**ORIGINAL ARTICLE**

# The biogeographical boundaries of northern Australia: evidence from ecological niche models and a multi-locus phylogeny of *Uperoleia* toadlets (Anura: Myobatrachidae)

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## ABSTRACT

**Aim** Our aim was to test hypothesized biogeographical barriers using small-bodied terrestrial *Uperoleia* frogs, to identify Pleistocene refugia and to define biogeographical units.

**Location** The Australian Monsoonal Tropics, defined as the open woodlands and savanna north of the inland arid and eastern winter-rainfall zones.

**Methods** A multi-locus molecular phylogeny of the *Uperoleia lithomoda*, *U. trachyderma* and *U. minima* species complex, with supporting morphological and acoustic data, was generated to test species boundaries and clarify distributions. Ecological niche modelling with current climate and Last Glacial Maximum climate data was used to identify biogeographical units, barriers to dispersal, and regions of stability that may have served as Pleistocene refugia.

**Results** Our combined data supported five clades that comprise four allopatric species. Ecological niche models of the resolved species suggest that the Kimberley Plateau represents a distinct bioregion, the Top End extends from the edge of the Kimberley Plateau to the Carpentarian Gap, and the transition from sandstone escarpments to flat, sandy soils represents a major barrier to dispersal between the Top End and the Northern Deserts. The Northern Deserts were found to comprise two distinct subregions. Population- and species-level divergences were evident in a north–south line in the Northern Territory, representing a newly identified biogeographical break. Putative Pleistocene refugia were predicted in the north-west Kimberley Plateau, the western half of the Top End, the Selwyn Range and western Cape York.

**Main conclusions** By combining detailed genetic, morphological and acoustic data with newly developed statistical methods, we have delineated species boundaries, identified cryptic species and provided a region-wide assessment of the biogeography of northern Australia. We have identified previously unrecognized biogeographical barriers, better defined biogeographical regions, and proposed new hypotheses about the effects of Pleistocene climate cycles on the present-day diversity of northern Australia. Our work provides a solid foundation for the investigation of biogeographical patterns in other taxa.

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## INTRODUCTION

Extant species provide important insights into biogeographical history. Using present-day observations, the processes leading to the generation of diversity can be investigated. For example, molecular studies have shown that both divergence across environmental gradients and allopatry caused by barriers of unsuitable habitat lead to speciation (Avise, 2000). Historical landscape evolution can also influence diversification through changes in available niches and by creating barriers to dispersal. The effect of landscape on diversification is often explained as the influence of explicit dispersal barriers such as mountains, drainage basins or arid zones (Scott *et al.*, 2004; Glor & Warren, 2011; Pepper *et al.*, 2011a,b). The geographical separation caused by these barriers of unsuitable habitat can drive speciation through genetic drift or changes in available niches, leading to reproductive isolation of the bisected units. Ideally, investigations of regional diversification begin with a comparison of wide-scale phylogenetic data sets with known geological and climatic history.

The Australian continent is best known for its deserts and extreme aridity. From the Oligocene to the Miocene (*c.* 23–10 Ma), however, Australia was climatically stable, warm and wet, with extensive flooding of inland basins and substantial rain forests. This period was followed by drying, resulting in the expansion of open woodlands (Martin, 2006). Starting *c.* 2.5 Ma, the glacial oscillations of the Pleistocene drove fluctuations of sea levels and the expansion of the arid zone associated with glacial maxima. While we know a great deal about the influence of climatic fluctuations on biotic diversification in the Australian Wet Tropics (e.g. Moritz *et al.*, 2009) and in arid (Byrne *et al.*, 2008; Pepper *et al.*, 2011a,b) and eastern winter-rainfall (Byrne *et al.*, 2011; Chapple *et al.*,

2011) systems, we know comparatively little about their influence on diversification in the Australian Monsoonal Tropics (AMT). The AMT (Fig. 1) is one of the largest biomes on the continent, and is the largest intact tropical savanna in the world (Bowman *et al.*, 2010). It is defined by a summer-wet season with widespread cyclonic rainfall, followed by a cooler dry winter season. Numerous biogeographical barriers across the AMT have been proposed based on a combination of climatic and topological factors (reviewed in Bowman *et al.*, 2010; summarized in Fig. 1 and Table 1). Recent studies have also demonstrated high levels of regional and micro-endemism across a number of taxonomic groups including mammals (Bowman *et al.*, 2010; Potter *et al.*, 2012), lizards (Fujita *et al.*, 2010; Melville *et al.*, 2011; Smith *et al.*, 2011) and frogs (Slatyer *et al.*, 2007). We briefly describe hypothesized AMT regions and the evidence for putative barriers to provide context for our study (Fig. 1, Table 1).

The Cape York region is delineated to the west by the Carpentarian Gap (MacDonald, 1969), a barrier over which population- and/or species-level divergence has been found repeatedly in many taxa (Braby, 2008; reviewed in Kearns *et al.*, 2011; Smith *et al.*, 2011). The Burdekin Gap forms the southern boundary of Cape York and is generally considered the major transition zone from the monsoonal/Wet Tropics to the southern winter-rainfall habitat (reviewed in Chapple *et al.*, 2011).

The Top End is a topographically complex region that is marked by scattered escarpments and has high levels of endemism (Crisp *et al.*, 2001; Bowman *et al.*, 2010); it is isolated to the west by the Victoria or Daly River catchments and to the south by the open woodlands of the Northern Deserts (Fig. 1, Table 1). Studies have found that the Arnhem
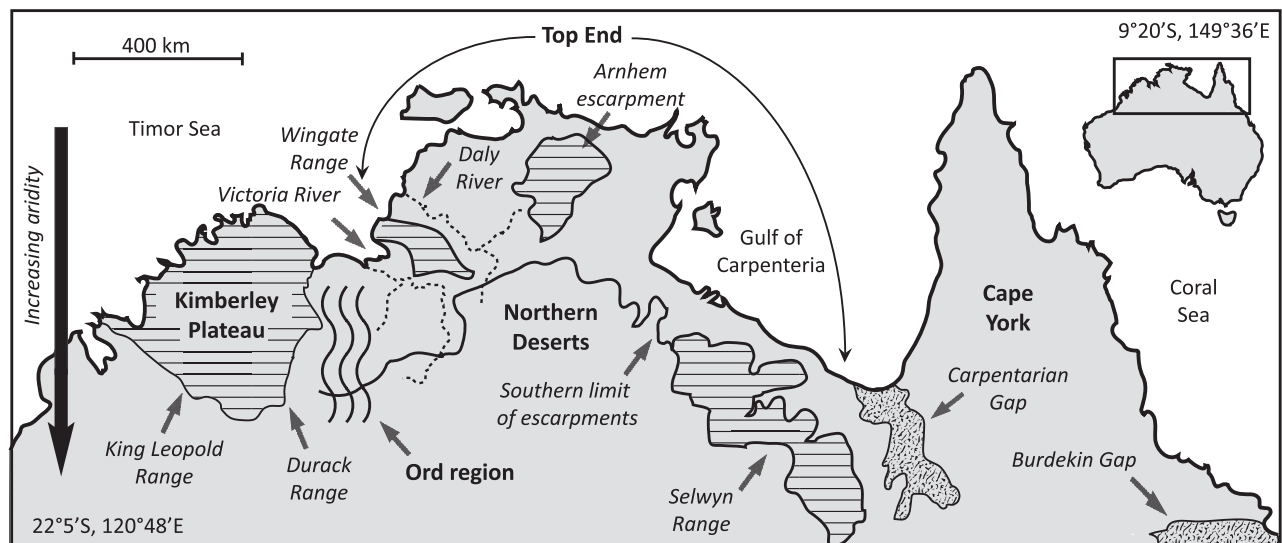


**Figure 1** Hypothesized biogeographical subregions and major barriers of the Australian Monsoonal Tropics. Major bioregions are in bold, and biogeographical barriers are in italics. Wavy lines represent the general hypothesized area of the Ord region; horizontal lines represent large uplifted escarpment blocks, dashed lines represent rivers, and mottled areas represent vegetative/substrate barriers. See Table 1 for details.

**Table 1** Description of hypothesized biogeographical regions and barriers in monsoonal Northern Australia (see Fig. 1).

| Region | Hypothesized extent | References |
|---|---|---|
| Cape York | Monsoonal region north of the eastern mesic zone and east of the Carpentarian Gap. | Reviewed in Kearns et al. (2011) |
| Northern Deserts | Topologically uncomplicated region of open woodland and savanna to the south of the escarpments of the Top End and north of the intensely arid inland dunefield deserts. | Cracraft (1991); this study |
| Top End | Topologically complex region of open woodland west of the Victoria or Daly River catchments, ranging to the Carpenterian Gap in the east. The southern boundary is poorly defined, but is generally associated with sandstone escarpments. | Reviewed in Bowman et al. (2010) |
| Ord region (sometimes called the Ord Arid Intrusion or the Bonaparte Gap) | (1) Low-lying region associated with the Ord River basin between the Kimberley Plateau and Top End. | Bowman et al. (2010); Eldridge et al. (2012) |
| | (2) Region of aridity running north to south from the Bonaparte Gulf to the inland deserts. | Melville et al. (2011); Eldridge et al. (2012) |
| | (3) Biogeographical break over the Victoria River floodplains. | Braby (2008); Toon et al. (2010); Melville et al. (2011) |
| | (4) Forms a single biogeographical unit with the Kimberley Plateau. | Cracraft (1991); Braby (2008) |
| Kimberley Plateau | Uplifted western portion of the underlying Kimberley Craton; region of open woodlands and dramatic escarpments. | Bowman et al. (2010); Eldridge et al. (2012) |

| Barrier | Description | |
|---|---|---|
| Carpentarian Gap | Series of claypans associated with the Flinders, Gillat and Norman rivers that divide the open woodlands of the Top End and Cape York. | MacDonald (1969); Bowman et al. (2010) |
| Burdekin Gap | Broad boundary of dry woodland and savanna that separates northern rain forest and monsoonal habitat from eastern mesic habitat. | Reviewed in Chapple et al. (2011) |
| Arnhem escarpment | Extensive series of escarpments located in the north-eastern Top End. Dramatically uplifted from surrounding regions. | Bowman et al. (2010) |
| Mid-Territory Break | A north–south line down the centre of the Northern Territory over which repeated genetic breaks have been found. Ecological causes are currently unclear. | This study |
| Wingate and associated ranges | An uplifted block of escarpments between the Daly and Victoria rivers. Eastern portion of the Kimberley Craton. | This study |
| Daly River | Major river that forms an extensive break in the surrounding sandstone escarpments. | Potter et al. (2012) |
| Victoria River | Major river that forms an extensive break in the surrounding sandstone escarpments. | Melville et al. (2011); Potter et al. (2012) |
| Durack Range | Eastern extent of the Kimberley Plateau; an uplifted region that divides the Kimberley from the low-lying Ord region to the east. | This study |
| King Leopold Range | Southern extent of the Kimberley Plateau; uplifted region that divides the Kimberley Plateau from the low-lying region to the south. | This study |

escarpment in the east and the region between the Arnhem escarpment and the Daly River harbour their own endemic taxa (Young et al., 2005; Potter et al., 2012). The Ord region is an area that has been poorly or inconsistently defined, and clear evidence of boundaries is lacking, but most consider it to be the region that divides the Kimberley Plateau from the Top End (Table 1).

The Kimberley Plateau is the uplifted western portion of the Kimberley craton, marked by the transition to the uplands of the King Leopold and Durack ranges (Fig. 1; Li, 2000). It is isolated to the south by sandplains and increasing aridity. The underlying Kimberley craton extends east through the Ord region to the Wingate Range, but is less uplifted in this region than it is on the plateau (Li, 2000). The Kimberley craton has been tectonically stable for c. 80 Myr (van Kranendonk et al., 2002) and is believed to have been climatically stable relative to the remainder of the continent during Pleistocene climate cycles (Byrne et al., 2008).

Recent evidence suggests that this region acted as a Pleistocene refugium (Pepper et al., 2011a).

Cracraft (1991) acknowledged that his proposed Northern Deserts region (Fig. 1, south of the solid line) was the least defined of all major Australian biogeographical regions, and this region has been largely absent from studies of the AMT and the arid zone. Recently obtained phylogenetic data, however, suggest that the Northern Deserts represents a fourth core region of the AMT, and that it is essential to understanding patterns of diversification within the biome (Fujita et al., 2010; Melville et al., 2011; Smith et al., 2011; this study).

Recent studies have gone some way towards testing whether these barriers have the predicted effects on species distributions; however, no studied groups have distributions that span the entire AMT region. Studies of species distributions, and of the biogeographical factors influencing them, are taking an increasingly integrative approach, combining

climate and landscape information in the form of ecological niche models (ENMs), with detailed phylogeographical information based on molecular data. ENMs estimate a subset of the conditions in which a species can survive and reproduce, extrapolated from known presence data and a given set of environmental variables (Peterson et al., 2011), and thereby can be used to identify regions of unsuitable habitat or barriers to dispersal (Glor & Warren, 2011). Here we apply ENM methods to a widely distributed species group of terrestrial frogs to provide a comprehensive assessment of putative biogeographical breaks across the entire AMT. Frogs are particularly sensitive to environmental change owing to the combination of possessing sensitive skin and having both aquatic and terrestrial life history stages, which makes them good model organisms for historical biogeographical studies. The influence of climate cycles on anuran diversification is well documented (Carnaval et al., 2009; Moritz et al., 2009; Hoskin et al., 2011). Our goals here were to generate detailed multi-locus phylogenetic and phylogeographical hypotheses to delimit species boundaries in a group of Australian frogs distributed in the AMT and then to use ecological niche modelling to generate hypotheses regarding the climate and landscape features influencing inter- and intraspecific distribution patterns for our group. We evaluated our results relative to previously hypothesized biogeographical barriers and provide a terminology framework for the region.

## MATERIALS AND METHODS

### Study taxa

The Uperoleia, or 'toadlets', are a large genus of Australo-Papuan myobatrachid frogs that include a number of species distributed in the AMT. In the AMT the species are associated with lowlands and breed in wet season ephemeral pools and flooded grasslands. Their small size and burrowing life style suggest low dispersal abilities, indicating that they are likely to be strongly influenced by changes in climate and landscape over evolutionary time. We reconstructed the phylogeographical history of the Uperoleia lithomoda Tyler, Davies & Martin, 1981, U. minima Tyler, Davies & Martin, 1981 and U. trachyderma Tyler, Davies & Martin, 1981 species complex, a group of closely related species endemic to the AMT. A previous molecular study showed that U. lithomoda and U. trachyderma are sister taxa and that U. minima is closely related to this group (Catullo et al., 2011). All three species are small (< 2.5 cm) burrowing frogs with a short, sharp click as a call. Some data on U. mimula Davies, McDonald & Corbin, 1986, an eastern Cape York species not targeted for this study, are included to clarify species boundaries.

### Samples, phylogenetic markers and molecular protocols

We first generated sequence data for the mitochondrial (mtDNA) genes 16S and ND2 for 789 individuals within the Uperoleia genus (not shown). We then mapped the distribution of each clade and used this as the basis for choosing a subset of 146 individuals representing our target species, as well as a representative individual of 17 out of 22 additional species in the genus. For 110 of these individuals we generated sequence data for five protein-coding nuclear (nDNA) genes (A2AB, BDNF, BMP2, NTF3 and RAG1). One U. lithomoda individual per location was sequenced for nDNA loci. GenBank accession numbers are listed in Appendix S1; Appendix S2 details primers and molecular protocols.

### Phylogenetic analyses

We used PartitionFinder 1.0.1 (Lanfear et al., 2012) to perform the combined selection of partitioning schemes and models of molecular evolution. Phylogenetic trees were estimated independently for the mtDNA and nDNA data sets. For Bayesian inference (BI) we used the message-passing interface (MPI) version of MrBayes 3 (Ronquist & Huelsenbeck, 2003), and for maximum likelihood (ML) we used RAxML-VI-HPC 7.2.8 (Stamatakis, 2006). For details of analyses, see Appendix S2. Spicospina flammocaerulea was the outgroup in all analyses, as it is the monotypic sister genus to Uperoleia (Read et al., 2001). We used the method of Shimodaira & Hasegawa (1999) to test for incongruence among the tree topologies recovered, using full optimization in paup* 4.0b10 (Swofford, 2002).

### Morphological data

We examined c. 20 adult specimens (listed in Appendix S1) for each group for the morphometric analyses. The characters measured were: snout–urostyle length (SUL), eye–naris distance (EN), interorbital distance (IO), head depth (HD) internarial distance (IN), eye length (EyeL), arm length (ArmL – elbow to third finger tip), tibia length (TL), and foot + tarsus length (knee to fourth toe tip). We examined the relationships among characters using discriminant function analysis (DFA) and principal components analysis (PCA) in the statistical software package jmp 9.0 (SAS Institute, Cary, NC, USA). We calculated standard principal components on the raw data with imputation of missing values so that all animals could be included, but only six data points were missing.

### Call data

We analysed the calls of 61 males represented by DNA sequence data (Appendices S1 & S3) using Raven Pro 1.3 (Bioacoustics Research Program, 2008). We also analysed three calls of U. lithomoda from eastern Cape York, and four calls of U. minima from the Kimberley Plateau. For each individual, we selected and analysed three representative calls to estimate average call characteristics. For each call we measured duration (to the nearest millisecond), pulse number, and maximum frequency (Hz) averaged over the whole call.

We analysed maximum frequency using a 1024 sample Hanning window (Catullo *et al.*, 2011). We analysed averaged call data for each individual using DFA and PCA statistics as in the morphological data. Analysis of call data indicated that at least one measured character did not overlap among species when calling temperature was not controlled for (Appendix S3).

## Ecological niche modelling

In order to estimate species distributions using the full known distribution, we combined locality data from three sources (Appendix S1): sequenced individuals from this study, locations of actively calling unvouchered males (R.A.C., unpublished data), and additional distributional data from Ozcam (http://ozcam.ala.org.au/, accessed November 2011), vetted by R.A.C. for taxonomic consistency and location accuracy. Owing to incongruence between mtDNA and nDNA tree topology (discussed below), we used a combination of genetic, morphological and acoustic data for species delineation (see Appendix S2). Resolved species for modelling were *U. minima*, *U. lithomoda*, *U. trachyderma* W (formally described as a new species in Catullo *et al.*, in press), and *U. trachyderma* E (the *U. trachyderma* holotype belongs to this lineage). We had location data for 410 individuals of *U. lithomoda*, 60 of *U. minima*, 50 of *U. trachyderma* E, and 38 of *U. trachyderma* W.

We generated ENMs for each of the target taxonomic units using the modelling approach implemented in Maxent 3.3.3 (Phillips & Dudík, 2008). To control for sampling bias and detectability, we limited pseudo-absence sampling to locations with myobatrachid frogs present, but not our target taxa. The final model included annual mean temperature, mean diurnal range, mean temperature of the driest quarter, mean temperature of the warmest quarter, precipitation seasonality, precipitation of the wettest quarter, precipitation of the driest quarter, surface geology, major vegetation groups, and elevation. We generated ENMs for the Last Glacial Maximum (LGM, 21 ka) using the climate layers listed above. Appendix S2 provides explicit details of modelling protocols.

We tested the significance of our models by comparing our model AUC (area under the curve of a receiver operating characteristic plot) values (a measure of how well a model predicts the distribution) against a null distribution of 100 sets of AUC values, based on random presence points generated using ENMTools 1.4.1 (Warren & Seifert, 2011). Recent work by Renner & Warton (2013) demonstrated that Maxent (Phillips & Dudík, 2008) uses a Poisson point process model, and suggests modifications to the software to improve model performance. When model parameter selection is cautious, however, Maxent outperforms nearly all other methods currently available. Thus ENMs and Maxent currently remain the most effective way of translating climate data into useable biogeographical information (Elith *et al.*, 2006; Wiens *et al.*, 2009).

## RESULTS

Coverage was nearly complete for all individuals and genes (100% for *16S*, *A2AB*, *BDNF* and *RAG1*, 98.7% for *ND2*, 98.4% for *NTF3* and 96.1% for *BMP2*). No more than a single gene was missing for any one individual. The nuclear alignment was 3069 bp, and the mitochondrial alignment was 1769 bp. Strong phylogenetic and phylogeographical structure in the *U. lithomoda/trachyderma/minima* group was inferred from our BI and ML analyses (Figs 2 & 3).

## Nuclear gene relationships and phylogeography

*Uperoleia trachyderma* is represented by a single clade; however, all individuals from the eastern half of the Northern Deserts, hereafter '*U. trachyderma* E' (Figs 2b & 3a, yellow), form a strongly supported monophyletic group [posterior probability (PP) = 1] within the broader *U. trachyderma* clade (Fig. 2b, yellow/green). The individuals from the western Northern Deserts, hereafter '*U. trachyderma* W' (Figs 2b & 3a, green), form a polytomy with *U. trachyderma* E, but with the broader *U. trachyderma* group weakly supported (PP = 0.85).

All Kimberley Plateau individuals, hereafter *U. minima*, form a strongly supported clade (PP = 1, Figs 2b & 3a, red). Samples from the Top End and Cape York, hereafter '*U. lithomoda* TE' and '*U. lithomoda* CY' (Figs 2b & 3a, dark and light blue, respectively), are part of a well-supported group that also includes the Cape York species *U. mimula*, *U. littlejohni* and *U. altissima* (PP = 1). These species form a soft polytomy. Most of the *U. lithomoda* TE individuals form a weakly supported clade (PP = 0.87). *Uperoleia mimula* individuals form a strongly supported clade (PP = 1; Figs 2b & 3a, orange).

## Mitochondrial relationships

The mtDNA and nDNA phylogenies were significantly different. The maximum clade credibility tree from the BI analysis of the nDNA was compared with an alternative topology that was consistent with the strongly supported maximum clade credibility BI topology found in the mtDNA data set. This alternative topology was rejected using the Shimodaira–Hasegawa test ($P < 0.0001$), indicating that the topologies based on the mtDNA and nDNA markers are significantly different.

The mitochondrial haplotypes for *U. minima* (crown support PP = 1; Fig. 2a, red) were broadly congruent with the distribution of *U. minima* nDNA haplotypes (Fig. 3a,b, red). However, the mitochondrial haplotype associated with all *U. trachyderma* W individuals (Fig. 2a, green) was also found in three samples from the Kimberley Plateau. The three Kimberley Plateau individuals that carry the *U. trachyderma* W mitochondrial haplotype (Fig. 3b, red and green half-diamonds) form a well-supported monophyletic group within the *U. trachyderma* W mtDNA clade (PP = 1). The
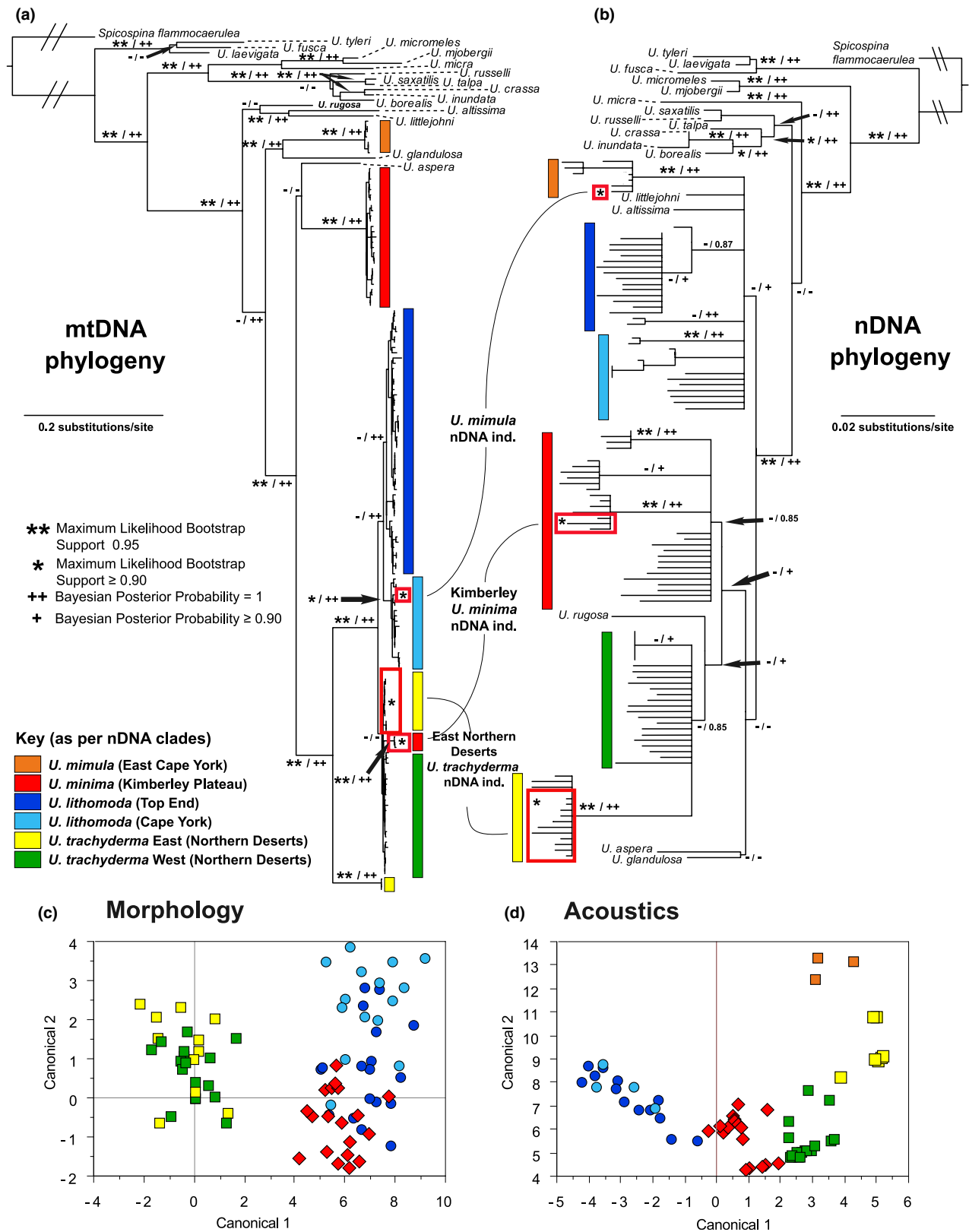
**Figure 2** (a) Mitochondrial and (b) nuclear gene phylogenies of *Uperoleia* toadlets in the Australian Monsoonal Tropics. Asterisks and pluses refer to maximum likelihood bootstrap support followed by Bayesian posterior probabilities. Colours indicate the region of specimen collection as well as final assigned species. Red boxes highlight individuals that are incongruent, and lines show the change of individual placements between the phylogenies. Discriminant function analysis results for (c) morphology and (d) acoustics are coloured according to the individual's nDNA clade (see text for details).
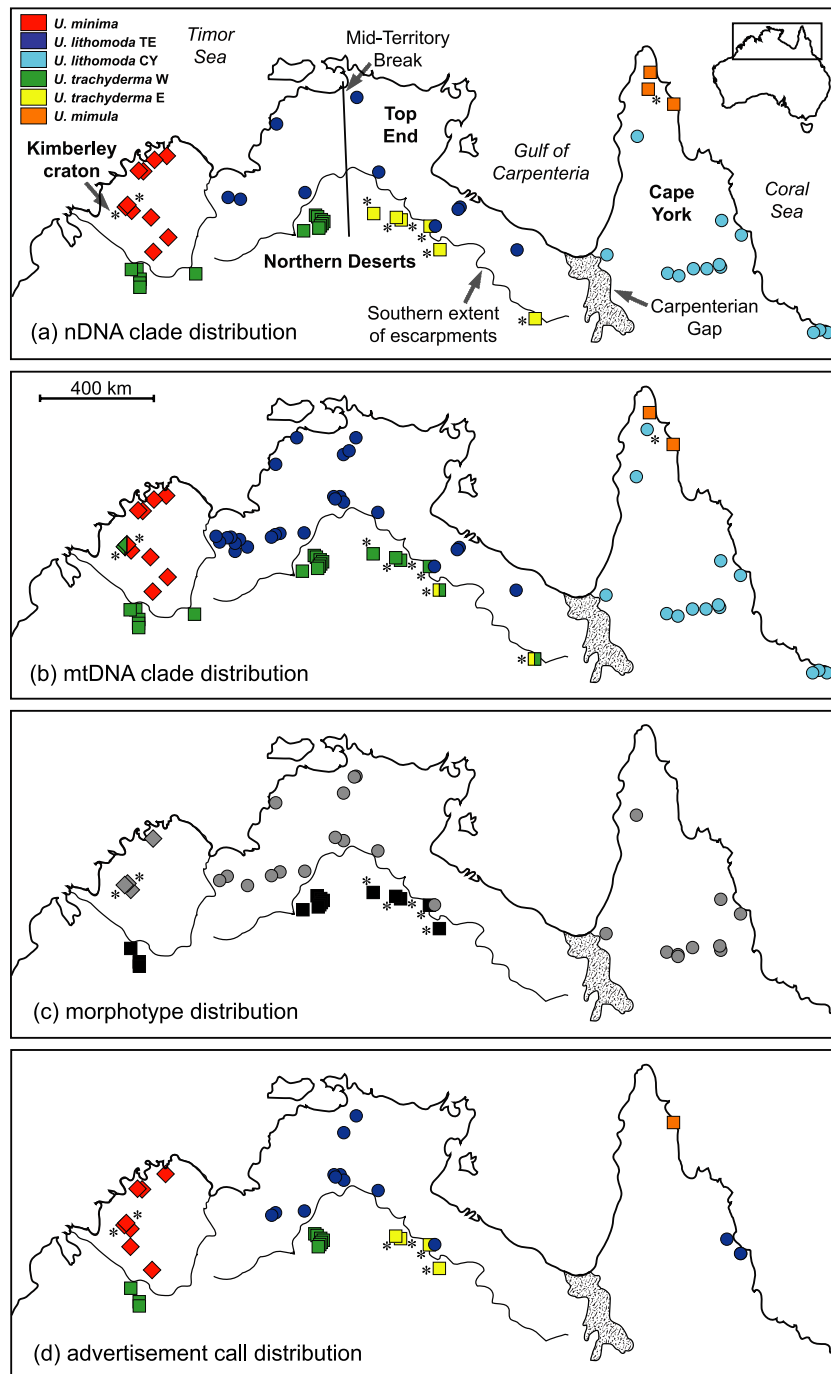
**Figure 3** Maps of the Australian Monsoonal Tropics showing the distribution of (a) nDNA clades, (b) mtDNA clades, (c) morphological variation and (d) acoustic variation of *Uperoleia* toadlets. Half-coloured shapes in (b) indicate locations with multiple mtDNA clades present. Locations with individuals showing a discordant gene pattern between the mtDNA and nDNA are marked by an asterisk. Grey and black in (c) indicate the distribution of the two discernible morphotypes, and blue in (d) indicates the two acoustically indistinguishable clades of *U. lithomoda*. Colours and shapes otherwise match those in Fig. 2.

*U. trachyderma* W mitochondrial haplotype was also found in most samples from the eastern Northern Deserts (PP = 0.88; Fig. 3b, green). The *U. trachyderma* E mitochondrial haplotype (Fig. 2a, yellow) is represented by samples collected at the far eastern end of the Northern Deserts (Fig. 3b, yellow) and is sympatric with the *U. trachyderma*

W mitochondrial haplotype (Fig. 3b, green and yellow half-squares).

Despite a significantly different phylogenetic placement from that in the nDNA phylogeny, *U. lithomoda* forms a strongly supported mtDNA clade (PP = 1; Fig. 2a). There is also strong support within the clade (PP = 1; Fig. 2a, dark blue and light blue) for Ord region/Top End versus Cape

York clades (Fig. 3b, dark blue and light blue circles, *U. lithomoda* TE and *U. lithomoda* CY, respectively). Four strongly supported mtDNA clades are present within the *U. lithomoda* TE clade (PP = 1; Fig. 4). One individual carrying a *U. lithomoda* CY mitochondrial haplotype is in the *U. mimula* nDNA clade (Figs 2a,b & 3a,b). All other samples of *U. mimula* (Figs 2a & 3b, orange), an east Cape York clade, form a well-supported clade (PP = 1; Fig. 3b, orange squares).

## Morphology

For 78 of 84 individuals the morphological data set was complete. For the remaining six, only head depth was not recorded. Analyses indicate the presence of two discernible morphotypes. The DFA (Figs 2c & 3c) and PCA (Appendix S3) distinguish one morphotype comprising individuals from the two *U. trachyderma* nDNA lineages from another comprising the nDNA lineages of *U. lithomoda* and *U. minima*. The *U. minima* nDNA individuals from the Kimberley Plateau carrying the *U. trachyderma* W mitochondrial haplotype are similar to the sympatric *U. minima* individuals and are easily distinguishable from the *U. trachyderma* W nDNA

individuals. These results are driven primarily by head depth and head shape (canonical variate 1) versus body size (canonical variate 2), with wider and thinner heads in the *U. trachyderma* clades (Appendix S3).

## Calls

All 62 individuals had complete data for call characteristics. DFA (Figs 2d & 3d) and PCA (Appendix S3) distinguished five acoustic groups that corresponded to the five major nDNA clades. The nDNA *U. minima* individuals from the Kimberley Plateau (including those with the *U. trachyderma* W mtDNA haplotype) were distinguishable from all other groups. The two *U. trachyderma* nDNA lineages were acoustically divergent from each other and from all other groups, consistent with the nDNA clade of the individuals. The two *U. lithomoda* mtDNA lineages were similar but could be distinguished from all other groups. *Uperoleia mimula* was divergent from all other clades. Canonical variate 1 primarily quantifies variation based on pulse number, and canonical variate 2 primarily quantifies call duration (Appendix S3). *Uperoleia lithomoda* has the greatest number of pulses, *U. mimula* has the longest call duration, *U. minima* and
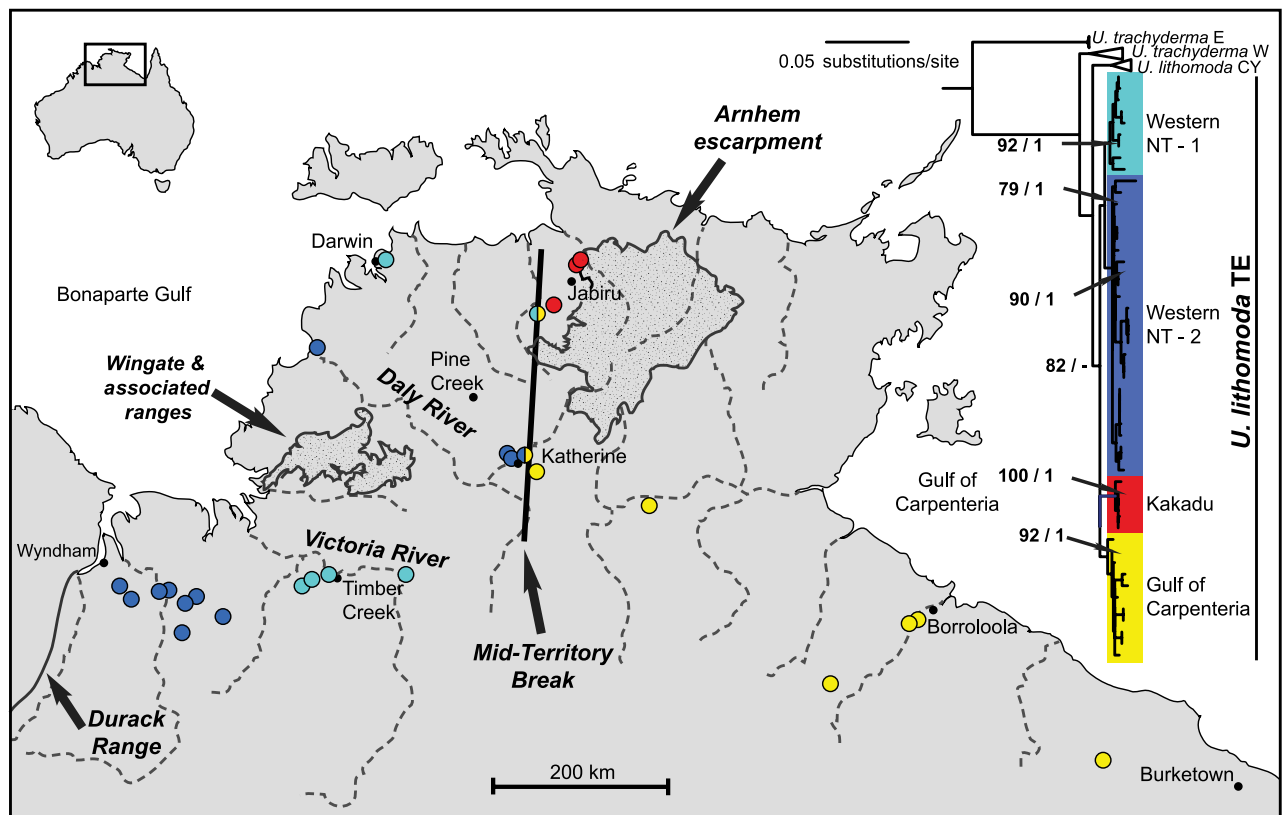


**Figure 4** Distribution map of the major clades of *Uperoleia lithomoda* TE (Top End) in the Australian Monsoonal Tropics. The phylogeny and colours on the right panel illustrate the four well-supported mtDNA clades in the Top End. Values represent maximum likelihood bootstrap support then Bayesian posterior probabilities. Colours on the map represent collection localities and the clade identified at that location. Where two clades are present in a location, this is represented by a circle with both colours. The bold dark bar represents the Mid-Territory Break, dashed lines represent rivers, and dark outlines represent escarpments.
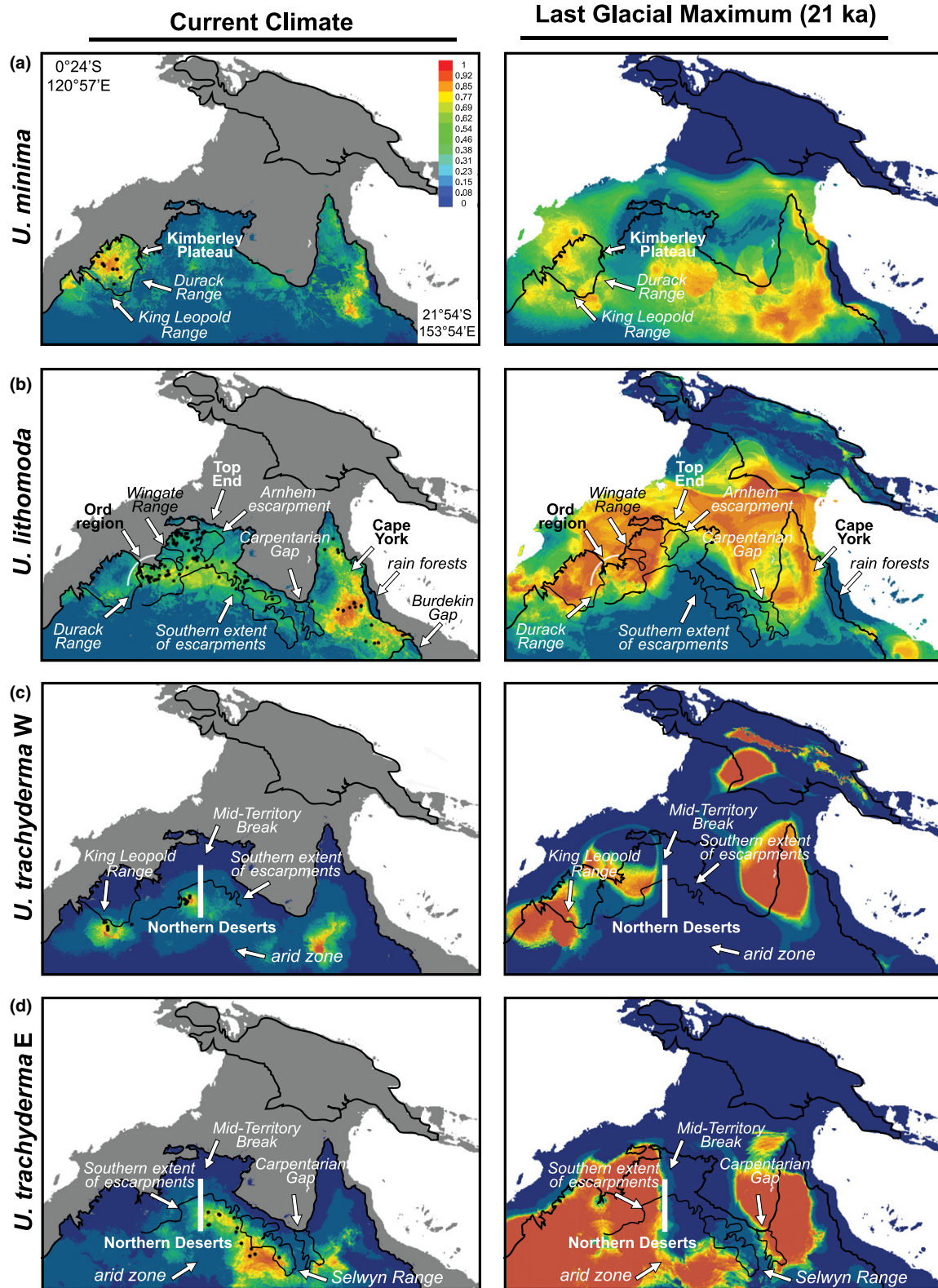
**Figure 5** Ecological niche models of current climate (left) and palaeoclimate (right) for each species of *Uperoleia* toadlets in the Australian Monsoonal Tropics. Black dots in the current climate represent model locations. Colours represent the predicted probability of presence from low (blue) to high (red) suitability. Bold outlines indicate the extent of the current landmass, and grey colours in the left column indicate the land extent during the Last Glacial Maximum (LGM). Detail on the models clarifies model-supported hypothesized barriers to species discussed in the text. Major bioregions are in bold and biogeographical barriers are in italics. Lines represent the ecological boundaries illustrated in Fig. 1.

*U. trachyderma* W have calls of similar pulse number and duration (but different pulse oscillation number, R.A.C., unpublished data), while *U. trachyderma* E has a greater pulse number and longer duration than *U. minima* or *U. trachyderma* W.

## Ecological niche modelling

All model AUC values were higher than every null AUC value ($P < 0.01$, Appendix S3), indicating that the ecological correlations between presence locations and predictor variables identified by Maxent significantly deviate from random (Raes & ter Steege, 2007).

The *U. minima* ENM predicts a region of suitable habitat that broadly corresponds to the uplifted Kimberley Plateau, with suitability ending at the interface of the King Leopold and Durack ranges and the surrounding low-lying regions, which isolate the Kimberley Plateau from other potential suitable regions (Fig. 5a).

The ENM for *U. lithomoda* (Fig. 5b) predicts suitability in the Ord region, in a band across the Top End, and into the Gulf of Carpentaria area. Lower suitability was predicted at the northernmost extent of the Top End, despite numerous samples from the area; this is likely to be an effect of the sampling bias method downweighting this area. The lowest-suitability areas in the Top End include the Wingate Range and east of the Arnhem escarpment. The model predicts a strong gap in suitability in the region of the Carpentarian Gap, high suitability on Cape York's western slopes, low suitability in the rain forest, and low suitability to the south at the Burdekin Gap.

The ENM for the *U. trachyderma* W predicts two disjunct regions of suitability in the western half of the Northern Deserts, and another disjunct area of suitable predicted habitat in Cape York (Fig. 5c). The area of unsuitable habitat at the eastern end of the species distribution corresponds to the drop-off in predicted suitability found in the *U. trachyderma* E model (Mid-Territory Break in Fig. 5c,d). The ENMs for both *U. trachyderma* clades have reduced suitability on the northern side, which broadly corresponds to the beginning of the sandstone escarpments of the Top End.

The ENM for *U. trachyderma* E shows a broad region of suitable habitat on the eastern half of the Northern Deserts, extending south of the Selwyn Range (Fig. 5d). Areas of predicted suitability are also present in southern Cape York, but this high suitability area is separated from the current distribution by a region of lower suitability associated with the Carpentarian Gap.

Projections of the current climate niche onto climate data for the LGM show that most of the Kimberley Plateau remained climatically suitable for *U. minima*, with the highest suitability in the north-west, but that it was surrounded by less suitable low-lying regions to the south and east (Fig. 5a). The ENM projection for *U. lithomoda* (Fig. 5b) shows that regions similar to the current niche remained in the western portion of the Top End and much of the Cape York Peninsula, with areas of low suitability in the Ord region and east Top End through to what is now the Gulf of Carpentaria. The *U. trachyderma* W model (Fig. 5c) predicts that parts of the current western Northern Deserts distribution remained relatively climatically stable. Projections for *U. trachyderma* E (Fig. 5d) suggest that suitable climates were available in western Cape York and the Gulf, south of the Selwyn Range, and in large portions of the western half of the monsoonal tropics.

## DISCUSSION

We have integrated genetic, morphological and acoustic data from a group of frogs with ENMs to evaluate the influence on diversification of previously identified putative biogeographical barriers. Despite incongruence between the nDNA and mtDNA phylogenies, our combined data support five clades representing four allopatric species. *Uperoleia minima* is endemic to the Kimberley Plateau. *Uperoleia lithomoda* is found in the Ord region, Top End and Cape York, with population structure between Cape York and the Ord region/Top End. *Uperoleia trachyderma* is divided into two relatively cryptic species distributed in the eastern and western portions of the Northern Deserts. Our results challenge all previous definitions of the Ord region and highlight the importance of including the Northern Deserts in studies of the AMT. This work also identifies new biogeographical barriers and putative Pleistocene refugia. Here we structure our discussion around each of the major AMT biogeographical regions or putative barriers as outlined in Fig. 1 and Table 1.

## Barriers isolating Cape York

The Carpentarian Gap was predicted in our models to be a major barrier to dispersal for *Uperoleia* species (Fig. 5b,d), supporting the molecular results of divergence between the *U. lithomoda* TE and *U. lithomoda* CY mtDNA clades (Figs 2a & 3b), and forming the eastern boundary of *U. trachyderma* E (Fig. 3a). Our work supports the hypothesis that changes in the Gulf of Carpentaria and the Carpentarian Gap have resulted in major vicariance events across multiple taxa (see Introduction). The Burdekin Gap was also predicted by our ENM as a region of low-suitability habitat for *U. lithomoda* (Fig. 5b). This provides support for this region representing the major biome-level transition zone from the monsoonal/Wet Tropics to winter-rainfall habitat (reviewed in Chapple et al., 2011).

## The Ord region: Kimberley Plateau or Top End affinities, or a unique bioregion?

Samples from the Ord region were in the *U. lithomoda* TE mtDNA clade (PP = 1), a clade that has a distribution across the entirety of the Top End. To determine whether the Victoria or Daly River catchments influence the current population structure of alluvial frogs in support of previous Ord

region hypotheses (Table 1), we mapped the four major mtDNA clades of *U. lithomoda* TE. We found no population structure associated with any of the hypothesized barriers (Fig. 1). Our data also did not support any of the four Ord region definitions (Table 1). The *U. lithomoda* and *U. minima* models (Fig. 5a,b) show that there is a strong suitability turnover between the Top End and Kimberley Plateau, which is coincident with the eastern boundary of the Kimberley Plateau (Durack Range) and thus incorporates the Ord region into the Top End.

We found little evidence in our data, or in other published studies, for broad-scale Ord region endemism, or for an overall pattern of species distributed across the Ord region–Kimberley Plateau (but not Top End). Exceptions exist, such as the Ord region endemic frog *Limnodynastes depressus* and the snake *Suta ordensis* (Cogger, 2000), or the Ord region–Kimberley Plateau species, including the tree frog *Litoria splendida* (Cogger, 2000) and boab trees (*Adansonia gregorii*; Boland *et al.*, 2006). However, many other studies show a variety of different patterns (Fujita *et al.*, 2010; Oliver *et al.*, 2010; Melville *et al.*, 2011; Smith *et al.*, 2011; Potter *et al.*, 2012.). Overall, we found little evidence that the Ord region is primarily associated with the Kimberley Plateau, or that it is a unique biogeographical region as previously defined.

Current terminology refers to the Ord as an 'arid intrusion' (Bowman *et al.*, 2010; Eldridge *et al.*, 2012); however, Guerschman *et al.* (2009) estimated actual evapotranspiration (a proxy for on-ground water availability) and showed that this area is not significantly different from the Kimberley Plateau or Top End. The study of Guerschman *et al.* (2009) indicated greater water availability than expected by precipitation, implying that the role of this region as a drainage basin may be highly important to ecosystem function. Climate layers also indicate that this region receives more than 700 mm of rainfall per year, indicating that the label 'arid' may be inaccurate (BIO12 – annual precipitation, WorldClim). Based on these data, we suggest the 'Ord region' as a more neutral descriptor. The presence of some Ord region endemics may be explained by the region's unique geology rather than climatic factors, a hypothesis that is yet to be tested.

### Barriers within the Top End

We found that the eastern edge of the Kimberley Plateau (Durack Range) represents the western boundary for our Top End species. Within the Top End, the model for *U. lithomoda* predicts slightly lower suitability on the Wingate Range (Fig. 5b), suggesting that the latter may be a driver of alluvial species assemblage transitions at the Ord region–Top End barrier. Our result differs substantially from the riverine barriers influencing rock wallaby distributions (Potter *et al.*, 2012), suggesting that biogeographical patterns may differ widely between species on the widespread savanna woodlands and those restricted to sandstone escarpments.

The *U. lithomoda* model (Fig. 5b) predicts lower suitability on the Arnhem escarpment, in agreement with studies showing that the region harbours a unique assemblage of flora (Bowman *et al.*, 1988, 2010) and fauna (Andersen *et al.*, 2001; Slatyer *et al.*, 2007). The possibility of the Arnhem and Wingate escarpments acting as vicariant barriers currently and during climate cycles, or creating speciation suture zones, should make them a priority for further study (Moritz *et al.*, 2009). The interaction of Pleistocene climate cycles with escarpments as barriers to dispersal could have played a role in the generation of population structure and the generation of new species, and represents a key topic for future study.

These data indicate that it is more appropriate to use the term 'Top End' for the broader biogeographical region and to restrict the term 'Arnhem' to the sandstone escarpments to the north-east, which have been suggested to represent an additional unique biogeographical region (Bowman *et al.*, 1988, 2010; Woinarski *et al.*, 2007). The terminology used has been variable in the literature (Fujita *et al.*, 2010; Melville *et al.*, 2011), but given the emerging complexity of the region we consider the term 'Top End' more appropriate than the Cracraft (1991) and Eldridge *et al.* (2012) definition.

### Defining the Kimberley

Our data clearly indicate the presence of only one of our target taxa, *U. minima*, on the Kimberley Plateau (Fig. 3). The ENM for *U. minima* (Fig. 5a) indicates that this region is determined largely by the extent of the uplifted escarpments and is separate from the Ord region. Although we had no samples encompassing the east–west Kimberley Divide, shown for wallabies (Potter *et al.*, 2012), our model did not suggest a major change in niche suitability along this line. Our LGM model's prediction of higher stability on the north-west of the plateau supports the idea that the Kimberley Plateau acted as a Pleistocene refugium. This stable region also agrees with the finding that the region has high endemism in all vertebrate taxa (Slatyer *et al.*, 2007; Powney *et al.*, 2010; Doughty, 2011).

### The Northern Deserts: one region or two?

Our phylogenetic, morphological and acoustic data support the presence of two parapatric species in the Northern Deserts (Fig. 3a). The northern boundary of the Northern Deserts ENMs broadly corresponds to the transition from rocky escarpments and soils and tall open woodland of the Top End to the level sandy soils and open woodland of the Northern Deserts. This result is supported by the distribution of Northern Deserts lineages of lizards (Fujita *et al.*, 2010; Melville *et al.*, 2011; Smith *et al.*, 2011), suggesting that geology may be a major factor driving distributional changes between the Top End and Northern Deserts. The southern boundary of ENMs for both Northern Deserts species broadly corresponds to latitude 18° S, which is in agreement

with the arid/monsoonal transition zone between lineages of Northern Deserts lizards and is strongly associated with the mean diurnal range in our models (Appendix S3).

We found a new biogeographical barrier between the two halves of the Northern Territory, which we refer to as the Mid-Territory Break. This boundary corresponds to species-level divergence between the two clades of *U. trachyderma* (Fig. 3a) and population-level divergence between the eastern and western *U. lithomoda* TE clades (Fig. 4). The ENMs for both these Northern Deserts species predict an abrupt change in niche suitability along this line, although the environmental factors in this transition remain unclear (Fig. 5c,d). The distinct transition in suitability down the middle of the Top End in the LGM models for *U. trachyderma* E and *U. trachyderma* W (Fig. 5c,d) is driven largely by changes in mean diurnal range (Appendix S3). The minimum temperature of the warmest season has been largely overlooked in relation to distributional changes through the Pleistocene; however, our data suggest that this may be a major limiting factor for tropical taxa.

An unexpected result was the prediction of the area south of the Selwyn Range as a Pleistocene refugium for *U. trachyderma* E (Fig. 5d), supporting studies indicating that mountain ranges in both monsoonal and arid areas of Australia have acted as refugia and influenced lineage persistence during the Pleistocene (Oliver et al., 2010; Pepper et al., 2011a). This suggests that the interaction between monsoonal and arid regions is complex and that speciation may occur repeatedly across this interface, as evidenced by the numerous mesic/arid transitions in *H. binoei* (Fujita et al., 2010).

## CONCLUSIONS

Tropical regions have been shown to harbour both significant endemism and cryptic species (Carnaval et al., 2009; Moritz et al., 2009; Hoskin et al., 2011), and our work shows that the AMT is no exception. Understanding the environmental barriers to dispersal and their effect on speciation is vital to exploring and documenting species diversity, especially when morphological crypsis masks the true diversity. We combined genetic, morphological and acoustic data to delineate species boundaries, identified cryptic species and provided a region-wide assessment of the biogeography of northern Australia. Through our work we have been able to identify previously unrecognized biogeographical barriers, better define biogeographical regions, and propose new hypotheses about the effects of Pleistocene climate cycles on the present-day diversity of northern Australia. Our study can serve as a springboard for future investigations of biogeographical patterns in other taxa.

## ACKNOWLEDGEMENTS

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Specimen details, GenBank accession numbers, locality details and OZCAM locality data.
**Appendix S2** Molecular and ecological niche modelling protocols.
**Appendix S3** Additional detailed genetic, morphological, acoustic and ecological niche modelling results.

## BIOSKETCH

**Renee Catullo** studies anuran evolution, systematics and biogeography, with a focus on the development and maintenance of diversity in the Australian Monsoonal Tropics.

Author contributions: R.A.C. and J.S.K. developed the project, and R.A.C., J.S.K. and P.D. obtained funding. Fieldwork and sample collection was conducted by R.A.C. and P.D.; R.A.C. completed the molecular and morphological data collection; R.A.C., R.L. and J.S.K. completed analyses. All authors contributed to writing the manuscript, with each reading and approving the final manuscript.

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