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Subspecies at crossroads: the evolutionary significance of genomic and phenotypic variation in a wide-ranging Australian lizard (*Ctenotus pantherinus*)

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Many subspecies were described to capture phenotypic variation in wide-ranging taxa, with some later being found to correspond to divergent genetic lineages. We investigate whether currently recognized subspecies correspond to distinctive and coherent evolutionary lineages in the widespread Australian lizard *Ctenotus pantherinus* based on morphological, mitochondrial and genome-wide nuclear variation. We find weak and inconsistent correspondence between morphological patterns and the presumed subspecies ranges, with character polymorphism within regions and broad morphological overlap across regions. Phylogenetic analyses suggest paraphyly of populations assignable to each subspecies, mitonuclear discordance and little congruence between subspecies ranges and the distribution of inferred clades. Genotypic clustering supports admixture across regions. These results undermine the presumed phenotypic and genotypic coherence and distinctiveness of *C. pantherinus* subspecies. Based on our findings, we comment on the operational and conceptual shortcomings of morphologically defined subspecies and discuss practical challenges in applying the general notion of subspecies as incompletely separated population lineages. We conclude by highlighting a historical asymmetry that has implications for ecology, evolution and conservation: subspecies proposed in the past are difficult to falsify even in the face of new data that challenge their coherence and distinctiveness, whereas modern researchers appear hesitant to propose new subspecies.

ADDITIONAL KEYWORDS: cryptic species – leopard skink – morphology – phylogeography – population genetics – Scincidae – speciation – taxonomy.

INTRODUCTION

Many species-level taxa with broad geographical distributions show phenotypic variation across their range. Zoologists have frequently used infraspecific categories (in particular, subspecies) to draw attention to such phenotypically distinctive populations that nonetheless appear insufficiently differentiated to warrant species status. With a long history in taxonomy, the subspecies category generally denotes a set of populations made up of phenotypically similar individuals that also cluster geographically (Mayr, 1963; Patton & Conroy, 2017). Importantly, most subspecies currently recognized were proposed to capture observable trait differences across the distribution of a taxon and are not explicitly designed to delimit evolutionary entities (Braby *et al.*, 2012). Nevertheless, genetic studies of a wide range of organisms have subsequently found that morphologically defined subspecies often correspond to phylogenetic lineages (e.g. Braby *et al.*, 2012; Sackett *et al.*, 2014; Kealley *et al.*, 2020; Marshall *et al.*, 2021). These subspecies might therefore warrant species recognition under widely used criteria for species delimitation (Dobzhansky, 1971; Cracraft, 1987; de Queiroz, 1998). Concomitantly, other studies have found that certain subspecies are, essentially, arbitrary groupings of continuous variation or lack phylogenetic cohesion (e.g. Burbrink *et al.*, 2000; Zink, 2004; Braby *et al.*, 2012). Therefore, some authors have contested the utility of subspecies, advocating either

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for discarding them (when they do not correspond to phylogenetic lineages) or for elevating subspecies to species (when they do) (Padial & De la Riva, 2020; Burbrink *et al.*, 2022). With the increasing availability of genetic datasets, many species descriptions, in groups ranging from European birds to Australian lizards, have involved testing whether subspecies demonstrate sufficient phylogenetic divergence to justify their elevation to species (e.g. Hutchinson & Donnellan, 1999; Collinson *et al.*, 2006; Hutchinson *et al.*, 2006; Kealley *et al.*, 2020; Pavia *et al.*, 2021).

The finding that many subspecies correspond to distinct phylogenetic lineages has often led to the (mostly unstated) assumption that most or all currently recognized subspecies are evolutionarily coherent and divergent (Zink, 2004; Braby *et al.*, 2012). This assumption has consequences for macroevolution, macroecology and conservation. For instance, some studies have considered subspecies to correspond to incipient species, using them to estimate rates of intraspecific lineage divergence and its relationship with diversification dynamics at the macroevolutionary scale (e.g. Haskell & Adhikari, 2009; Phillimore, 2010; Van Holstein & Foley, 2020). Moreover, subspecies are often the focus of protective legislation and conservation programmes, in some cases under the premise that they represent evolutionary potential and thus increase the resilience and persistence of species (Haig *et al.*, 2006; Braby *et al.*, 2012). However, whether currently recognized subspecies correspond to evolutionary lineages is unknown in many (and probably most) groups of organisms.

In this article, we address the evolutionary significance of morphologically defined subspecies in one of the most widespread taxa of Australia, the leopard skink *Ctenotus pantherinus* (Peters, 1866) (Fig. 1A). These lizards occur across Australia in most of the central arid zone, monsoonal tropical grasslands to the north and mediterranean and temperate woodlands and grasslands to the west

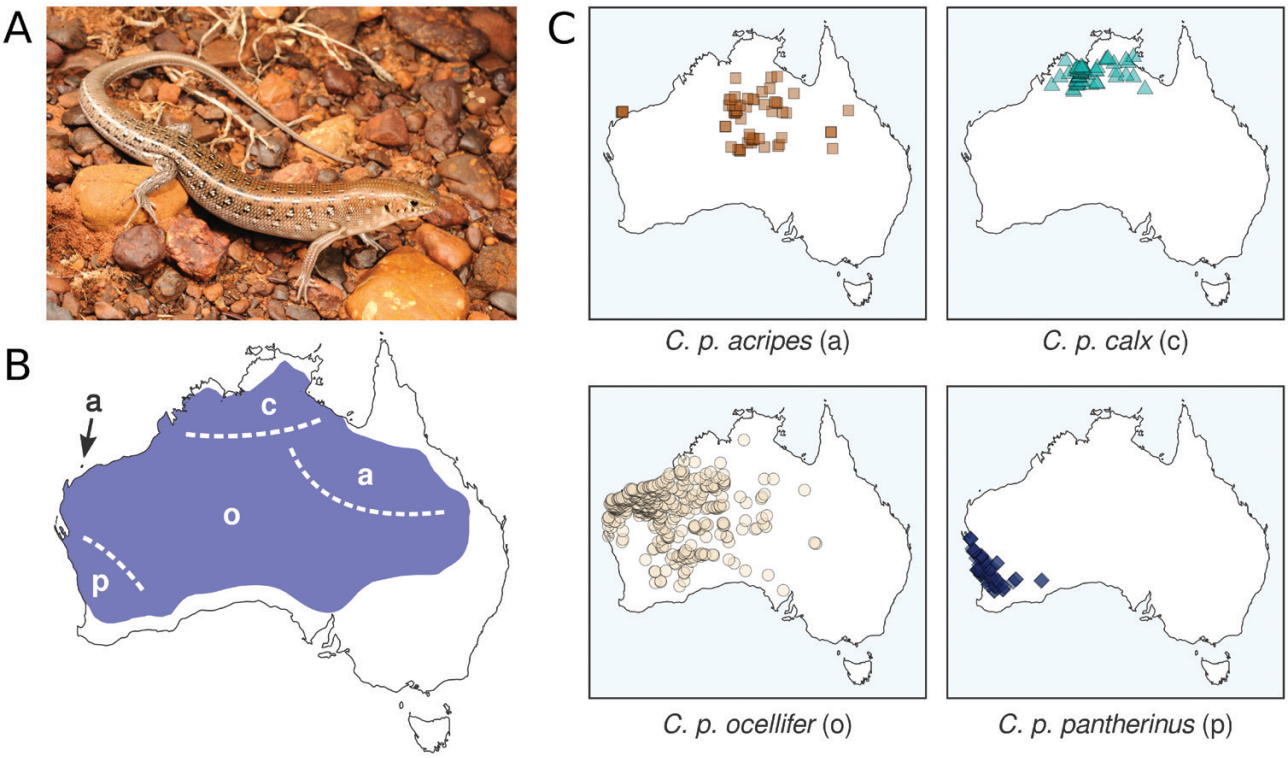

Figure 1. A, Illustrative picture of *C. pantherinus* in life (subspecies *C. p. ocellifer*), courtesy of Eric Vanderduys. B, C, distributions of currently recognized *Ctenotus pantherinus* subspecies. B, presumed distributions of *C. pantherinus* subspecies as typically presented in field guides and taxonomic compendiums (based on Ehmann & Strahan, 1992; Storr *et al.*, 1999). Subspecies are as follows: *C. p. acripes* (a), *C. p. calx* (c), *C. p. ocellifer* (o) and *C. p. pantherinus* (p). Note the disjunct distribution of *C. p. acripes*, whose type locality is on a Western Australian island (Barrow Island; arrow). C, sampling localities of 1464 voucher specimens split by subspecies assignment as in the original museum records (for details on how we compiled these data, see the Material and methods section). Note that subspecies ranges as commonly understood (B) often disagree with those suggested by museum records.

and east. This distribution spans ~5 000 000 km^2, > 65% of the total area of the Australian continent (Roll *et al.*, 2017). Four regional subspecies were described in *C. pantherinus* four to five decades ago based on morphological attributes (Storr, 1969, 1970, 1975). However, the coherence and distinctiveness of these subspecies have not been revisited ever since, despite much improved geographical sampling and the availability of genetic samples of *C. pantherinus* in recent years. This situation is representative of many other Australian squamate reptiles. Typically, subspecies descriptions in Australian squamates rely on one or a few arbitrary traits (e.g. the presence of stripes or the shape of specific scales) and rarely provide details on trait variation within and between populations. Furthermore, the basis for recognizing species vs. subspecies is rarely discussed (e.g. Storr, 1969, 1970, 1975; Horner, 2005).

In groups ranging from lizards (e.g. Hutchinson *et al.*, 2006; Rabosky *et al.*, 2014; Kealley *et al.*, 2020) to birds (e.g. Pavia *et al.*, 2021), mammals (e.g. Patton & Conroy, 2017; Balakirev *et al.*, 2019), butterflies (Braby *et al.*, 2012) and plants (Chase *et al.*, 2018, 2021), genetic data have become a crucial aid to interpretating patterns of phenotypic trait variation as reflective of evolutionary divergence or simply polymorphisms. Nevertheless, it can be difficult to assess whether morphologically defined groups correspond to genetic lineages when morphological variation has been characterized incompletely (Cadena & Zapata, 2021). In the case of Australian squamates, most species and subspecies were described at a time when knowledge of geographical variation was limited owing to the difficulty of accessing remote and sparsely populated regions of the interior. More recently, increased specimen sampling has revealed continuous or subtle variation in characters presumed to diagnose species and subspecies (e.g. Hutchinson *et al.*, 2006; Rabosky *et al.*, 2014; Kealley *et al.*, 2018; Doughty *et al.*, 2018). Therefore, proper assessment of whether morphologically defined taxa correspond to separately evolving lineages will probably require, in most cases, a combined reassessment of morphological and genetic variation.

The concept and application of subspecies have been debated intensely (Hillis, 2020; Padial & De la Riva, 2020; de Queiroz, 2020; Reydon & Kunz, 2021; Burbrink *et al.*, 2022). Some authors advocate the traditional definition of subspecies as mere groups of phenotypically similar populations, and many morphologically defined subspecies are still in use (Patton & Conroy, 2017). Others expect subspecies also to correspond to phylogenetic lineages (Zink, 2004; Sackett *et al.*, 2014; Brenneman *et al.*, 2016; Trujillo & Hoffman, 2017). This requirement approximates subspecies to the concept of evolutionarily significant units in conservation biology (Braby *et al.*, 2012; Coates *et al.*, 2018), although subspecies definitions invariably presuppose the existence of phenotypic variation (i.e. there are no 'cryptic subspecies'). Other authors have argued that the requirement for phylogenetic coherence equates subspecies to species, with the two categories differing solely in the relative degree of divergence, which is, essentially, arbitrary (Patton & Conroy, 2017; Padial & De la Riva, 2020). Lastly, some authors have suggested redefining the subspecies concept to accommodate cases where genetic clines, divergence with gene flow and secondary contact lead to phenotypic intergradation among populations. In this view, subspecies are redefined as incompletely separated population lineages (Frost & Hillis, 1990; Hillis, 2020; de Queiroz, 2020, 2021). This proposal requires subspecies to have properties indicative of incipient yet incomplete evolutionary separation. We can expect these properties to include: (1) the correspondence with a phylogenetic lineage; (2) the presence of distinctive phenotypic characters; (3) geographical structure in phenotype and genotype; and (4) the presence of genetic exchange among closely related diverging populations, a crucial property to distinguish subspecies (incompletely separated lineages) from species (separately evolving lineages). In principle, these properties provide a framework for subspecies recognition, invalidation or elevation to species. However, it is unclear whether most subspecies currently in use meet these criteria.

The four regional subspecies currently recognized in *C. pantherinus* (Fig. 1B) are, purportedly, diagnosed by differences in body size, coloration and scalation (Storr *et al.*, 1999). Based on improved geographical sampling relative to when the subspecies were proposed (Storr, 1969, 1970, 1975), we characterize patterns of morphological variation in *C. pantherinus* and revisit the correspondence between character states and the presumed subspecies ranges. We then test whether populations assignable to each subspecies based on presumed ranges are genetically coherent and divergent from other subspecies. With this goal, we perform phylogenetic and genotypic clustering analyses using mitochondrial and genome-wide nuclear loci. Finally, we examine the extent to which phenotypic, genetic and geographical patterns are concordant, testing whether the proposed subspecies might correspond to independently evolving species. Based on our findings, we discuss the conceptual and operational limitations of morphologically defined subspecies and the challenges to categorization of early diverging lineages in taxonomy, including the concept of 'incomplete lineage separation' (de Queiroz, 2020) to guide the designation of subspecies.

MATERIAL AND METHODS

SUBSPECIFIC TAXONOMY OF *C. PANTHERINUS*

Populations currently ascribed to *C. pantherinus* were first described by Peters (1866) as *Lygosoma pantherinum* and by Boulenger (1896) as *Lygosoma ocellatum* (modified to *Lygosoma ocelliferum* in the same volume). Among them, *L. ocelliferum* was the first to be transferred to the newly proposed genus *Ctenotus* by Storr (1964). Soon afterwards, Storr (1969) noted that populations of *C. ocelliferum* were closely related to, but larger in size than, *C. pantherinus*, incorporating the former into the latter as the subspecies *C. p. ocellifer*. Under this scheme, *C. p. pantherinus* was restricted to populations from south-west Australia, with *C. p. ocellifer* occupying the arid zone. Storr (1970) assigned the northernmost populations of *C. pantherinus* in the Northern Territory and Western Australia to *C. p. calx* based on larger body size, smoother palmar and plantar scales and more rounded keels on the subdigital lamellae. Lastly, *C. p. acripes* was described by Storr (1975) based on specimens from Barrow Island in Western Australia. Storr *et al.* (1978a) later assigned mainland populations from the Northern Territory and Queensland to this same subspecies based on sharing spiny foot scales, despite large distances from the type locality of the subspecies. More recently, Storr *et al.* (1999) remarked that those eastern mainland populations of *C. p. acripes* might correspond to an undescribed species different from the nominal population from Barrow Island > 1500 km away.

This arrangement of four subspecies has been broadly reproduced in field guides and taxonomic compendiums for nearly 50 years, albeit in the form of verbal accounts (e.g. Cogger, 2014; Wilson & Swan, 2020) or general outlines on a map (see Fig. 1B; Ehmann & Strahan, 1992; Storr *et al.*, 1999). To provide a better outline of the geographical distribution of the four subspecies as currently recognized, we obtained georeferenced records for 1464 specimens that were assigned to a subspecies (by the original collectors or museum staff) from the Western Australian Museum, the Museum and Art Gallery of the Northern Territory and Museums Victoria, obtained through the Online Zoological Collections of Australian Museums (Wallis, 2006; available at: https://ozcam.org.au). We were unable to include *C. pantherinus* records from other collections that appear not to recognize the subspecies (e.g. Queensland Museum, South Australian Museum and Australian National Wildlife Collection).

ASSESSMENT OF MORPHOLOGICAL DISTINCTIVENESS

To test whether the characters proposed to diagnose subspecies in *C. pantherinus* show geographical structure and concordance with putative subspecies ranges, we performed morphological examinations on 145 specimens deposited in the Western Australian Museum (WAM), the University of Michigan Museum of Zoology (UMMZ) and the South Australian Museum (SAM). We scored the following characters used in the subspecies descriptions (Fig. 2): snout–vent length; contact of nasal and prefrontal scales; number of supralabial scales; number of midbody scale rows; number of subdigital lamellae (i.e. scales) on the fourth toe (counted for both toes, then averaged); degree of keeling of the subdigital lamellae (single fine keel, single broad keel or fine medial keel flanked by smaller parallel keels); condition of the palmar and plantar scales (smooth, pyramidal or with a spiny projection); presence (or absence) of dark longitudinal dorsal stripes; degree of dark pigmentation around the pale dorsal spots, the ocelli; and coloration of the hindlimbs (spotted, striped or a combination). Contact of nasals and prefrontals and the number of supralabials were nearly invariable and not commented on further.

We then mapped the geographical distribution of character states and assessed the degree of morphological distinctiveness and overlap across regions. For the second goal, we plotted a morphospace defined by two axes from non-parametric multidimensional scaling on the character data as implemented in the *vegan* package (Oksanen *et al.*, 2007) in R v.4.1.2 (R Core Team, 2021).

Our morphological examinations largely failed to recover previously suggested geographical patterns of phenotypic variation, instead finding character polymorphism within regions and individual localities, and thus questioning the morphological distinctiveness of each subspecies (see Results). As a result, we were unable to assign the museum specimens we examined unequivocally to a subspecies using morphological information alone, without considering locality data. This uncertainty limits our capacity to compare characters between the subspecies of *C. pantherinus* as defined traditionally. Thus, we performed tentative assignments of individuals to subspecies based on their sampling localities relative to subspecies ranges as currently understood (Fig. 1B). Although less direct, this approach allowed us to assess morphological distinctiveness across the geographical regions historically associated with each subspecies.

SAMPLING OF GENETIC DATA

To characterize genetic structure patterns and phylogenetic relationships, we obtained genetic data from 125 specimens sampled at 68 localities spanning nearly the entire distribution of *C. pantherinus*. As outgroups, we included representatives of other major *Ctenotus* species groups and the closely related genus

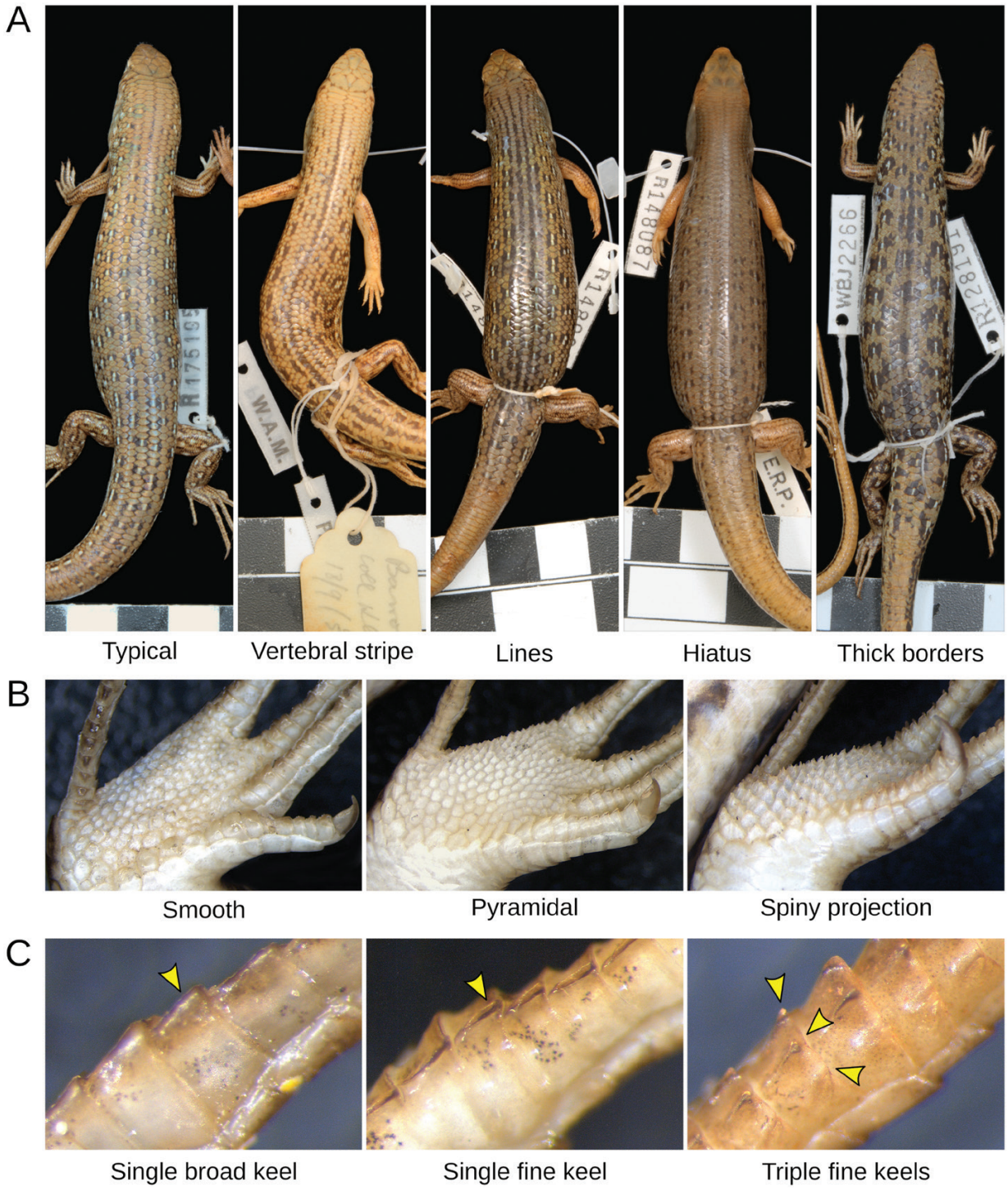

Figure 2. Selected phenotypic characters examined in museum specimens of *Ctenotus pantherinus* and their variation. A, dorsal coloration (from left to right): typical ocellated pattern, vertebral stripe, longitudinal lines, hiatus of ocelli on the vertebral region and ocelli with thick dark borders. B, condition of the plantar scales: smooth, pyramidal and with a spiny projection. C, condition of the subdigital lamellae (yellow arrows): single broad keel, single fine keel and fine medial keel flanked by two smaller parallel keels. Variation among the character states shown was often near-continuous and difficult to categorize.

Lerista Bell, 1833, namely *Ctenotus duricola* Storr, 1975, *Ctenotus essingtonii* (Gray, 1842), *Ctenotus inornatus* (Gray, 1845), *Ctenotus nasutus* Storr, 1969, *Ctenotus nigrilineatus* (Storr, 1990), *Ctenotus rubicundus* Storr, 1978b, *Ctenotus schomburgkii* (Peters, 1863), *Ctenotus taeniolatus* (White, 1790), *Ctenotus youngsoni* Storr, 1975, *Lerista bipes* (Fischer, 1882) and *Lerista ips* Storr, 1980. The Supporting Information (Table S1) presents detailed sample information for previously and newly generated DNA sequences, including museum vouchers, locality information and GenBank and Sequence Read Archive accessions.

To characterize genetic structure based on the nuclear genome, we incorporated double-digest restriction site-associated data (ddRAD) (Peterson *et al.*, 2012) generated by broad-scale evolutionary investigations of Australian sphenomorphine skinks (Singhal *et al.*, 2017, 2018; Prates *et al.*, 2021) and available in the Sequence Read Archive (BioProjects PRJNA755251 and PRJNA382545). Briefly, DNA extractions were digested with the restriction enzymes EcoRI and MspI, tagged with individual barcodes, PCR-amplified, multiplexed, and sequenced on an Illumina platform. We then used the *ipyrad* v.0.9.71 pipeline (Eaton & Overcast, 2020) to demultiplex and assign reads to individuals based on sequence barcodes (allowing no nucleotide mismatches from individual barcodes), perform *de novo* read assembly (minimum clustering similarity threshold = 0.90), align reads into loci, and call single nucleotide polymorphisms (SNPs) while enforcing a minimum Phred quality score (= 33), minimum sequence coverage (= 6×), minimum read length (= 35 bp) and maximum proportion of heterozygous sites per locus (= 0.5), and ensuring that variable sites had no more than two alleles within an individual (i.e. a diploid genome). The final dataset was composed of 85 743 SNPs, each present in ≥ 50% of the sampled individuals. Sampling of nuclear data included four putative *C. p. acripes* samples, one *C. p. calx*, 61 *C. p. ocellifer* and six *C. p. pantherinus*.

To characterize genetic structure based on the mitochondrial genome, we PCR-amplified, sequenced, edited and aligned a 1143 bp fragment of the cytochrome *b* (*Cytb*) gene, following standard protocols described by Rabosky *et al.* (2009). Sampling of mitochondrial data included eight putative *C. p. acripes* samples, five *C. p. calx*, 96 *C. p. ocellifer* and seven *C. p. pantherinus*, according to currently recognized subspecies distributions. Newly generated mitochondrial sequences were uploaded to GenBank (accession numbers ON035994–ON036035).

Inference of phylogenetic lineages

To assess whether populations from regions historically associated with each subspecies correspond to evolutionary lineages, we inferred phylogenetic relationships based on both the nuclear (ddRAD) and mitochondrial (*Cytb*) datasets (analysed separately). As in the morphological analyses, we performed tentative assignments of individuals to subspecies based on their sampling localities relative to subspecies ranges as currently understood (Fig. 1B). This approach aimed to circumvent the following issues: (1) the only partial overlap between individuals included in the morphological and genetic analyses owing to poor preservation, being a juvenile, and challenges to loan and access specimens owing to restrictions related to the coronavirus disease of 2019; and (2) our failure to assign museum vouchers to subspecies owing to intrasite polymorphism and unclear character states (see Results). We adopted an individual-based approach for phylogenetic inference under maximum likelihood, which allowed us to assess whether individuals assigned to the same subspecies were phylogenetically clustered. With this goal, we used RAxML-HPC v.8.2.12 (Stamatakis, 2014) through the CIPRES Science Gateway (Miller *et al.*, 2010), using the GTRCAT model of nucleotide evolution and estimating node support based on 1000 bootstrap replicates. All phylogenetic analyses included both SNPs and invariant sites.

Assessment of genetic admixture

To assess the degree of genetic admixture between the major lineages we inferred in *C. pantherinus*, we estimated the ancestry proportions of individuals. Ancestry proportions were estimated through genotypic clustering under sparse non-negative matrix factorization (sNMF), a method that does not rely on traditional population genetic model assumptions (Frichot *et al.*, 2014). Given that sampling imbalance can result in the spurious grouping of intensely sampled localities (Puechmaille, 2016; Lawson *et al.*, 2018), we limited the maximum number of samples per collecting site to five. We then removed SNPs with a minimum allele frequency < 0.05 to improve inference of population structure (Linck & Battey, 2019) and minimize spurious SNPs from sequencing errors (Ahrens *et al.*, 2018) using VCFtools v.0.1.16 (Danecek *et al.*, 2011). To ensure independence of SNPs, we extracted a single SNP per ddRAD locus. Lastly, we ran sNMF using the *LEA* R package (Frichot & François, 2015) to infer the best-fitting number of clusters (*K*). We compared the fit of schemes under *K* = 1–10, with 20 replicates for each value of *K*.

Our phylogenetic analyses based on the ddRAD data suggested that a sample from the Kimberley region was divergent from other main clades of *C. pantherinus* (see Results). Given the potential effects of undersampling individual genetic groups on genotypic clustering

analyses (Puechmaille, 2016; Lawson *et al.*, 2018), we reassembled the ddRAD dataset (from the demultiplexing step in *ipyrad*) without including this sample, then reran sNMF following the steps detailed above.

RESULTS

Subspecies distributions from museum specimens

The first step of this investigation was to map the distributions of the four subspecies of *C. pantherinus* based on hundreds of museum records using the assignments made by original collectors and museum staff. This exercise revealed conflicting assignments to subspecies of specimens from the same geographical regions, resulting in unclear subspecies ranges (Fig. 1C). For instance, specimens from neighbouring sites in northern and central Australia have been assigned to *C. p. ocellifer*, *C. p. calx* or *C. p. acripes*; the geographical limits between *C. p. ocellifer* and *C. p. pantherinus* in south-western Australia are unclear; and *C. p. acripes* has a disjunct distribution with populations from a Western Australian continental island (Barrow Island) separated by > 1500 km from the closest mainland population, with another subspecies (*C. p. ocellifer*) present in the intervening regions. Such blurry, overlapping or disjunct distributions call into question whether these subspecies correspond to geographically coherent units. Moreover, these patterns might call into question the utility of the characters presumed as diagnostic. Limited diagnosability might explain why some major collections (e.g. Queensland Museum, South Australian Museum and Australian National Wildlife Collection) appear not to recognize subspecies in *C. pantherinus* at all.

Morphological patterns and support for the subspecies

Our examination of morphological characters purported to diagnose subspecies confirmed that they varied across the distribution of *C. pantherinus* (Fig. 2; Supporting Information, Table S2), with specific character states often clustering in geographical space (Fig. 3). For instance, broadly keeled digits occurred mainly in specimens from the Kimberley region in northern Australia, although they were also present in at least one locality on the western Australian coast. Likewise, specimens from south-western Australia tended to show relatively lower midbody scale counts, although this variation appeared to be clinal relative to neighbouring regions to the north and east. Lastly, body size variation followed some of the previously reported patterns (Storr *et al.*, 1999); specimens from the Kimberley tended to have larger average adult body sizes, whereas those from the south-west tended to have smaller sizes, although this variation also appeared mostly clinal (Fig. 3).

In contrast, other characters showed largely idiosyncratic geographical patterns, contradicting previous suggestions of character state restriction to specific regions (Storr *et al.*, 1999). For instance, vertebral stripes were not restricted or dominant in the south-west but were also recorded in isolated localities in south-western, north-western and central Australia, where unstriped individuals predominated (Fig. 3). Likewise, palmar and plantar scales with a spiny projection were not restricted to Barrow Island, occurring in specimens from the south-west, Pilbara and central arid zone. In the north (e.g. Kimberley), individuals showed the smooth, pyramidal or spiny condition, often being polymorphic within the same collecting site (Fig. 3). Lastly, some of the character states we encountered were not reported previously. This was the case for subdigital lamellae with three keels (Fig. 2), which we found to be restricted to the Pilbara region (Fig. 3). Nevertheless, single-keeled digits were also common throughout this region.

The spatial distribution of morphological character states was consistent, in part, with the distributions of the four currently recognized subspecies (Table 1). For instance, broadly keeled digits and large body sizes were common in the general region where *C. p. calx* was thought to occur (Kimberley). Likewise, lower midbody scale counts and dorsal spots with thick dark outlines were largely concentrated around the proposed range of *C. p. pantherinus* (south-western Australia). Nevertheless, these correspondences were limited and included many exceptions. For instance, besides broadly keeled digits, we found finely keeled digits in the Kimberley (20% of specimens), inconsistent with the diagnosis of *C. p. calx*. On Barrow Island, the type locality of *C. p. acripes*, both single- and three-keeled specimens were recorded (44% and 56% of the specimens, respectively), although single keels are purportedly diagnostic for this subspecies. A vertebral stripe, supposedly diagnostic for *C. p. pantherinus*, had limited occurrence in south-western populations (31% of the specimens) and also occurred in distant sites within the range of the supposedly unstriped *C. p. ocellifer* (15% of the specimens). Although mostly restricted to the south-west, (previously unreported) spots with thick dark outlines occurred in only 47% of the specimens from this region. Spiny plantar scales occurred much more extensively than on Barrow Island or in the north-east arid zone (Fig. 3; Table 1), regions associated with *C. p. acripes*. As a result of these and other inconsistencies, we failed to assign any given individual confidently to a subspecies based on morphological characters alone; all the more so

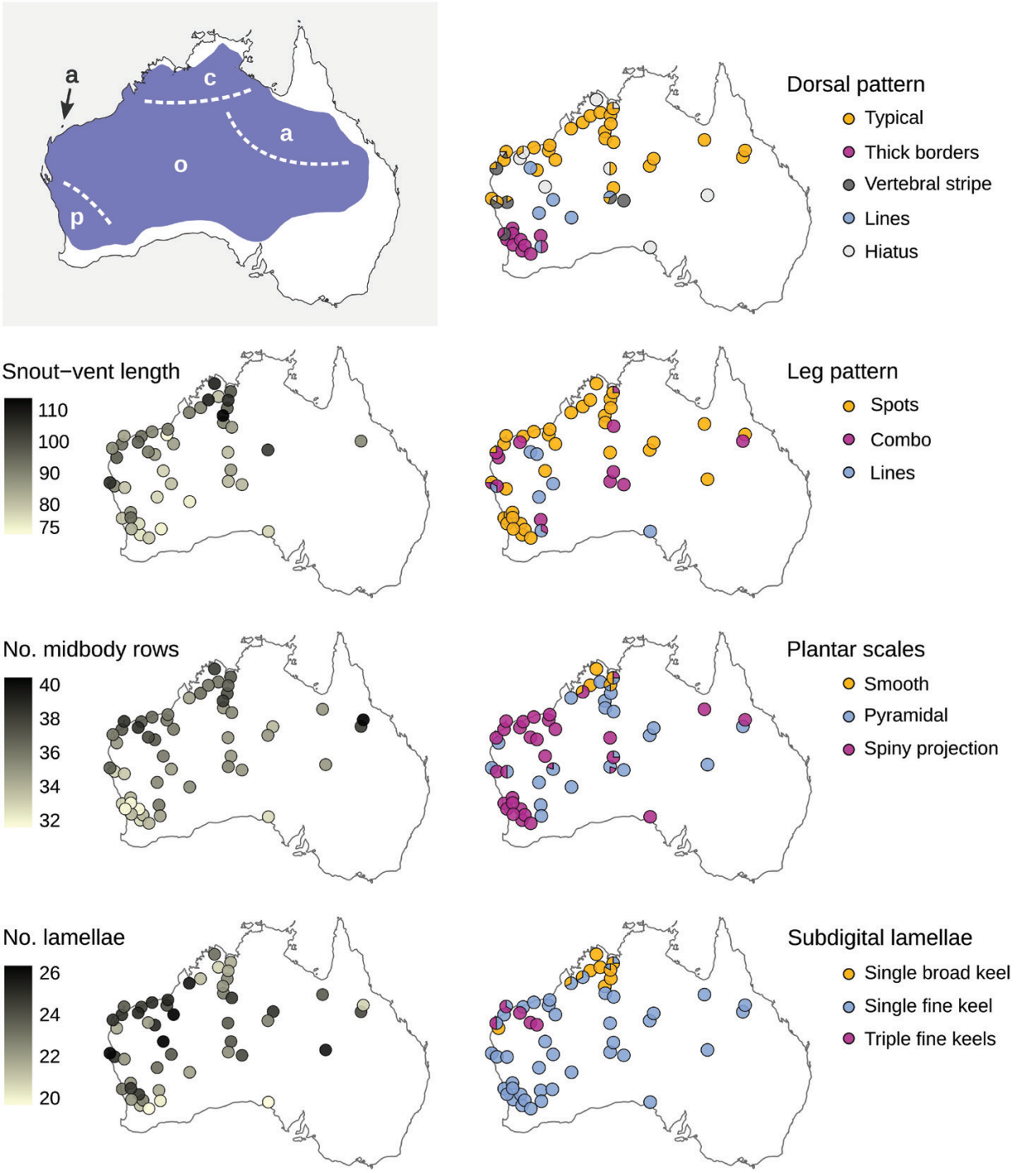

Figure 3. Geographical distribution of the characters proposed to diagnose subspecies in *Ctenotus pantherinus*. The top left panel indicates the presumed ranges of the four subspecies as in Figure 1B: *C. p. acripes* (a), *C. p. calx* (c), *C. p. ocellifer* (o) and *C. p. pantherinus* (p). For the quantitative characters (remaining left panels), colours of circles indicate average trait values in a locality. Juveniles (< 75 mm) were not included in the snout–vent length map. For the qualitative characters (right panels), pie charts indicate the relative frequency of alternative character states in a locality. Some character states tended to be more frequent in certain regions, yet many specimens deviated from these regional trends.

Table 1. Summary of phenotypic character states by tentative subspecies assignment (based on presumed subspecies ranges; see main text for details) in museum specimens of *Ctenotus pantherinus*. Values within parentheses indicate ranges.

Tentative subspecies	*N*	Snout–vent length (mm)	Number of midbody scale rows	Number of subdigital lamellae	Dorsal pattern (proportion of individuals)				
					Lines	Typical	Vertebral hiatus	Vertebral stripe	Thick border
C. p. acripes	18	86.6 (75–95)	38.3 (35–40)	23.4 (20.5–26.5)	0.00	0.76	0.12	0.12	0.00
C. p. calx	20	98.2 (83–111)	36.9 (35–40)	21.6 (18.5–24.0)	0.00	0.90	0.10	0.00	0.00
C. p. ocellifer	75	86.9 (76–100)	35.5 (32–39)	23.5 (19.5–28.5)	0.26	0.47	0.11	0.15	0.01
C. p. pantherinus	32	86.4 (75–108)	33.8 (31–38)	23.1 (18.0–27.5)	0.00	0.19	0.03	0.31	0.47

Tentative subspecies	Leg pattern (proportion of individuals)			Plantar scales (proportion of individuals)			Subdigital lamellae (proportion of individuals)		
	Combo	Lines	Spots	Pyramidal	Smooth	Spiny	Single broad	Single fine	Triple fine
C. p. acripes	0.06	0.00	0.94	0.06	0.00	0.94	0.00	0.44	0.56
C. p. calx	0.05	0.00	0.95	0.35	0.50	0.15	0.80	0.20	0.00
C. p. ocellifer	0.26	0.30	0.44	0.51	0.00	0.49	0.03	0.84	0.13
C. p. pantherinus	0.25	0.09	0.66	0.22	0.00	0.78	0.00	1.00	0.00

when the specimens were examined without prior knowledge of their geographical locality.

In agreement with these findings, a morphospace defined by two axes from non-parametric multidimensional scaling suggested that populations assignable to each subspecies based on purported distributions showed broad morphological overlap (Fig. 4A), with samples from different regions frequently grouping together. Localized clusters of points corresponding to a single subspecies primarily consisted of samples collected at the same site (e.g. *C. p. calx* samples in the lower left portion and *C. p. ocellifer* in the lower right portion of Fig. 4A). Conversely, specimens corresponding to a given subspecies often did not cluster in morphological space.

Phylogenetic patterns and support for the subspecies

A phylogenetic analysis based on genome-wide ddRAD markers inferred five well-supported (bootstrap support > 70) major nuclear clades within *C. pantherinus* (Fig. 5A). These major nuclear clades were geographically coherent and non-overlapping. Specifically, nuclear clade 1 was represented by a single sample from the Kimberley in northern Australia; nuclear clade 2 occurred along the edge of the arid zone in eastern Australia; nuclear clade 3 was largely restricted to the south-west; nuclear clade 4 occurred largely in the Pilbara region and adjacent sites in the north-west; and nuclear clade 5 was distributed throughout the central arid zone (Fig. 5C).

A phylogenetic analysis based on *Cytb* inferred three well-supported major mitochondrial clades within *C. pantherinus* (Fig. 5B). Similar to the nuclear results, these clades were geographically coherent, each restricted to a non-overlapping region of Australia. Specifically, mitochondrial clade 1 was restricted to two neighbouring sampling localities in south-western Australia; mitochondrial clade 2 occurred along the western coast and into the Pilbara; and mitochondrial clade 3 spanned the core of the central arid zone while extending into the tropical grasslands of northern and eastern Australia (Fig. 5D). Significantly, samples from the type locality of *C. p. acripes* (Barrow Island; asterisks in Fig. 5B) clustered among samples from the adjoining Pilbara region.

Patterns of phylogenetic structure were often congruent across nuclear and mitochondrial analyses. For instance, both analyses clustered samples from most of the arid zone, suggesting limited genetic structure over large areas. Nevertheless, nuclear and mitochondrial analyses also yielded conflicting patterns. In particular, individuals clustering in the same major mitochondrial clade were often inferred as nested within multiple nuclear clades and vice versa.

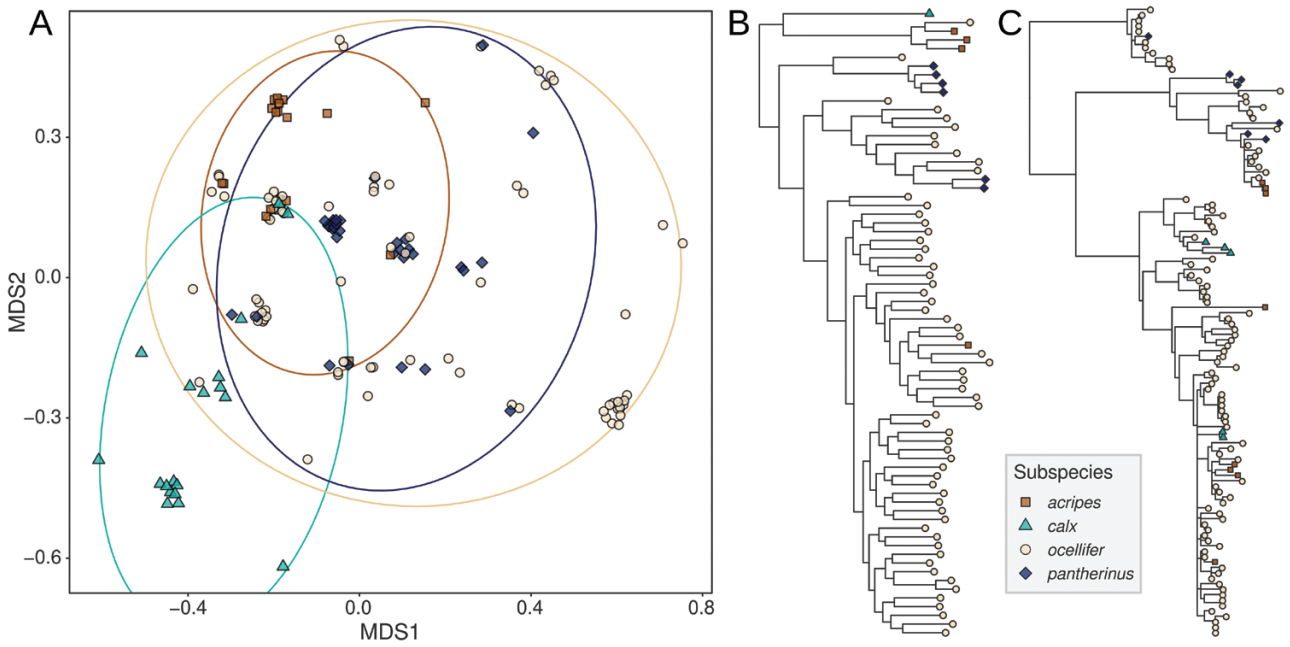

Figure 4. Evidence of weak and inconsistent phenotypic and phylogenetic coherence and distinctiveness of *Ctenotus pantherinus* subspecies. A, morphospace defined by two axes from non-parametric multidimensional scaling on seven characters scored from museum specimens (N = 145). Many specimens putatively corresponding to the same subspecies (based on geographical ranges) did not group in morphological space, whereas samples assignable to different subspecies often grouped together. B, C, both the nuclear (B) and mitochondrial (C) phylogenetic analyses suggest paraphyly of subspecies. For detailed phylogenetic trees and corresponding clade distributions, see Figure 5.

For instance, samples from mitochondrial clade 2 from Australia's south-western interior (Fig. 5B) grouped in the nuclear tree with a subset of south-western coastal samples from mitochondrial clade 3, forming nuclear clade 3 (Fig. 5A). Other samples from mitochondrial clade 3 from the Pilbara formed nuclear clade 4. These mitonuclear discordances frequently involved individuals from geographically adjacent regions.

Similar to the morphological patterns, the geographical distribution of major phylogenetic groups was congruent with expectations from subspecies ranges only in part. For instance, samples from south-western Australia, supposedly occupied by *C. p. pantherinus*, grouped together in nuclear clade 3 (Figs 4B, 5A). However, this clade also included a sample from the arid zone (the easternmost sample in nuclear clade 3; Fig. 5B), generally associated with *C. p. ocellifer*. Additionally, samples assigned geographically to *C. p. pantherinus* were recovered in two non-sister mitochondrial clades (1 and 2), clustering with samples assignable to *C. p. ocellifer* and *C. p. acripes* (Figs 4C, 5B). Likewise, our only nuclear sample from the range of *C. p. calx* in the Kimberley (north-western Australia) was found to be highly divergent from the other clades (Figs 4B, 5A). However, in the mitochondrial tree (Figs 4C, 5B), the more numerous samples assignable to *C. p. calx* branched in two places, intermixed with more southern specimens assignable to *C. p. ocellifer* and *C. p. acripes*. Samples from the presumed range of *C. p. acripes* in the north-east of Australia grouped together in the nuclear tree, yet the clade formed by them also included a sample from the range of *C. p. ocellifer* to the south (Figs 4B, 5A). Moreover, *C. p. acripes* was recovered in four different mitochondrial clades, nested among *C. p. ocellifer* samples (Figs 4C, 5B). Lastly, samples from the range of *C. p. ocellifer* in the arid zone were inferred in four major nuclear clades (Figs 4B, 5A) and all three major mitochondrial clades (Figs 4C, 5B). This pattern of paraphyly of *C. p. acripes*, *C. p. ocellifer* and *C. p. pantherinus* in the nuclear tree and of all four subspecies in the mitochondrial tree provides only weak and inconsistent phylogenetic support for the subspecies.

Evidence of genetic admixture

Consistent with the phylogenetic analyses, estimates of ancestry coefficients supported genetic structure across the range of *C. pantherinus*. Genotypic clustering analyses inferred four major clusters, whose sample composition and geographical distributions closely matched those of the major nuclear clades (Fig. 6). One notable difference was that the only

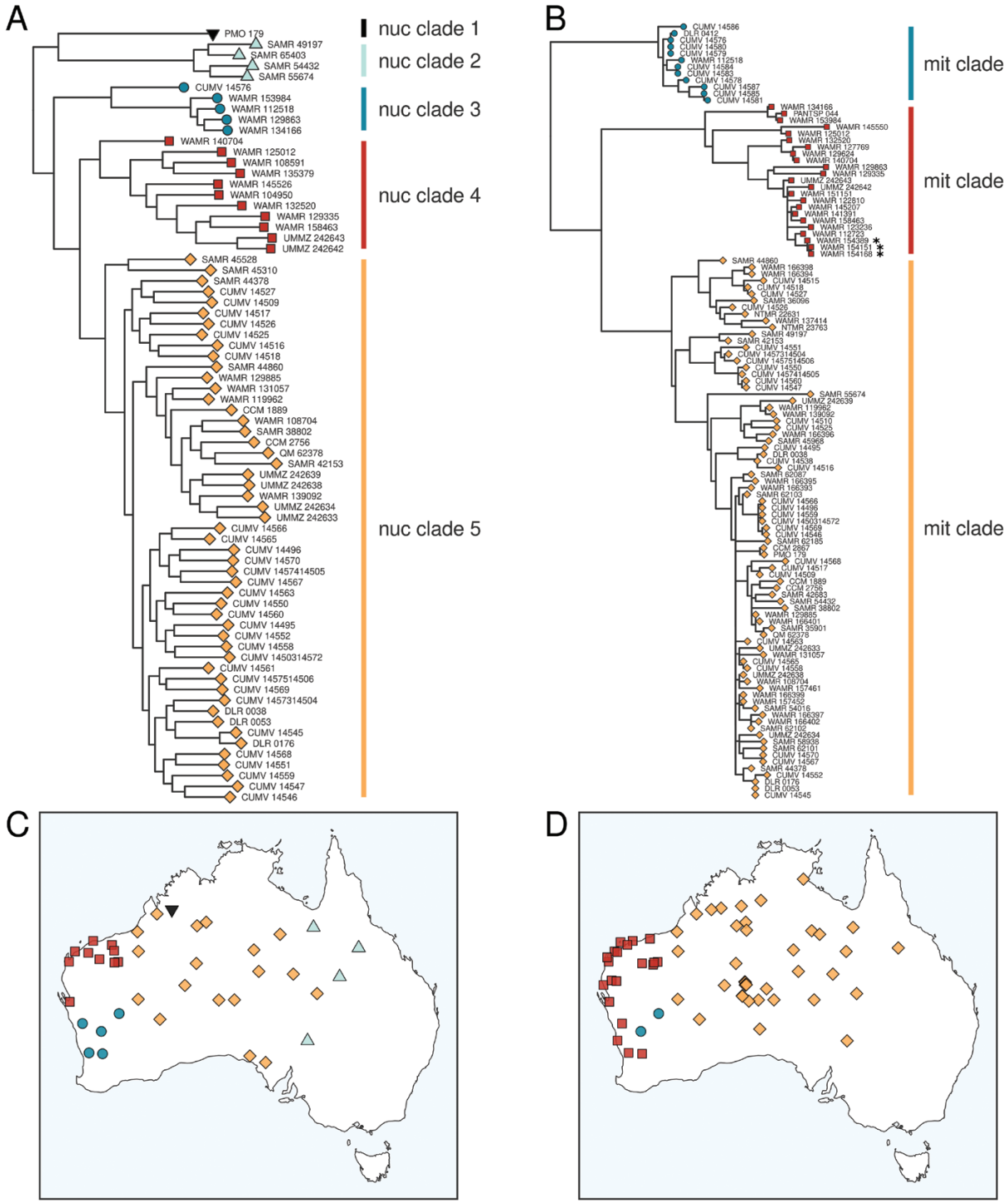

Figure 5. Phylogenetic relationships in *Ctenotus pantherinus* and geographical distribution of inferred clades. A, phylogenetic tree based on a dataset including 85 743 nuclear single nucleotide polymorphisms from a double-digest restriction site-associated data (ddRAD) approach. B, tree based on the cytochrome *b* mitochondrial marker. Asterisks indicate samples from the type locality of *C. p. acripes*, a taxon that we deem invalid (see main text). C, geographical distributions of major nuclear clades. D, distributions of major mitochondrial clades. Nuclear and mitochondrial trees show multiple points of discordance and limited correspondence to putative population assignments to subspecies.

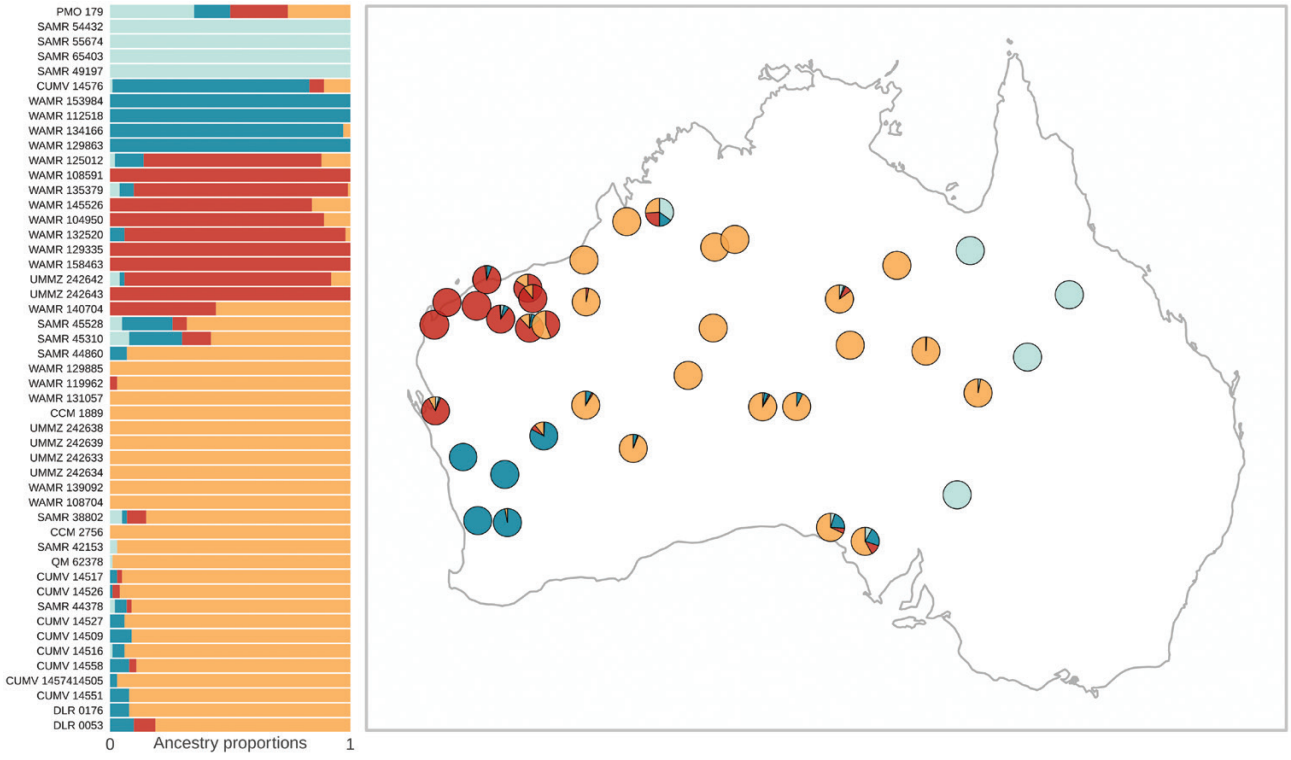

Figure 6. Genotypic clustering and admixture in *Ctenotus pantherinus*. Bars depict the relative proportion of alleles in each individual corresponding to the inferred genotypic clusters (i.e. ancestry proportions of individuals). Pie charts on the map indicate the average ancestry proportions corresponding to each cluster at each site (based on all individuals at that site). The clusters closely match the sample composition and geographical distribution of major clades from the nuclear phylogenetic analysis (Fig. 5). Multicoloured pies indicate admixture among clusters.

sample from the Kimberley region, corresponding to nuclear clade 1, was not found in a separate cluster; instead, it showed nearly equal ancestry proportions from all four clusters. This result might challenge the distinctiveness of *C. p. calx* in nuclear DNA. However, it seems more likely that this pattern indicates failure to detect a truly distinctive cluster owing to a small sample size (Puechmaille, 2016; Lawson *et al.*, 2018). These analyses inferred admixture among genotypic clusters, particularly in western and southern Australia, suggesting genetic exchange among incipient lineages. A genotypic clustering analysis based on a ddRAD dataset assembled without the divergent Kimberley sample yielded the same results (Supporting Information, Fig. S1).

DISCUSSION

Based on comprehensive geographical sampling of morphological, mitochondrial and genome-wide nuclear variation, we investigated whether currently recognized subspecies corresponded to distinctive and coherent evolutionary lineages in *C. pantherinus*. We found weak and inconsistent correspondence between morphological patterns and presumed subspecies ranges, with character polymorphism within regions and broad morphological overlap across regions. Likewise, genetic data suggested paraphyly of subspecies, mitonuclear discordance and admixture across regions.

Below, we discuss the implication of these results for the evolutionary history and taxonomy of *C. pantherinus*. We then relate our findings to the use of the subspecies category more generally and discuss the conceptual and operational challenges to the categorization of early diverging lineages in taxonomy.

SUPPORT FOR *C. PANTHERINUS* SUBSPECIES

Our morphological examinations confirmed that coloration and scalation characters broadly used in *Ctenotus* taxonomy varies across the range of *C. pantherinus*. Some character states were more common in certain regions, including broadly keeled digits, lower midbody scale counts, larger average adult sizes and dorsal spots with thick outlines. This variation was consistent, in part, with the

presumed distributions of certain subspecies, namely *C. p. calx* and *C. p. pantherinus*. Nevertheless, many individuals in a given region deviated from the most frequent phenotype and corresponding phylogenetic lineage, whereas character combinations presumed as diagnostic also occurred outside the recognized subspecies ranges. Such regional variation appears to have been overlooked or unreported in the subspecies descriptions, potentially owing to limited sampling. In the case of the continuous characters, spatial variation often appeared clinal. Moreover, despite broad geographical trends when we examined certain characters in isolation, the morphological distinctiveness of regional populations faded when multiple characters were considered jointly. Concentrating on single characters and overlooking their intrasite and intraregion variation appears to have overestimated the phenotypic coherence and distinctiveness of the subspecies of *C. pantherinus*.

Other patterns of morphological variation did not conform to subspecies definitions. We found some character states to be infrequent in regions where these states were once thought to predominate, whereas others occurred more extensively than previously reported. Most of the character variation we encountered was continuous (e.g. degree of spininess of the plantar scales and coloration traits; Fig. 2), making it difficult to score these characters objectively. This difficulty might explain the pattern of conflicting voucher assignments among collectors and museum staff (Fig. 1) and our partial failure to recover previously reported spatial patterns (Fig. 3; Table 1). Remarkably, some of the subspecies did not appear morphologically distinct at all (*C. p. acripes* and *C. p. ocellifer*). These findings might stem from broader specimen collections now available relative to when the subspecies were described. For instance, < 100 specimens of *C. pantherinus* were housed at the Western Australian Museum at the time *C. p. calx* was described based on nine of them. Likewise, ~200 specimens of *C. pantherinus* were available in the same museum when *C. p. acripes* was described, with that description incorporating 28 specimens from a single site (Barrow Island). Today, this museum houses > 1300 *C. pantherinus* specimens. This increased sampling has revealed variation that contradicts the presumed coherence and distinctiveness of subspecies.

Similar to the morphological patterns, the correspondence of genetic clades to currently recognized subspecies was weak and inconsistent. Samples from the presumed range of a subspecies were partitioned into multiple clades, particularly in the nuclear analysis. Conversely, clades that corresponded roughly to a subspecies included samples from another presumed distribution of a subspecies. Notably, the mitochondrial analysis grouped two to three subspecies together and inferred all subspecies as paraphyletic. This finding appears unexpected, because shorter coalescent times in mitochondrial markers (relative to nuclear DNA) make them well suited to the identification of shallow divergences, such as those seen within species (Palumbi *et al.*, 2001). Instead, the results are consistent with mitochondrial haplotype sharing among populations of the same species. Nuclear estimates of ancestry coefficients and widespread mitonuclear discordance provide further support for a pattern of broad genetic admixture and introgression, as reported in other species (e.g. Toews & Brelsford, 2012; Pereira *et al.*, 2016). Therefore, although the genetic data support the presence of multiple incipient lineages within *C. pantherinus*, these lineages do not appear to be evolving independently, providing no support for their designation as species-level taxa.

Challenges to the taxonomic categorization of early diverging lineages

Genetic admixture and introgression between major genetic groups in *C. pantherinus* arguably provide evidence of incomplete evolutionary separation, consistent with certain conceptualizations of subspecies (Frost & Hillis, 1990; Hillis, 2020; de Queiroz, 2020, 2021). Genetic exchange across incipient lineages might explain why many sampled localities were polymorphic and a pattern of widespread character state sharing across regions. In the presence of gene flow, populations can develop misaligned phenotypic and neutral genetic transitions in space (Lipshutz *et al.*, 2019). Therefore, individuals with conflicting phenotypic and genetic patterns, as seen in *C. pantherinus*, might be typical of the early stages of lineage divergence (Zamudio *et al.*, 2016).

Facing such instances of regional variation, some authors have proposed the recognition of a subspecies when an arbitrary proportion (e.g. 75%) of the individuals in a region exhibit a given trait (Amadon, 1949; Patten & Unitt, 2002). This proposal illustrates that, if we decide to categorize incipient lineages in taxonomy, they can be described at best in terms of the most frequent phenotypes and clade memberships. Under such a scheme, an unknown, variable and potentially large proportion of individuals will be misclassified or classified ambiguously. We might expect the spatial limits of subspecies ranges to be unclear, not only because of phenotypic or genetic clines but, perhaps primarily, owing to fallible morphological and genetic diagnoses. As suggested by empirical analyses of mammals (e.g. Patton & Conroy, 2017), birds (e.g. Patten & Unitt, 2002), butterflies (reviewed by Braby *et al.*, 2012) and our analyses of *C. pantherinus*, the evolutionary processes that

characterize early lineage divergence might preclude the unequivocal assignment of many individuals to taxa. It seems likely that researchers will continue to disagree on whether this limitation undermines or justifies the utility of subspecies in taxonomic practice (Patton & Conroy, 2017).

A recent debate on subspecies has revolved around redefining this category to indicate incompletely separated population lineages (Hillis, 2020; de Queiroz, 2020, 2021). The case of *C. pantherinus* suggests that, despite the conceptual appeal of this redefined subspecies concept, it might be unclear how to use it to guide taxonomic practice. It can be challenging to determine whether a pair of population lineages is separated completely or incompletely. For instance, closely related populations frequently show varying degrees of genetic divergence and admixture (Singhal & Moritz, 2013; Dufresnes *et al.*, 2015). This pattern raises the question of what degree of genetic divergence or reduction in gene flow might warrant the recognition of species or subspecies (Padial & De la Riva, 2020). To circumvent this issue, we might consider as conspecific those population lineages showing any level of incomplete separation. We might then propose subspecies to indicate identifiable genetic and morphological subgroups. However, many divergent lineages experience rampant genetic introgression; arguably, the most direct indication of incomplete lineage separation. Often, these lineages belong to distant (e.g. genus-level) clades and differ starkly in morphology, ecology and behaviour (e.g. hybridizing ducks or canids) (Johnsgard, 1960; Monzón *et al.*, 2014). Despite their apparent incomplete separation, such lineages would hardly be considered conspecific. These examples illustrate some of the challenges in translating conceptual definitions of taxonomic categories into empirical taxon delimitation. Such challenges apply to both subspecies and species (de Queiroz, 2007).

Traditional subspecies are difficult to test and falsify

This study also highlights another peculiarity of subspecies: a historical asymmetry, whereby subspecies proposed in the past are hard to test and falsify (Burbrink *et al.*, 2022), contrasting with an apparent hesitation from the taxonomists of today to propose new subspecies. In reptiles, for instance, subspecies descriptions peaked around the 1960s but declined sharply thereafter despite, or maybe because of, a rapid increase in specimen collection towards the end of the 20th century (Uetz & Stylianou, 2018). Subspecies proposed decades ago can be difficult to falsify and discard owing to typically vague morphological definitions and deference to the opinions of previous workers about population distinctiveness. Given that subspecies are nested in a developmentally and ecologically constrained species, the phenotypic differences invoked to define subspecies are necessarily subtle. Furthermore, as in *C. pantherinus*, subspecies were often described focusing on one or a few characters from relatively little material, thereby sampling gaps might have exacerbated the perception of distinctiveness (Braby *et al.*, 2012). Given that trait variation can be seen even when incompletely characterized, and owing to broad acceptance of partly speculative geographical ranges (e.g. Fig. 1), even vaguely defined subspecies continue to be recognized in taxonomic treatments and field guides. In contrast, present-day taxonomists appear unlikely to propose subspecies based on evidentiary standards typical in the 1960s–1980s. As a case in point, a recent study on Australian frill-necked lizards (*Chlamydosaurus kingii* Gray, 1825) described clinal variation in frill colour over the distribution of this species (Pepper *et al.*, 2017). However, no subspecific taxa have been proposed to accommodate this variation, despite the presence of concomitant (albeit shallow) genetic differentiation. In contrast, recent evidence of limited or inconsistent distinctiveness in phenotype and genotype does not appear to bear on the rejection of many historically proposed subspecies (Zink, 2004).

Contrasting with the view that subspecies must correspond to lineages, some researchers advocate for using subspecies to denote groups of phenotypically similar populations regardless of evolutionary relationships (Patton & Conroy, 2017). This perspective is at odds with principles of scientific thought that trace back to Darwin, whereby taxa at all levels of biological classification should reflect phylogenetic relationships (Darwin, 1859; de Queiroz & Gauthier, 1992). This criterion also applies to the species category, because broadly applied concepts define species as phylogenetic lineages or predict that they will become lineages through sustained reproductive isolation (Hennig, 1966; Dobzhansky, 1971; Cracraft, 1987; de Queiroz, 1998; Harrison & Larson, 2014). In this regard, it is worth noting that the nature of species as lineages is unaffected by the inference of paraphyly in gene genealogies (e.g. from incomplete lineage sorting or introgression; Padial & De la Riva, 2020). In contrast, a strictly morphological subspecies concept disregards the otherwise universal criterion of phylogeny, and thus potential incongruences between phylogeny and phenotype (Burbrink *et al.*, 2000). Under this concept, 'subspecies' might evoke similarly named categories, such as subgenus or subfamily, but is the only one not required to denote a clade. As such, 'subspecies' can be a misleading term because it is considered a taxonomic

category but lacks the defining property of all other taxonomic categories.

Additionally, by overlooking the evolutionary coherence of populations, morphological subspecies definitions are unfalsifiable. As illustrated by *C. pantherinus*, such definitions require only that individuals from distinct locations tend to differ in a given trait, even if trait variation within and across locations hampers subspecies diagnosability (Patten & Unitt, 2002; Braby *et al.*, 2012). Thus, limited internal coherence does not appear to challenge morphologically defined subspecies, contrasting with taxonomic groupings based on evolutionary relationships. Nevertheless, some authors have argued that even morphologically diagnosable subspecies are not biologically meaningful unless their defining characters reflect evolutionary separation (Mayr, 1963; Reydon & Kunz, 2021). Otherwise, the characters used to identify subspecies are essentially arbitrary, and infinite partitions could be proposed within any species (Mayr, 1963; Wilson & Brown, 1953). Given the long-lasting contentions on how to designate subspecies, it might be clearer to simply annotate phenotypic variation patterns across species ranges as relevant (e.g. Owen, 1963a, b). This approach does not artificially impose discrete taxonomic structures on clinal or other continuous patterns of variation (Wilson & Brown, 1953; Mayr, 1963; Owen, 1963b).

Finally, some authors advocate using geographical range as a 'diagnostic character' of subspecies (Patton & Conroy, 2017). Although this often allows fieldworkers to assign taxonomic labels to specimens more easily, this solution is inherently circular and leads, in a similar manner, to taxonomic entities that cannot be falsified. Moreover, as illustrated by *C. pantherinus*, reliance on geographical location for subspecies assignment can result in groupings of individuals that lack morphological and genetic coherence. Other studies have found that subspecies defined primarily based on geographical ranges are not phylogenetically divergent from those in neighbouring regions, as is the case of insular populations of primates in eastern Africa (Penna *et al.*, 2022).

Implications for the infraspecific taxonomy of *C. pantherinus*

Patterns of genetic and morphological variation appear to contradict the presence of independently evolving lineages and thus unrecognized species diversity within *C. pantherinus*. Additionally, our results provide weak to no support for the currently recognized subspecies and their presumed distributions. The data at hand challenge the distinction of *C. p. acripes* (Barrow Island and north-eastern Australia) from *C. p. ocellifer* (arid zone) owing to extensive paraphyly and morphological overlap. Nuclear (but not mitochondrial) markers and a couple of morphological characters appear consistent only in part with *C. p. pantherinus* (south-west) and *C. p. calx* (north). However, intrasite polymorphism is prevalent, and morphological characters proposed as diagnostic broadly occur outside the purported range of a subspecies. In the face of these patterns, we struggled to assign individuals to subspecies based on morphological characters alone. Moreover, we failed to match the genetic lineages and spatial character transitions we have identified with subspecies ranges as currently understood.

Arguably, the four traditional subspecies capture certain aspects of phenotypic or genetic variation across the range of *C. pantherinus*. Nonetheless, it seems unlikely that a modern taxonomist with access to the data presented here would converge on the proposed subspecies definitions. Even if particular coloration and scalation traits show some degree of geographical structure, no partition of characters is diagnostic of regional populations, owing to local polymorphisms and shared character states across regions. Moreover, genetic data do not support a partition of monophyletic units corresponding to phenotypes. Taken together, these results suggest that it is appropriate to synonymize *Lygosoma ocelliferum* (= *Ctenotus pantherinus ocellifer*), *Ctenotus pantherinus calx* and *Ctenotus pantherinus acripes* with *Ctenotus pantherinus*, and we do so formally here. Should future evidence support the recognition of new or redefined taxa (including species), the northern and south-western names are available. However, it seems unlikely that the name *C. p. acripes* might be found to correspond to a distinctive unit. At this time, recognizing *C. pantherinus* as a wide-ranging species devoid of subspecific taxa appears best to convey the evolutionary history of the species and often spatially idiosyncratic patterns of phenotypic variation. We believe this fundamentally conservative arrangement is preferable to recognizing subspecies whose unclear boundaries, distributions and morphological diagnoses have baffled many biologists working in the field and in natural history collections (Fig. 1).

Patterns of partly decoupled morphological and genetic transitions across the range of widespread species, as seen in *C. pantherinus*, provide opportunities to investigate the factors behind lineage and trait divergence (Lipshutz *et al.*, 2019). To identify the incipient lineages involved properly, present-day researchers will need to reassess many taxa that were proposed using small datasets and under fundamentally different views about the nature of species and other taxonomic categories (Zink, 2004; Padial & De la Riva, 2020; de Queiroz, 2020).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

Double-digest restriction site-associated data are available in the Sequence Read Archive (BioProjects PRJNA755251 and PRJNA382545). Newly generated mitochondrial data are available in GenBank (ON035994–ON036035). R and UNIX shell scripts used to prepare the data and perform all analyses are available online through GitHub (https://github.com/ivanprates/pantherinus_ZJLS).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Specimen information. Tentative subspecies assignments based on presumed geographical distributions.
Table S2. Morphological character data. Tentative subspecies assignments based on presumed geographical distributions.
Figure S1. Genotypic clustering and admixture in *Ctenotus pantherinus* based on a double-digest restriction site-associated data (ddRAD) dataset assembled without a divergent Kimberley sample (see main text). Bars depict the relative proportion of alleles in each individual corresponding to the inferred genotypic clusters (i.e. ancestry proportions of individuals). Pie charts on the map indicate the average ancestry proportions corresponding to each cluster at each site (based on all individuals at that site). Multicoloured pies indicate admixture among clusters. This analysis yielded the same results as the one including all sampled individuals (Fig. 6), including the best-fitting number of clusters (four), their sample composition and admixture patterns.