marked with several, irregular, broad, dark bars. Evenly cream over the majority of the ventral surface. The chin and throat region has a light brown wash and some degree of fine dark speckling. Speckling is obvious over the entire throat and chin in some individuals ($N = 5$) and restricted to the chin area in others ($N = 3$). Fine brown mottling covers the lateral surfaces, underside of lower hindlimbs and feet, posterior thigh, and groin. In some males, the fine mottling gives way to immaculate cream on the latter half of the flanks. Underside of discs cream and underside of hands ranging from cream to mottled brown. Eye has fine venation throughout, a dark horizontal bar passing through the pupil, and a pale upper iris. *In life*: Dorsal colour and pattern highly variable, ranging from even brown or tan (Fig. 2A), through mottled grey, fawn or brown (Fig. 2B), to blotched tan, brown and green. Ventral surfaces cream to white with a faint to obvious light brown or grey wash on the throat and chin (particularly in males). Dark speckling on the chin, and on the entire throat in some individuals (particularly in males). Nuptial pads on males usually black but occasionally grey. Pupil edged by a horizontal dark brown strip and iris cream or grey with a distinctly green upper crescent, and covered in fine brown venation.

COMPARISON

Litoria myola sp. nov. is only likely to be confused with other Australian members of the '*L. eucnemis*' species group (*L. genimaculata* and *L. eucnemis* Lönnberg, 1900). Members of this group can be distinguished from other Australian frogs by having a serrated ridge along the trailing edge of the feet and forearms (Barker, Grigg & Tyler, 1995). *Litoria myola* sp. nov., *L. genimaculata*, and *L. eucnemis* also have green coloration in the upper iris (in life) and males lack a vocal sac (Tyler, 1971), which is present in most other Australian *Litoria*. Amongst these three species, *L. eucnemis* is restricted to northern Cape York Peninsula and has a distinctive call of a series of short growls (vs. a series of soft 'tocs') (Richards *et al*., 1993). *Litoria myola* sp. nov. is most likely to be confused with *L. genimaculata*, with which it co-occurs in the Kuranda area. From this species, *L. myola* sp. nov. differs in having a distinctive call of faster note ('toc') rate, shorter duration and higher dominant frequency, and also in being smaller in all aspects of male body size (see Diagnosis). These differences are evident between *L. myola* sp. nov. and Australian *L. genimaculata* in general, and are particularly pronounced between *L. myola* sp. nov. and the northern lineage of *L. genimaculata* with which it co-occurs (see Diagnosis). Although *L. myola* sp. nov. was not compared with New Guinean populations of *L. genimaculata* in the present study, the New Guinean populations appear to be similar in morphology and call to the Australian populations of *L. genimaculata* (Richards *et al*., 1993; Cunningham, 2001). Therefore, it would appear that the characters outlined above would distinguish *L. myola* sp. nov. from both Australian and New Guinean populations of *L. genimaculata*. Additionally, the taxonomy of *L. genimaculata* is currently under revision to reflect the distant (and deeply paraphyletic) genetic relationship between the Australian and New Guinean populations of *L. genimaculata* across the '*L. eucnemis*' species group (Moritz *et al*., 1997; Cunningham, 2001).

ETYMOLOGY

The specific epithet is in recognition of Myola, a locality where this species occurs. This name is believed to be of aboriginal origin, although the language and

dialect are not recorded (http://www.nrm.qld.gov.au/property/placenames). The common name 'Kuranda tree frog' refers to the township around which the distribution is centred.

Genetics

Litoria myola sp. nov. co-occurs with the northern lineage of *L. genimaculata* (Fig. 1), from which it is genetically highly distinct at both mitochondrial (13% COI mtDNA sequence divergence) and nuclear loci (Hoskin *et al*., 2005). *Litoria myola* sp. nov. is genetically similar (0.1% net COI mtDNA sequence divergence) to, and nested within, the southern lineage of *L. genimaculata* (Hoskin *et al*., 2005).

Call

The courtship call of *L. myola* sp. nov. is an excited, short call consisting of rapidly uttered notes (Fig. 3). Each note consists of two pulses heard as a single 'toc'. The call has the following characteristics (mean followed by range in brackets, $N = 19$): duration 0.85 s (0.57–1.35), number of notes 7 (5–11), note rate 8.8 notes s^{-1} (6.7–11.4), and dominant frequency 1.79 kHz (1.53–2.07). There is no frequency modulation in the call (i.e. all notes of each call are of similar dominant frequency). The call of *L. myola* sp. nov. is significantly different to that of *L. genimaculata*, being of faster note rate, shorter duration, and generally higher dominant frequency (Table 1, Fig. 4; Hoskin *et al*., 2005). *Litoria myola* sp. nov. also occasionally utters a longer call of slower note rate, which is used during aggressive encounters between males. This call appears similar to the aggressive call of *L. genimaculata*, although too few recordings were made to assess this in detail. Like other members of the '*L. eucnemis*' species group (Tyler, 1971), *L. myola* sp. nov. lacks a vocal sac and the call is of relatively low volume.

Reproductive isolation from *L. genimaculata*

Hoskin *et al*. (2005) conducted experimental crosses between the northern and southern lineages of *L. genimaculata*. These crosses included males and females of *L. myola* sp. nov., which was termed the 'iS' population of the southern lineage. The trials suggest that successful breeding in *L. genimaculata* is only possible between northern lineage females and southern lineage (including *L. myola* sp. nov.) males. Reciprocal crosses, southern lineage (including *L. myola* sp. nov.) females with northern lineage males, resulted in fertilized clutches that hatched out but died at an early larval stage. *Litoria myola* sp. nov. only co-occurs with the northern lineage of *L. genimaculata*; therefore, the potential for hybridization between *L. myola* sp. nov. and *L. genimaculata* appears to be limited to *L. myola* sp. nov. males mating with *L. genimaculata* females.

Hybridization appears to occur very rarely, due to call divergence and associated mate choice. Mate choice trials have been conducted in which gravid *L. genimaculata* (then including *L. myola* sp. nov. as the 'iS' population of the southern lineage) females were given a choice between alternative male calls in a laboratory mate choice chamber (Hoskin *et al*., 2005). These trials revealed significant premating isolation between *L. myola* sp. nov. and the co-occurring northern lineage of *L. genimaculata*. This is supported by: (1) a preliminary genetic analysis of the contact zone that suggested hybridization between *L. myola* sp. nov. and *L. genimaculata* is very low, with an estimated 0–1.4% of individuals being hybrids at mixed sites (Hoskin *et al*., 2005), and (2) field observations of pairs in amplexus at mixed sites in the Kuranda area, with no mixed pairings in the 10 pairs observed (six *L. myola* sp. nov. pairs and four *L. genimaculata* pairs). The laboratory-based call trials also revealed highly significant premating isolation between *L. myola* sp. nov. and the genetically similar southern lineage of *L. genimaculata*.

Distribution

Litoria myola sp. nov. has a very small distribution, being known from short sections of 13 streams draining into the Barron River in the Kuranda area (between the localities of Kuranda, Fairyland, Myola, Mantaka, Kowrowa, and Oak Forest) in north-eastern Queensland (Fig. 6). The distribution is bound to the north, east and south by the northern lineage of *L. genimaculata* (which also occurs at most *L. myola* sp. nov. sites), and to the west by the limit of rainforest distribution (Figs 1, 6; see also 'iS' population in Hoskin *et al*., 2005). All sites are between 320 and 360 m a.s.l.

Habitat and habits

The habitat and habits of *L. myola* sp. nov. appear to be similar to those of *L. genimaculata*. All records of *L. myola* sp. nov. are from rainforest along permanent and ephemeral streams (Fig. 7). Rainforest along the streams ranges from mesophyll vine forest to rainforest regrowth dominated by *Acacia* and *Calamus*. *Litoria myola* sp. nov. is a stream breeder. Stream substrate at the sites ranges from rock and gravel (Fig. 7A) through to coarse sand (Fig. 7B), and stream gradient at all sites is low. Males were only encountered along the streams, at high density at some sites (discussed below). Females were rarely encountered,

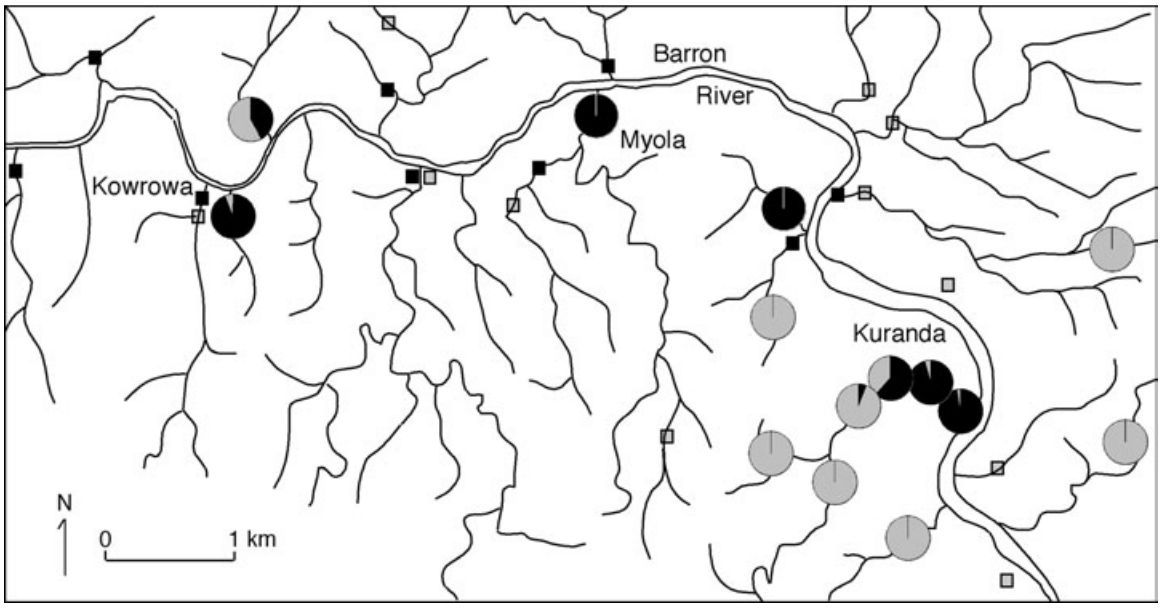

Figure 6. The distribution of ***Litoria myola* sp. nov.** and *Litoria genimaculata* in the Kuranda area. The pie charts show the proportion of ***L. myola* sp. nov.** (black) and *L. genimaculata* (grey) individuals on five streams. Nearby sites on the streams are grouped together to provide a consistent scale. Sample size for the pie charts averages 20 individuals. The squares show additional sites where ***L. myola* sp. nov.** (black squares) and *L. genimaculata* (grey squares) have been recorded but relative proportions of each have not been determined. All *L. genimaculata* are northern lineage individuals. Major stream catchments flowing into the Barron River are marked. The sections of stream without records of either species are either unsuitable habitat or have not yet been surveyed. Sites surrounding this area are occupied by northern lineage *L. genimaculata* or are unsuitable habitat (Fig. 1).

Figure 7. Rainforest stream habitat, with rocky (A) and sandy (B) substrate, Kuranda, north-east Queensland. ***Litoria myola* sp. nov.** and *Litoria genimaculata* are present at both sites.

with most found on the streams, primarily as gravid individuals (17 out of 21), and always where *L. myola* sp. nov. males were calling. Occasionally, females were sighted perched high in trees, and several females were encountered a considerable distance from the streams. Metamorphs were rarely encountered (on streamside vegetation) and juveniles were not observed. Therefore, as is in *L. genimaculata* (Richards & Alford, 2005; K. R. McDonald, unpubl. data; C. J. Hoskin, unpubl. data), *L. myola* sp. nov. utilizes streams as breeding habitat, primarily in spring and summer, and nonbreeding adults and subadults utilize the surrounding rainforest. Given the apparent rarity of nonbreeding individuals on the streams, and how infrequently the species is encountered in the surrounding rainforest, it would appear that *L. myola* sp. nov. utilizes the mid and upper forest levels when not breeding.

Breeding was observed during the summer wet season between October and March. Breeding activity (male calling intensity and female presence on the streams) is greatest in the nights prior to and following heavy rain. Males call throughout the night from elevated perches, primarily around riffle zones and small waterfalls. Perch height is usually between 30 cm and 1.5 m, but sometimes up to 5 m above the ground. Courtship calling is occasionally interrupted by periods of aggressive calling between neighbouring males. Aggressive calling intensifies as the males approach each other and on several occasions aggressive calling bouts were observed to result in wrestling. Such wrestling has been previously reported in *L. genimaculata* (Richards & James, 1992; Richards & Alford, 2005). At mixed sites wrestling was observed between *L. myola* sp. nov. males, between *L. genimaculata* males, and on one occasion between a male *L. myola* sp. nov. and a male *L. genimaculata*. Males display dark, fleshy nuptial pads on the thumbs during the breeding season and amplexus is axillary. Sexual dimorphism is pronounced, with males being on average 63% of the SVL and 22% of the weight of females. This is equivalent to the sexual size dimorphism seen in *L. genimaculata* (Table 1; McDonald *et al*., 1999; Richards & Alford, 2005). The only clutch data for *L. myola* sp. nov. is a clutch of 509 pigmented eggs (15% of the females body weight) that was laid as a cohesive gelatinous clump. The diameter of each egg was approximately 2 mm. This is similar to clutch data for *L. genimaculata* (described under the name *L. eucnemis* in Davies, 1989; C. J. Hoskin & K. R. McDonald, unpublished data).

Sixteen species of frog were recorded in rainforest in the Kuranda area during fieldwork between 2001 and 2007 but only six of these species were observed sharing breeding habitat (rainforest streams) with *L. myola* sp. nov. These species were: *L. genimaculata*, *Litoria jungguy* Donnellan & Mahony, 2004/*Litoria wilcoxii* Günther, 1864 (these two species could not be diagnosed in the field), *Rana daemeli* Steindachner, 1868, *Mixophyes coggeri* Mahony, Donnellan, Richards & McDonald, 2006, *Litoria rheocola* Liem, 1974 and *Nyctimystes dayi* Günther, 1897. The first four species were those most often recorded calling alongside *L. myola* sp. nov., while *L. rheocola* and *N. dayi* were very rarely recorded in the Kuranda area.

DISCUSSION

Populations size, monitoring and conservation of *L. myola*

Surveys for, and monitoring of, *L. myola* were conducted during summer wet seasons from 2001 to 2007. Most stream catchments in the Kuranda area were surveyed for *L. myola* during this period. Monitoring was performed by opportunistically revisiting five of the known *L. myola* breeding sites and assessing presence/absence and abundance based on calling males. *Litoria myola* has a very restricted and fragmented range. Despite the extensive survey effort the species was only recorded from short sections of 13 streams within a small area (Fig. 6). Calling males were located on all of these streams so all are assumed to be breeding sites. *Litoria myola* was abundant on only one stream, where it occurred at a density of up to 50 males per 100 m of stream, a density similar to or higher than recorded at sites across the range of *L. genimaculata* (Laurance, McDonald & Speare, 1996; Richards & Alford, 2005; K. R. McDonald, unpubl. data; C. J. Hoskin, pers. observ.). Although *L. myola* was consistently recorded at high density on this stream, the four smaller monitored populations were inconsistently detected and declined in abundance from common to rare over the monitoring period. The decline appeared to be due an extended drought between 2002 and 2005, during which most streams in the area stopped flowing and several were completely dry for consecutive years. This was particularly the case for streams on the drier western end of the species' range and populations at these sites may be particularly susceptible to dry periods as they breed in ephemeral streams on sandy soils in marginal rainforest habitat. Intensive surveys in the region in early 2007, following a year of reasonably high rainfall, detected *L. myola* at all known sites (13 streams), although it remained rare at four of the five monitoring sites.

Based on stream surveys of mature males, and assuming an equal sex ratio, the total breeding population was estimated to be less than 1000 individuals in the summer of greatest abundance and considerably less in other summers. This consisted of a population on one stream estimated at 500 mature individuals and smaller populations of between ten and 100 individuals across the other streams. The sites where *L. myola* has been recorded are generally unprotected strips of riparian rainforest along streams whose catchments have been heavily altered by rural and urban development. Disturbance to upstream sections of the stream catchments has the potential to detrimentally impact the breeding habitat of *L. myola* by affecting stream flow, water quality, or sedimentation. Over the survey period the *L. myola* sites were subject to considerable disturbance from clearing, road construction, dam construction, and run-off of sediment, chemicals and rubbish from the catchments. The degree of connectivity between the populations in each of the catchments is not known.

Given the genetic similarity between *L. myola* and *L. genimaculata*, the effect of disease and parasites on *L. myola* would be predicted to be similar to that seen

in *L. genimaculata*. *Litoria genimaculata* is currently common throughout the Wet Tropics but underwent population declines in the early 1990s (Laurance *et al*., 1996; McDonald & Alford, 1999). Concurrent declines in several other Wet Tropics stream breeding frog species resulted in complete disappearance of some species and the decline of others from upland areas (Laurance *et al*., 1996; McDonald & Alford, 1999). A chytridiomycete fungus (*Batrachochytrium*, 'chytrid') has been identified as the most likely proximate cause of these declines (Berger *et al*., 1998), and chytrid is known to be a source of mortality in *L. genimaculata* (Speare & Berger, 2005). Populations of *L. genimaculata* appear to have recovered to predecline levels across the Wet Tropics (McDonald & Alford, 1999; Richards & Alford, 2005). The effect of chytrid on *L. myola* is not known but is assumed to be similar to its effect on *L. genimaculata*.

Litoria myola is parasitized by a Dipteran fly (*Batrachomyia* sp.). Species of *Batrachomyia* have been recorded to parasitize a number of Australian frog species (Lemckert, 2000; Schell & Burgin, 2001). The larvae live in the subcutaneous lymph spaces of the frog, feeding on blood before dropping to the ground to pupate (Skuse, 1889). Amongst the Wet Tropics frog species, *Batrachomyia* is most prevalent in *L. genimaculata* (7.8% of males) and *L. myola* (3.4% of males) (Hoskin & McCallum, in press; C. J. Hoskin, K. R. McDonald & H. McCallum, unpubl. data). *Batrachomyia* larvae were not found in *L. myola* females. Infected *L. myola* males had a single larva located on one of the shoulders or on the back of the head. *Batrachomyia* parasitism appears to have little impact on *L. myola* health and survival, as the body condition of parasitized males is not significantly lower than that of unparasitized males (Hoskin & McCallum, in press), and scars from larva are occasionally found on healthy males calling on the streams.

Hybridization between *L. myola* and the surrounding populations of *L. genimaculata* is very limited (Hoskin *et al*., 2005). Interaction between these two species as it currently stands is not a threat; indeed, it appears to be the driving force for speciation of *L. myola* from *L. genimaculata* (Hoskin *et al*., 2005). However, the reproductive isolation (and competitive interaction) between these two species may be in part density or habitat dependent, and if this were so, the integrity of *L. myola* could be compromised by reductions in population size or translocations of *L. myola* frogs or tadpoles out of, or *L. genimaculata* into, the Kuranda area.

The extent of occurrence of *L. myola* is 13.5 km^2 (calculated as a minimum convex polygon that includes all records) and the area of occupancy is 3.5 km^2 (calculated by plotting records on a grid and summing all 0.25 km^2 grid cells that contain a record). *Litoria myola* is threatened by: (1) clearing of rainforest (including regenerating rainforest); (2) impacts to the streams in terms of water flow, water quality, and sedimentation; and (3) fragmentation of habitat and breeding populations. The species is potentially threatened by: (1) altered levels of hybridization and/or competition with *L. genimaculata* due to captive breeding and release or movement of individuals of either species into or out of the Kuranda region; (2) frog chytrid fungus; and (3) stochastic events. Management of *L. myola* should focus on protection and revegetation of the stream habitat and surrounding rainforest throughout its range, and include strict control of impacts from the catchments that may affect water quality, water flow and sedimentation. *Litoria myola* should be recognized as a 'Critically Endangered' (B1, 2) species under IUCN guidelines (IUCN Species Survival Commission, 2001) due to its restricted distribution (extent of occurrence less than 100 km^2 and area of occupancy less than 10 km^2), small and fragmented population, and observed population decline.

Contact zones as areas of evolutionary novelty

The distribution of *L. myola* is of particular interest in the context of Wet Tropics biogeography and conservation prioritization. This is the only vertebrate species known to be restricted to the central Wet Tropics area, between the mountains of the Carbine Tableland in the north and the Bellenden Ker Range in the south (Williams, Pearson & Walsh, 1996; Moritz *et al*., 2005). *Litoria myola* is unusual amongst the Wet Tropics rainforest vertebrate species (along with *Phyllurus gulbaru* Hoskin, Couper & Schneider, 2003 in the southern Wet Tropics) in being restricted to an area not predicted to have been a historical rainforest refuge. The majority of narrowly distributed Wet Tropics rainforest species are restricted to upland areas predicted to have been historical refugia (Williams *et al*., 1996; Moritz *et al*., 2001; Yeates, Bouchard & Monteith, 2002; Moritz *et al*., 2005). The distribution of these species, in conjunction with patterns of species richness and phylogenetic diversity, has resulted in historical refugia being recognized as areas of conservation priority, and in general these areas are currently well protected (Moritz *et al*., 2001, 2005; Moritz, 2002; Yeates *et al*., 2002).

The central Wet Tropics area (northern Atherton Tableland, Lamb Range and Black Mountain Corridor) is recognized as a suture zone – an area in which multiple species have deeply divergent lineages in secondary contact (Phillips *et al*., 2004). Contact zones have the potential to be regions of evolutionary novelty where phenotypic variation and new species can arise relatively rapidly (Arnold, 1997; Endler, 1998;

Barton, 2001; Hoskin *et al*., 2005). For this reason, the conservation importance of the Wet Tropics suture zone has been suggested previously (Moritz *et al*., 1997; Moritz, 2002). The recognition of *L. myola* shows that Wet Tropics contact zones are indeed areas where rapid phenotypic evolution and speciation is possible in an otherwise phenotypically conservative landscape (Schneider & Moritz, 1999; Phillips *et al*., 2004; Hoskin *et al*., 2005). The Wet Tropics suture zone is generally well protected; however, the centre of the zone (the Kuranda area) is an area undergoing continued development in which the fragments and gullies of rainforest are generally poorly protected or connected. Protection and connection of rainforest in the Kuranda area has both the immediate value of securing *L. myola* and also the long-term value of maintaining the integrity of the area of overlap between deeply divergent lineages of this and other Wet Tropics species.

The discovery of *L. myola*, along with recent discoveries in peripheral rainforest areas of the Wet Tropics (Hoskin, Couper & Schneider, 2003), highlights the conservation importance of areas outside the high diversity, high profile historical refugia. Although currently of lower diversity and often degraded condition, these areas may harbour much of the evolutionary potential of the Wet Tropics.

Taxonomic resolution of the remaining populations of *L. genimaculata*

Having described *L. myola*, do the remaining populations of the northern and southern lineages of *L. genimaculata* represent a single species? Genetic divergence between the northern and southern lineage is high (13% COI mtDNA) (Schneider *et al*., 1998; Cunningham, 2001; Hoskin *et al*., 2005), and of a level seen between recognized sister species of the '*L. eucnemis*' group (Moritz *et al*., 1997; Cunningham, 2001). This divergence is most likely responsible for the asymmetric postzygotic isolation between the lineages (Hoskin *et al*., 2005), most probably due to cytonuclear incompatibility. The two lineages cannot be reliably distinguished in the field by any trait. There are no significant morphological differences between the lineages, either at Contact A or elsewhere in the Wet Tropics, and there appears to be no ecological divergence between the lineages (Hoskin *et al*., 2005). Limited morphological divergence has been detected between the lineages of other Wet Tropics vertebrate species investigated to date (Schneider & Moritz, 1999; Schneider *et al*., 1999; Cunningham, 2001), perhaps due to a lack of divergent selection during the period of isolation in refugia (Schneider & Moritz, 1999; Schneider *et al*., 1999). Male calls differ significantly between the *L. genimaculata* lineages in a multivariate analysis of three primary characters (Hoskin *et al*., 2005), with the calls of southern lineage males being generally of faster note rate, shorter duration and lower dominant frequency. However, the range of variation in the individual call characters within each lineage, and the large overlap in the range of each character between the two lineages, prevents accurate identification in the field. More importantly, females of both lineages from Contact A do not show significant positive assortative mating when the calls are used in laboratory-based mate choice trials (Hoskin *et al*., 2005). Field observations of female choice at Contact A are limited to two northern females found in amplexus at mixed sites: one was with a northern male and the other with a southern male (in contrast 10 amplectant pairs were found at mixed sites in Contact B, all correctly paired by species). A preliminary genetic analysis suggested that hybridization is limited where the lineages co-occur at Contact A (3.1–6.8% hybrids), but is significantly higher than in the contact zone between the northern lineage and *L. myola* (Contact B, 0–1.4%) (Hoskin *et al*., 2005).

Therefore, the northern and southern lineages of *L. genimaculata* display deep genetic divergence, asymmetric postzygotic isolation and some call differences, but there is no detectable morphological or ecological divergence, females show limited premating isolation where the lineages overlap, and there is some degree of hybridization in this area (Hoskin *et al*., 2005). This leads to the conclusion that the remaining Australian populations of *L. genimaculata* represent a single paraphyletic species, pending further genetic and phenotypic analyses of Contact A. A similar conclusion was drawn for the only other Wet Tropics species (the skink *Carlia rubrigularis* Ingram & Covacevich, 1989) in which the contact zone between the northern and southern lineages has been assessed in detail (Phillips *et al*., 2004). Genetic analysis of the *C. rubrigularis* contact zone inferred substantial (probably asymmetric) postzygotic isolation but limited prezygotic isolation between the lineages and concluded that the lineages should be retained as a single species (Phillips *et al*., 2004).

Further analysis of the contact zone between the lineages of *L. genimaculata* using additional nuclear markers will improve the accuracy of estimates of hybridization, and the proportion of F_1 and backcross hybrids (Hoskin *et al*., 2005). This is of importance as the survival, phenotype and breeding success of F_1 and backcross hybrids will further clarify whether the two lineages represent distinct species. Hybridization may be limited to producing F_1 offspring or, alternatively, hybrids may breed successfully, potentially leading to introgression between the lineages. Such analyses do not appear possible in another area where the two lineages abut, in coastal ranges south-east of Contact A

(Fig. 1), as the joint out-flow of the Mulgrave and Russell Rivers appears to separate the northern lineage in the Malbon Thompson Range from the southern lineage in the Graham Range.

Litoria genimaculata is also distributed widely across New Guinea (Richards *et al*., 1993) and the taxonomic relationship between the Australian and New Guinean populations is currently being revised to reflect their distant and deeply paraphyletic genetic relationship (Moritz *et al*., 1997; Cunningham, 2001).

Conflicting data: the value of multiple lines of evidence for delimiting species

On the face of it the deep genetic split, partial postzygotic isolation and significant call divergence between the northern and southern lineages of *L. genimaculata* suggest two species. However, there is no morphological or ecological divergence and the call divergence between the lineages does not translate to significant premating isolation in laboratory-based mate choice trials, which is supported by the field observation of, and genetic evidence for, hybridization where the two lineages overlap (Hoskin *et al*., 2005). This leads to the tentative conclusion that the two lineages represent a single species. In contrast, *L. myola* is a phenotypically highly distinct species that is significantly reproductively isolated from both lineages of *L. genimaculata*, despite the fact that it is genetically very similar to the southern lineage of *L. genimaculata* (< 0.1% net divergence, with shared haplotypes; Hoskin *et al*., 2005). The evolutionary hypothesis for this taxonomic conclusion is that the genetic and call divergence between the lineages of *L. genimaculata* reflects gradual divergence during an extended period of isolation in historical rainforest refugia, whereas, the phenotypic divergence of *L. myola*, despite little genetic divergence, reflects relatively rapid speciation by reinforcement of this small population in the contact zone (Hoskin *et al*., 2005). This unusual speciation event presents an interesting systematic situation in which the data do not support the recognition of two genetically highly divergent lineages, which differ in mating call, as separate species, but do support species status of a genetically indistinguishable, but phenotypically distinct, population within one of those lineages.

The importance of call divergence on reproductive isolation is of particular interest in this case. The call divergence between *L. myola* and *L. genimaculata* is reflected in highly significant premating isolation in laboratory-based trials, whereas, the call divergence between the lineages of *L. genimaculata* is not (Hoskin *et al*., 2005). Call divergence was determined by a multivariate analysis of multiple characters seen as potentially important for mate choice or species recognition. The relative importance of individual characters for premating isolation in these frogs is not known, but other anuran studies have shown that some characters are more important in female choice than others, and that the relative importance of characters varies across species and even populations within species (Gerhardt & Huber, 2002). Therefore, a measure of overall call divergence does not necessarily reflect premating isolation as it is dependent on which characters are driving call divergence and their impact on mate choice. This may explain why premating isolation is high between *L. myola* and *L. genimaculata* but apparently weak between lineages of *L. genimaculata*. Divergence of *L. myola* call due to reinforcement should have involved divergence of call characters of greatest effect in the avoidance of hybridization, whereas, divergence associated with historical isolation between the two lineages of *L. genimaculata* may or may not have involved the characters of greatest importance for lineage discrimination. This shows that significant divergence in a phenotypic trait may not reflect significant premating isolation, even when the trait is a recognized mate choice trait (e.g. frog call), and even when accompanied by 'species level' genetic divergence.

Debate continues regarding how to delimit species boundaries (Sites & Marshall, 2003, 2004; Coyne & Orr, 2004), focusing on the value of different types of genetic, phenotypic and ecological data and the importance of relative levels of divergence in these data (Turner *et al*., 2001; Jockusch & Wake, 2002; Weins & Penkrot, 2002), the relevance of monophyly (Avise & Ball, 1990; Patton & Smith, 1994; Crandall *et al*., 2000; Jockusch & Wake, 2002), and the use of experimental tests and contact zone genetic studies to assess reproductive isolation (Turner *et al*., 2001; Phillips *et al*., 2004). The *L. genimaculata*/*L. myola* research of the present study and elsewhere (Hoskin *et al*., 2005) incorporates many of these issues and supports other studies (Turner *et al*., 2001; Jockusch & Wake, 2002; Phillips *et al*., 2004) in demonstrating the value of multiple lines of data combined with direct and genetic tests of reproductive isolation in delimiting species boundaries.

ACKNOWLEDGEMENTS

I thank Craig Moritz, Keith McDonald, Megan Higgie, Michael Cunningham, Ben Phillips, Jeremy Austin, Gaynor Dolman, Claire Larroux, Patrick Couper, Jacque Milton, Steve Williams, and Hamish McCallum for their help with this work. I also thank the reviewers for constructive comments. This research was supported by a National Science Foundation grant (to Craig Moritz), the Cooperative Research Centre for Tropical Rainforest Ecology and Manage-

ment (CRC TREM), the University of Queensland, and Queensland Parks and Wildlife Service. Research was conducted under Queensland Parks and Wildlife Service and State Forests permits, and in conjunction with Queensland Parks and Wildlife Service, Atherton.

REFERENCES

Arnold ML. 1997. *Natural hybridization and evolution*. New York, NY: Oxford University Press.

Avise JC, Ball MR Jr. 1990. Principles of geneological concordance in species concepts and biological taxonomy. In: Futuyma D, Antonovics J, eds. *Oxford surveys in evolutionary biology*. New York, NY: Oxford University Press.

Barker J, Grigg GC, Tyler MJ. 1995. *A field guide to Australian frogs*. Sydney: Surrey Beatty and Sons.

Barton NH. 2001. The role of hybridization in evolution. *Molecular Ecology* **10:** 551–568.

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H. 1998. Chytridiomycosis causes amphibian mortality associated with population crashes in the rain forests of Australia and Central America. *Proceedings of the National Academy of Science of the United States of America* **95:** 9031–9036.

Bowerman BL, O'Connell RT. 1990. *Linear statistical models: an applied approach*. Belmont, CA: Duxbury.

Cogger HG. 2000. *Reptiles and amphibians of Australia*, 6th edn. Sydney, Reed New Holland.

Coyne JA, Orr HA. 2004. *Speciation*. Sunderland, MA: Sinauer.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK. 2000. Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution* **15:** 290–295.

Cunningham M. 2001. Vicariance, speciation and diversity in Australopapuan rainforest frogs. DPhil Thesis, University of Queensland.

Davies M. 1989. Developmental biology of the Australopapuan hylid frog *Litoria eucnemis* (Anura: Hylidae). *Transactions of the Royal Society of South Australia* **113:** 215–220.

Endler JA. 1998. The place of hybridization in evolution. *Evolution* **52:** 640–644.

Gerhardt HC, Huber F. 2002. *Acoustic communication in insects and anurans*. Chicago, IL: University of Chicago Press.

Hoskin CJ. 2004. Australian microhylid frogs (*Cophixalus* and *Austrochaperina*): phylogeny, taxonomy, calls, distributions and breeding biology. *Australian Journal of Zoology* **52:** 237–269.

Hoskin CJ, Couper PJ, Schneider CJ. 2003. A new species of *Phyllurus* (Lacertilia: Gekkonidae) and a revised phylogeny and key for the Australian leaf-tailed geckos. *Australian Journal of Zoology* **51:** 153–164.

Hoskin CJ, Higgie MA, McDonald KR, Moritz C. 2005. Reinforcement drives rapid allopatric speciation. *Nature* **437:** 1353–1356.

Hoskin CJ, McCallum H. In press. Phylogeography of the parasitic fly *Batrachomyia* in the Wet Tropics of north-east Australia, and susceptibility of host frog lineages in a mosaic contact zone. *Biological Journal of the Linnean Society*, in press.

Hugall A, Moritz C, Moussalli A, Stanisic J. 2002. Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). *Proceedings of the National Academy of Science of the United States of America* **99:** 6112–6117.

IUCN Species Survival Commission. 2001. *IUCN red list categories*, Version 3.1.Gland: IUCN Council.

Jockusch EL, Wake DB. 2002. Falling apart and merging: diversification of slender salamanders (Plethodontidae: *Batrachoseps*) in the American West. *Biological Journal of the Linnean Society* **76:** 361–391.

Joseph L, Moritz C, Hugall A. 1995. Molecular support for vicariance as a source of diversity in rainforest. *Proceedings of the Royal Society London Series B, Biological Sciences* **260:** 177–182.

Laurance WF, McDonald KR, Speare R. 1996. Epidemic disease and the catastrophic decline of Australian rain forest frogs. *Conservation Biology* **10:** 406–413.

Lemckert F. 2000. Parasitism of the common eastern froglet *Crinia signifera* by flies of the genus *Batrachomyia* (Diptera: Chloropidae): parasitism rates and the influence on frog condition. *Australian Zoologist* **31:** 492–495.

McDonald KR, Alford RA. 1999. A review of declining frogs in Northern Queensland. In: Campbell A, eds. *Declines and disappearances of Australian frogs.* Canberra: Environment Australia.

Moritz C. 2002. Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology* **51:** 238–254.

Moritz C, Richardson KS, Ferrier S, Monteith GB, Stanisic J, Williams SE, Whiffin T. 2001. Biogeographic concordance and efficiency of taxon indicators for establishing conservation priority in a tropical rainforest biota. *Proceedings of the Royal Society of London Series B, Biological Sciences* **268:** 1875–1881.

Moritz C, Hoskin C, Graham CH, Hugall A, Moussalli A. 2005. Historical biogeography, diversity and conservation of Australia's tropical rainforest herpetofauna. In: Purvis A, Gittleman JL, Brooks T, eds. *Phylogeny and conservation*. Cambridge: Cambridge University Press, 243–264.

Moritz C, Joseph L, Cunningham M, Schneider CJ. 1997. Molecular perspectives on historical fragmentation of Australian tropical and subtropical rainforest: implications for conservation. In: Laurance WF, Bieregard RO, eds. *Tropical rainforest remnants: ecology, management and conservation of fragmented communities*. Chicago, IL: University of Chicago Press, 442–454.

Patton JL, Smith MF. 1994. Paraphyly, polyphyly, and the nature of species boundaries in pocket gophers (genus *Thomomys*). *Systematic Biology* **43:** 11–26.

Phillips BL, Baird SJE, Moritz C. 2004. When vicars meet: a narrow contact zone between morphologically cryptic phy-

logeographic lineages of the rainforest skink, *Carlia rubrigularis*. *Evolution* **58:** 1536–1549.

Richards SJ, Alford RA. 2005. Structure and dynamics of a rainforest frog (*Litoria genimaculata*) population in northern Queensland. *Australian Journal of Zoology* **53:** 229–236.

Richards SJ, James C. 1992. Foot-flagging displays of some Australian frogs. *Memoirs of the Queensland Museum* **132:** 302.

Richards SJ, McDonald KR, Ingram GJ. 1993. Recognition of *Litoria eucnemis* (Lönnberg) (Anura: Hylidae) in Australia. *Memoirs of the Queensland Museum* **34:** 94.

Savage JM, Heyer WR. 1997. Digital webbing formulae for anurans: a refinement. *Herpetological Review* **28:** 131.

Schell CB, Burgin S. 2001. *Batrachomyia strigapes* (Diptera) parasitism of *Uperoleia laevigata* (Anura). *Journal of Parasitology* **87:** 1215–1216.

Schneider CJ, Moritz C. 1999. Rainforest refugia and evolution in Australia's Wet Tropics. *Proceedings of the Royal Society of London Series B, Biological Sciences* **266:** 191–196.

Schneider CJ, Cunningham M, Moritz C. 1998. Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforest of Australia. *Molecular Ecology* **7:** 487–498.

Schneider CJ, Smith TB, Larison B, Moritz C. 1999. A test of alternative models of diversification in tropical rainforests: ecological gradients vs. refugia. *Proceedings of the National Academy of Science of the United States of America* **96:** 13869–13873.

Sites JW Jr, Marshall JC. 2003. Delimiting species: a renaissance issue in systematic biology. *Trends in Ecology and Evolution* **18:** 462–470.

Sites JW Jr, Marshall JC. 2004. Operational criteria for delimiting species. *Annual Review of Ecology, Evolution and Systematics* **35:** 199–227.

Skuse FAA. 1889. Description of a new genus (*Batrachomyia*, W. S. Macleay, MS), and two new species of dipterous insects parasitic upon Australian frogs. *Proceedings of the Linnean Society of New South Wales* **4:** 171–177.

Speare R, Berger L. 2005. *Chytridiomycosis status of wild amphibians in Australia.* Available at: http://www.jcu.edu.au/school/phtm/PHTM/frogs/chy-au-status. htm.

Turner GF, Seehausen O, Knight ME, Allender CJ, Robinson RL. 2001. How many species of cichlid fishes are there in African lakes? *Molecular Ecology* **10:** 793–806.

Tyler MJ. 1971. The phylogenetic significance of vocal sac structure in hylid frogs. *Miscellaneous Publications of the University of Kansas Museum of Natural History* **19:** 319–360.

Weins JJ, Penkrot TA. 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Scleroporus*). *Systematic Biology* **51:** 69–91.

Williams SE, Pearson RG, Walsh PJ. 1996. Distributions and biodiversity of the terrestrial vertebrates of Australia's Wet Tropics: a review of current knowledge. *Pacific Conservation Biology* **2:** 327–362.

Yeates D, Bouchard P, Monteith GB. 2002. Patterns and levels of endemism in the Australian Wet Tropics rainforest: evidence from insects. *Invertebrate Systematics* **16:** 605–619.

Zweifel RG. 1985. Australian frogs of the family Microhylidae. *Bulletin of the American Museum of Natural History* **182:** 265–388.