A new lizard species (Scincidae: *Ctenotus*) highlights persistent knowledge gaps on the biodiversity of Australia's central deserts

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Abstract

Australia harbors the most diverse lizard assemblages on Earth, yet the biodiversity of its vast arid zone remains incompletely characterized. Recent sampling of remote regions has revealed new species with unique phenotypes and unclear evolutionary affinities. Here, we describe a new species of scincid lizard that appears to be widely distributed across the Great Victoria Desert and adjacent regions. The new species was previously overlooked among specimens of the wide-ranging desert taxon *Ctenotus schomburgkii* but is distinguished from it by coloration and scalation characters. Phylogenetic analyses based on mitochondrial and genome-wide nuclear loci confirmed that the new species is highly divergent from *C. schomburgkii*, with which it appears to be sympatric across much of its range. In addition to the new species, our survey of genetic variation within *C. schomburgkii* as currently recognized revealed three additional lineages that approach one another in southern and northwestern Australia, and which may also represent distinct species. These results suggest that our knowledge of the extraordinary biodiversity of arid Australia remains incomplete, with implications for the conservation and management of this unique fauna. The targeted collection of voucher specimens in undersampled regions, coupled with population genetic screening of lineage diversity, will be crucial for characterizing species boundaries and understanding the composition of Australia's vertebrate communities.

Keywords: Cryptic species, population genetics, phylogeography, arid zone, *Ctenotus schomburgkii*.

Introduction

Australia's arid lands harbor the most diverse lizard assemblages on Earth (Pianka 1969, 1972; Roll et al. 2017; Tejero-Cicuéndez et al. 2022). Nevertheless, new species continue to be described every year. In some cases, the recent increase in taxonomic diversity has resulted from phylogeographic and population genetic analyses that revealed multiple evolutionary lineages within formerly wide-ranging arid zone taxa (Oliver et al. 2009; Doughty et al. 2012, 2018; Mecke et al. 2013; Rabosky et al. 2017; Kealley et al. 2018; Eastwood et al. 2020). However, species with distinctive phenotypes and unclear phylogenetic affinities continue to be encountered through field surveys in remote desert regions that remain undersampled. A remarkable example is the large elapid snake *Oxyuranus temporalis*, the first specimen of which was only collected in 2006 (Doughty et al. 2007). Recently described desert species highlight our inadequate knowledge of the composition of Australian squamate assemblages, which in turn limits our understanding of biogeographic patterns and the evolution of regional biotas (Pepper and Keogh 2021; Prates et al. 2021).

Australia's tremendous squamate diversity is disproportionately concentrated in just a few major clades, with nearly half of the species corresponding to scincid lizards (Cogger 2014). Among them is the genus *Ctenotus*, with 107 recognized species-level taxa (Uetz et al. 2021). *Ctenotus* is a conspicuous and often dominant component of Australian arid zone assemblages, with areas known to harbor up to 14 species in fine-scale sympatry (Pianka 1969, 1972, 1986). As such, they have become central to studies of ecological community structure and assembly (Pianka 1972, 1986; James and Shine 2000; Rabosky et al. 2007, 2011). While *Ctenotus* has been relatively well-studied from an ecological perspective, species diversity in these lizards remains incompletely characterized. *Ctenotus* was first recognized as a distinct genus by Storr (1964), and knowledge of its diversity was limited before a series of revisions based on external morphological attributes such as color pattern, scalation, and limb proportions (Storr 1969, 1970, 1971, 1973, 1975, 1981). Storr's work is a successful example of the traditional taxonomic approach in describing a newly sampled biota, and most of his species remain valid. Nevertheless, recent studies incorporating molecular data have found that certain nominal taxa contain multiple divergent genetic lineages, with targeted morphological examinations supporting the recognition of new species (Rabosky et al. 2017). Similarly, comprehensive comparative analyses of *Ctenotus* have identified potentially unrecognized species diversity based on marked genetic differentiation (Singhal et al. 2017; Prates et al. 2021). Much of this undescribed diversity occurs in Australia's arid zone, highlighting persistent knowledge gaps about species boundaries and distributions in the world's most diverse lizard fauna.

In this contribution, we dissect the potential for unrecognized diversity within what is currently regarded as a single species, *Ctenotus schomburgkii* (Peters 1863). Previous studies of this wide-ranging taxon reported extensive variation in coloration (Storr et al. 1999) and spatial genetic structure (Singhal et al. 2018; Prates et al. 2021). To assess whether this variation reflects unrecognized species diversity, we combine morphological, mitochondrial, and genome-wide nuclear data. We identify a highly divergent and morphologically diagnosable phylogenetic lineage whose distribution appears centered on the Great Victoria Desert. This form was previously assigned to *C. schomburgkii* due to a superficial

morphological similarity between both, combined with a poor understanding of phenotypic variation within *C. schomburgkii*. We also infer the phylogenetic relationships of the new species relative to other taxa thought to be closely allied to *C. schomburgkii* based on morphological and molecular evidence (Storr et al. 1999; Singhal et al. 2017; Prates et al. 2021). In doing so, we provide a test of whether Storr's phenetic species groups, erected as aides to species identification in *Ctenotus* lizards, also reflect evolutionary relationships.

Material and Methods

Sampling of genetic data

We inferred phylogenetic relationships among samples of *C. schomburgkii* and closely related taxa based on both mitochondrial and genome-wide nuclear loci. We incorporated sequences from the following taxa thought to be closely related to *C. schomburgkii* based on morphological attributes: *C. schomburgkii* (116 specimens), *C. allotropis* (two), *C. brooksi* (12), *C. euclae* (seven), *C. mesotes* (two), *C. strauchii* (22), *C. taeniatus* (seven), and *C. tantillus* (one). These taxa correspond to the morphologically defined *C. schomburgkii* species group (Storr et al. 1999). We also included taxa that might be related to this group based on molecular analyses (Singhal et al. 2017, 2018; Prates et al. 2021), namely *C. rosarium* (one specimen), *C. calurus* (nine), and *C. zebrilla* (five). For all these taxa, we examined the voucher specimens corresponding to our genetic samples to control for identification errors, except for three unavailable vouchers of *C. allotropis* and *C. rosarium*.

Our molecular sampling also included representatives of other major molecular *Ctenotus* clades (Prates et al. 2021), as follows: *C. atlas* (two), *C. australis* (two), *C. catenifer* (one), *C. ehmanni* (one), *C. essingtonii* (two), *C. halysis* (three), *C. inornatus* (two), *C. labillardieri* (two), *C. leae* (18), *C. leonhardii* (two), *C. nasutus* (two), *C. nigrilineatus* (two), *C. pantherinus* (two), *C. rufescens* (one), *C. striaticeps* (one), *C. taeniolatus* (two), and *C. youngsonii* (one). To root the resulting phylogenies, we used representatives from the genus *Lerista*, namely *L. bipes* (two) and *L. ips* (two). In total, 234 specimens were sampled for genetic data. Table S1 presents detailed sample information for previously and newly generated sequences, including museum vouchers, locality information, and GenBank and Sequence Read Archive accessions.

To infer phylogenetic relationships based on the mitochondrial genome, we PCR-amplified, sequenced, edited, and aligned an 1143 base pair-long fragment of the *cytochrome B* gene following standard protocols for Australian scincid lizards (Rabosky et al. 2009).

To infer phylogenetic relationships 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). To generate this ddRAD dataset, genomic DNA was digested with the restriction enzymes EcoRI and MspI, tagged with individual barcodes, PCR-amplified, multiplexed, and sequenced on an Illumina platform. We used the *ipyrad* v. 0.9.71 pipeline (Eaton and Overcast 2020) to de-multiplex 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 (= 6x), minimum read length (= 35 bp), and maximum proportion of heterozygous sites per locus (= 0.5), while ensuring that variable sites had no more than two alleles within an individual (i.e., a diploid genome). The final dataset was composed of 3,321 loci, each present in at least 30% of the sampled individuals.

Phylogenetic analyses

We inferred evolutionary relationships based on both the mitochondrial and nuclear (ddRAD) datasets. We first inferred phylogenetic lineages using an individual-based approach under Maximum Likelihood for the mitochondrial and nuclear datasets separately. To this goal, we implemented 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 1,000 bootstrap replicates. We also compared the nuclear RaxML results to a species-tree inferred under a multispecies coalescent framework using SVD Quartets (Chifman and Kubatko 2014) as implemented in the command line version of PAUP v. 4 (Swofford 2002). In this analysis, we sampled all possible quartets and estimated node support based on 1,000 bootstrap replicates.

Morphological analyses

Our molecular phylogenetic analyses revealed that certain specimens from Australia's arid zone previously assigned to *C. schomburgkii* comprise a different and highly divergent lineage (see Results). To assess whether this lineage is morphologically distinct from the nominal *C. schomburgkii*, we performed an initial assessment of morphological variation in *C. schomburgkii* and similar taxa based on museum vouchers with corresponding sequences in our genetic datasets (n = 172; listed in Table S1). To inform our comparisons, we relied on the range of scalation and meristic variation reported for *C. schomburgkii* (Storr 1969, 1970, 1971, 1973, 1975; Storr et al. 1999). Morphological characters were scored following previous studies of *Ctenotus* (Storr 1971; Couper et al. 2002), except for the terminology of the scalation around the eye, as we considered the more anterior of Storr's (e.g., 1971) presubocular scales as a lower preocular (Greer and Cogger 1985; Couper et al. 2002; Hutchinson et al. 2006). As a result, our scoring of presubocular scales is one lower than in Storr's descriptions.

These initial examinations of the specimens sequenced revealed consistent differences in coloration pattern between a highly divergent lineage and the remaining samples assigned to *C. schomburgkii* (see Results). To search for additional yet unsequenced specimens of this newly identified and phenotypically distinctive lineage, we then screened the coloration pattern of specimens assigned to *C. schomburgkii* deposited at the South Australia Museum (SAMA), Western Australian Museum (WAM), Museum and Art Gallery of the Northern Territory (MAGNT), University of Michigan Museum of Zoology (UMMZ), and Cornell University Museum of Vertebrates (CUMV), totaling around 1700 vouchers. This screening revealed a total of 30 specimens of the candidate new species.

Throughout this manuscript, geographic distributions were annotated with the relevant IBRA 7 bioregions and subregions (1995; Australian Government 2021).

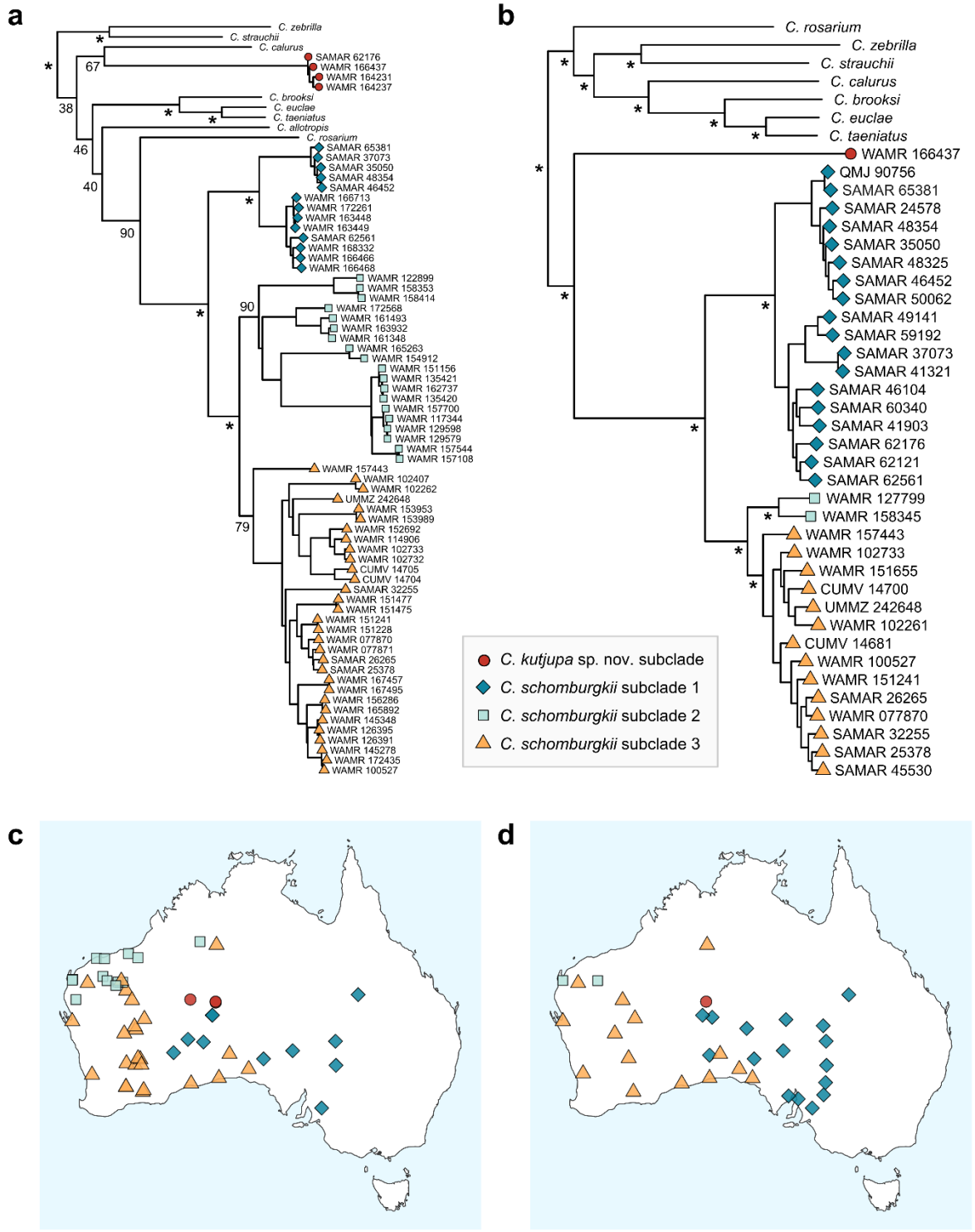
Results

Phylogenetic structure within C. schomburgkii

Samples assigned to *C. schomburgkii* formed four major subclades in both the mitochondrial (Fig. 1a) and nuclear (Fig. 1b) RAxML analyses: one occurring in the interior central and eastern arid zone, potentially including the type locality of *C. schomburgkii* (Buchsfelde, South Australia); another in the southwestern arid zone and along the southern coast; a third centered mostly in the Pilbara region in Australia's northwest; and a fourth in the central deserts (Central Ranges bioregion) surrounding the limits between the states of South Australia (SA), Western Australia (WA), and Northern Territory (NT). This fourth subclade was found as sister to all the other samples of *C. schomburgkii* in the nuclear tree, the split between the two being one of the deepest divergences in the entire clade (Fig. 1b). The mitochondrial data for this distinctive nuclear lineage showed an even more distant relationship to *C. schomburgkii*, being closer to *C. calurus* (Fig. 1a).

Our examination of museum specimens confirmed that the highly divergent lineage from the Central Ranges bioregion is morphologically distinct from typical *C. schomburgkii* based on coloration and scalation patterns (see below). Based on the sampling localities of the genotyped specimens, as well as other specimens that show the same suit of distinctive characters, it is clear that this lineage occurs in broad sympatry with *C. schomburgkii* in the Great Victoria Desert and Central Ranges bioregions (Australian Government, 2021). The genetic and morphological distinction of this lineage and *C. schomburgkii* despite ample opportunities for gene flow supports that the former corresponds to a yet unnamed species, which we formally describe below.

Figure 1. Evolutionary relationships between samples assigned to *Ctenotus schomburgkii* based on the *cytochrome B* mitochondrial marker (a) and the nuclear ddRAD data (b). For clarity, a maximum of two samples per locality per taxon is shown on trees, and other taxa in the *C. schomburgkii* species group are shown as a single terminal. Nodal bootstrap support values > 95 are indicated with an asterisk, and support values within major subclades are omitted. Maps indicate the geographic distributions of mitochondrial (c) and nuclear (d) subclades.



Evolutionary relationships of C. schomburgkii *and related taxa*

Nuclear phylogenetic analyses under both the concatenated (RaxML; Fig. 2) and multispecies coalescent (SVD Quartets; Fig. S1) approaches yielded the exact same topology, except for a single relationship (see below). These analyses found the new species and most taxa regarded as closely related to *C. schomburgkii* based on morphological similarity, and thus assigned to the *C. schomburgkii* species group (Storr et al. 1999), to be nested in the

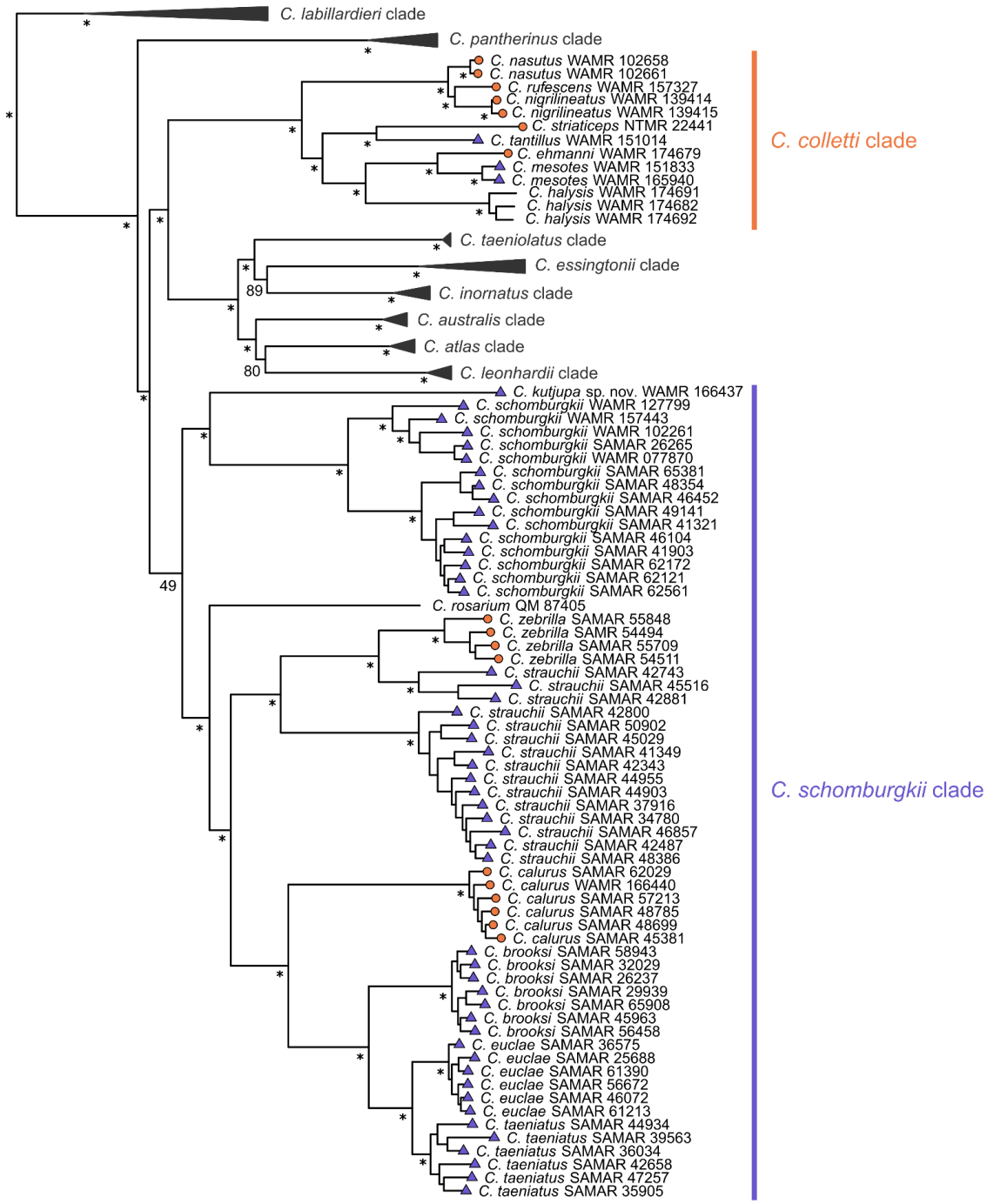
same highly supported clade. This was the case of *C. schomburgkii*, *C. brooksi*, *C. euclae*, *C. taeniatus*, and *C. strauchii*. However, the morphologically defined *C. schomburgkii* species group was not monophyletic. The major *Ctenotus* clade that included most taxa in the *C. schomburgkii* group also included *C. calurus* and *C. zebrilla*, both formerly associated with the *C. colletti* species group based on dorsal coloration pattern (Storr et al. 1999). This clade also included a similarly small-bodied taxon, *C. rosarium*, not previously regarded as related to the *C. schomburgkii* group (Couper et al. 2002).

At the same time, *C. mesotes* and *C. tantillus*, formerly assigned to the *C. schomburgkii* group based on their morphological similarity to *C. schomburgkii* (Storr et al. 1999; Horner 2009), branched within another major clade of *Ctenotus*. This second clade mostly included taxa assigned to the *C. colletti* group, namely *C. nasutus*, *C. nigrilineatus*, *C. rufescens*, and *C. striaticeps* (Fig. 2; Fig. S1). These emerging patterns indicate that the morphological attributes used to distinguish the *C. schomburgkii* and *C. colletti* species groups are not always accurate indicators of phylogenetic relationships.

These two major clades, which we refer to as the *C. schomburgkii* and *C. colletti* clades of *Ctenotus*, were found as successive branches in the concatenated RaxML tree rather than as sister clades (Fig. 2). Specifically, the *C. schomburgkii* clade was sister to a large clade that included all the other *Ctenotus* except for the *C. labillardieri* and *C. pantherinus* clades. In turn, the *C. colletti* clade was the next branching clade, sister to a clade composed of the remaining *Ctenotus* (i.e., the *C. atlas*, *C. australis*, *C. essingtonii*, *C. inornatus*, *C. leonhardii*, and *C. taeniolatus* clades). By contrast, the SVD Quartets species-tree (Fig. S1) found the *C. schomburgkii* and *C. colletti* clades to be sisters, albeit with low bootstrap support (= 64).

Within the *C. schomburgkii* clade, *C. taeniatus* and *C. euclae* formed a clade sister to *C. brooksi*, supporting previous findings of monophyly of the *C. brooksi* complex and a sister relationship between this clade and *C. calurus* (Hutchinson et al. 2006).

Figure 2. Evolutionary relationships of taxa assigned to the *Ctenotus schomburgkii* morphological species group based on 468,161 base pairs across 3,321 restriction-site associated DNA loci. Purple triangles on tips indicate taxa traditionally assigned to the *C. schomburgkii* group based on morphology; orange circles indicate taxa previously assigned to the *C. colletti* group. For clarity, a maximum of 15 samples per taxon is shown. Nodal bootstrap support values > 95 are indicated with an asterisk, and within-taxon support values are omitted.



A new species of Ctenotus

***Ctenotus kutjupa* sp. nov.**

(Fig. 3, 4a–b)

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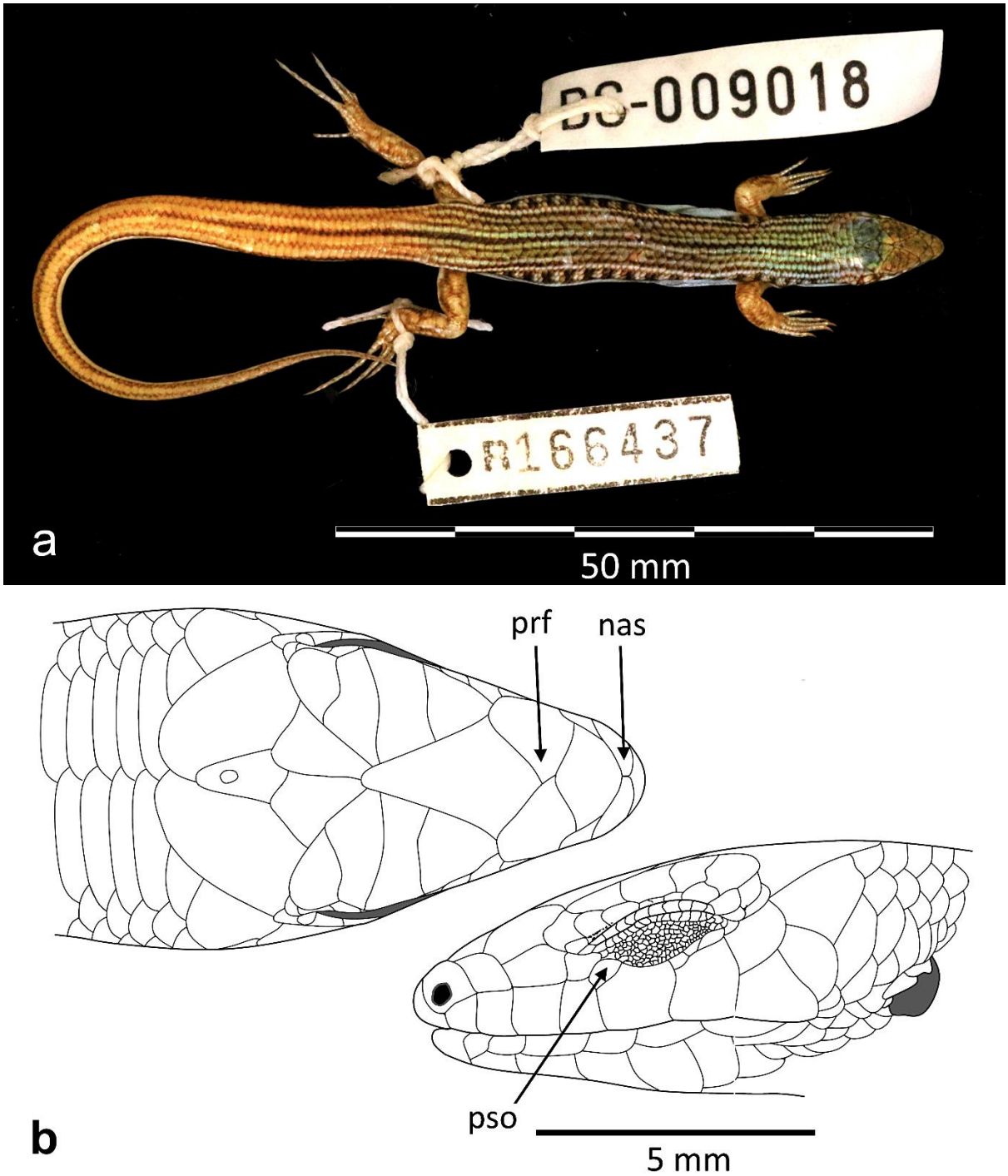
Ctenotus schomburgkii (part) (Robinson et al. 2003)

Ctenotus schomburgkii (part) (Foulkes and Thompson 2008)

Holotype: WAM R166437 (Fig. 3a; GenBank accession KJ506010; Sequence Read Archive accession SRX2727531), 5.3 km SSE Pungkulpirri Waterhole, WA, 24° 42’ 26” S, 128° 45’ 37” E, on 22 September 2006, by S. C. Donnellan, P. Doughty, and M. Hutchinson.

Paratypes: SAMA R18215, 40 km SSW of Wyola Lake, SA, 29° 30’ 00” S, 130° 08’ 00” E; R36119, 1 km down Giles Rd, Yulara Townsite, NT, 25° 14’ 00” S, 131° 12’ 00” E; R46128–29, 27 km NE of Pipalyatjara, SA, 26° 02’ 58” S, 129° 24’ 39” E; R48782, 6.6 km WNW of Mt Lindsay (Wataru), SA, 27° 03’ 39” S, 129° 49’ 45” E; R51751, Anne Beadell Highway, 11.2 km E Vokes Hill Corner, SA, 28° 33’ 42” S, 130° 47’ 40” E; SAMA R62233 (Fig. 3b), from the Anne Beadell Highway, 300 m E of the WA-SA border, SA, 28° 30’ 28” S, 129° 00’ 17” E; R62176 (GenBank accession KJ505036), 3 km SE Mt Ant, WA, 24° 46’ 57” S, 128° 46’ 44” E; R62312–13, Anne Beadell Highway, 26.3 km E of the WA-SA border, SA, 28° 30’ 24” S, 129° 15’ 43” E. NTM R14234, Amata Rd (site 5), Uluru National Park, NT, 25° 20’ 00” S, 130° 47’ 00” E; R17505–10, Uluru National Park, NT, 25° 15’ 00” S, 131° 00’ 00” E; R32339, Petermann Ranges, NT, 25° 01’ 00” S, 129° 23’ 00” E; R36149, SE of Reedy Rockhole, Kings Canyon, NT, 24° 15’ 00” S, 131° 34’ 00” E. WAM R51077, 22 km NE White Cliffs HS, 28° 20’ 00” S, 123° 06’ 00” E; R53490, Red Hill, 92 km E of Laverton, 28° 18’ 00” S, 123° 11’ 00” E; R85273, 85297, 4 km ESE of Big Shot Bore, 28° 24’ 00” S, 123° 04’ 00” E; R147767, 7–8 km WNW Point Salvation, 28° 12’ 00” S, 123° 35’ 00” E; R155723, 7–8 km WNW Point Salvation, 28° 14’ 00” S, 123°36’ 00” E; R163450, Neale Junction, 28° 18’ 09” S, 126° 17’ 57” E; R164231 (GenBank accession OM966792), Mina Mina, Clutterbuck Hills, Gibson Desert, 24° 31’ 01” S, 126° 12’ 46” E; R164237 (GenBank accession OM966793), Mina Mina, Clutterbuck Hills, Gibson Desert, 24° 31’ 06” S, 126° 13’ 12” E; R175023 (Fig. 4a), Beyondie Lakes, Little Sandy Desert, 24° 43’ 44” S, 120° 17’ 46” E.

Figure 3. a) Holotype of *Ctenotus kutjupa* sp. nov., WAM R166437. Picture by Paul Doughty. b) Head scales of paratype SAMA R62233, showing the diagnostic scalation combination of the prefrontals (prf) and nasals (nas) in medial contact and the presence of a presubocular scale (pso).



Etymology: The word *kutjupa*, meaning "the other one" or "another one" in its noun form, makes reference to the discovery of the new species among collections of *C. schomburgkii*. We use this word, shared by several Western Desert languages (e.g., Maralinga Tjarutja, Yankunytjatjara, Pitjantjatjara, Ngaanyatjarra), to acknowledge that this lizard belongs to the country where these languages are spoken. The specific epithet is a noun in apposition to the genus.

Diagnosis: A small species of *Ctenotus*, characterized by the unique combination of sharp-edged and spinose subdigital lamellae, a presubocular scale between the lower preocular and the subocular supralabial, and prefrontal and nasal head shields usually in medial contact (Fig. 3b). The color pattern typically includes seven narrow dark dorsal stripes, including a vertebral stripe that becomes more prominent on the (unbroken) tail and runs almost the full length of the tail in most individuals (Fig. 3a). The new species is further characterized by the combination of a dark upper lateral zone with a single series of large pale spots with an ascending series of black and whitish blotches that curve dorsally behind the eye (Fig. 4a–b, e).

Description: Adult snout-vent length (mm) 35.5–45.0, ($\bar{x}$ = 39.7, n = 24). Smallest individual 28.0 mm. Tail length (%SVL) 143–192 ($\bar{x}$ = 160.1, n = 16). Hind limb length (% SVL) 42.1–50.0 ($\bar{x}$ = 45.3). Head length (%SVL) 20.0–24.7 ($\bar{x}$ = 22.8, n = 30). Head length/head width (%) 143–183 ($\bar{x}$ = 158.9, n = 30). Nasals in point to broad medial contact (Fig. 3b) (2 of 30 narrowly separated). Prefrontals usually in point to broad medial contact (4 of 30 narrowly separated). Supraoculars four, first three in contact with frontal, second and third subequal. Supraciliaries 7–8 (mode 7), the count depending on whether two or three small supraciliaries separate the initial three from the terminal two. Posterior loreal tapering only slightly anteriorly and less than twice as long as high. Seven supralabials. One rectangular presubocular lies between the lower preocular and the fifth (subocular) supralabial, contacting the fourth supralabial. Ear lobules usually two, obtusely pointed, the lower larger than the upper, or a single large blunt lobule occupies the place of the two. Midbody scale rows 24–28 ($\bar{x}$ = 26.2, mode 26, n = 30). Toes compressed; 22–26 ($\bar{x}$ = 23.6, n = 29) lamellae under fourth toe, each forming a laterally compressed, sharp-edged keel with a small apical spine. Plantar scales obtusely pointed, sometimes with two to four scales similar in shape to the subdigital lamellae running on to the plantar surface from the base of the fourth toe.

Dorsal background color sandy orange in life, most individuals with seven black to dark gray longitudinal dorsal stripes (Fig. 3a, 4a–b). The vertebral stripe, and a laterodorsal and a dorsolateral stripe on each side, are formed by dark pigment in the overlapping corners of adjacent scale rows. A paravertebral stripe on each side is formed by a black line running down the middle of the first scale row (Fig. 4e). In a few individuals the paravertebral and laterodorsal dark lines are more irregular and may coalesce or braid together. The black dorsolateral stripe forms the dorsal margin of a white dorsolateral stripe that runs down the middle of the third scale row. The white dorsolateral line is bordered below by a blackish upper lateral zone that covers the fourth and fifth scale rows and bears a single series of large pale orange blotches. A mid-lateral white stripe bordered below by black forms the lower boundary of the dark upper lateral zone, which runs from the groin to the ear. Anteriorly to the ear, the white stripe continues to the posterior supralabial or breaks up. The scales of the temporal region have a distinctive crescentic mark that highlights the posteroventral margin of the eye (postsuboculars and adjacent portions of the temporal scale), usually bounded by an ascending series of dark postocular blotches (on the penultimate supralabial, primary temporal and base of the upper secondary temporal), in turn bordered posteriorly by the pale centers of the terminal supralabial, lower secondary temporal and upper secondary temporal. A weak dark zone runs from the preocular scales to the nasal. Vertebral stripe continues

along the tail almost its full length, and often becomes wider on the tail before narrowing again distally. Lateral markings merge on the tail into a light-edged dark lateral stripe that also runs most of the length of the tail. Limbs with a blackish reticulum over a sand-orange background. Underside pure white.

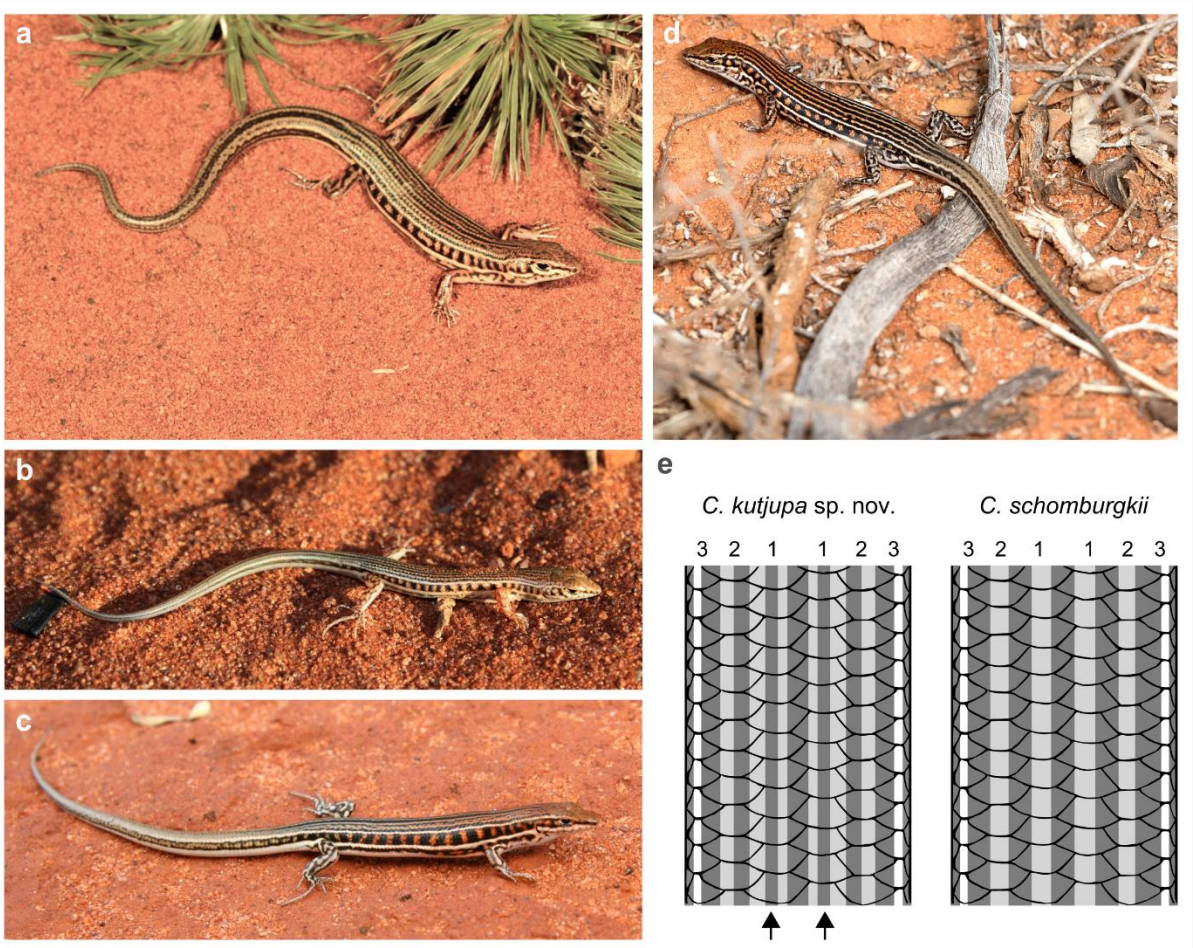
Species comparisons: Taxa morphologically similar to the new species are all members of the *C. schomburgkii* species group, established and expanded by Storr (Storr 1969, 1971, 1981). Species in this group are small *Ctenotus* with keeled and spinose subdigital lamellae, a dorsal pattern including longitudinal lines, and a lateral pattern including spots or bars.

The new taxon is superficially most similar to *C. schomburgkii* (Fig. 4c–d), with which it has been confused. However, *C. kutjupa* is distinguished from *C. schomburgkii* by having seven well-defined dark dorsal stripes (versus five in *C. schomburgkii*) where both co-occur. This is due to the presence (in *C. kutjupa*) of two median paravertebral stripes that run down the midline of each of the paravertebral scale rows (Fig. 4e). *Ctenotus kutjupa* is further distinguished from *C. schomburgkii* by having prefrontals and nasals usually in strong medial contact (versus rarely so for either pair in *C. schomburgkii*); lack of a strong continuation of the white midlateral stripe along the upper labials (versus continuation present in *C. schomburgkii*), instead showing a crescentic whitish marking that curves up behind the eye; and by having a dark vertebral stripe that becomes more prominent on the tail and continues for almost all of its length in most individuals (versus a vertebral stripe that fades out on the tail and terminates at about the halfway point in *C. schomburgkii*). While *C. kutjupa* appears to attain a smaller maximum body size than *C. schomburgkii*, meristic and morphometric measurements overlap broadly between the two species. For instance, body size (SVL) ranges 35.5–45.0 mm in *C. kutjupa* versus 25–52 mm in *C. schomburgkii*.

The presence of a post-ocular pale crescent marking in *C. kutjupa* resembles members of the *C. brooksi* complex (Hutchinson et al. 2006), but *C. kutjupa* differs from them by the presence of a presubocular scale between the lower preocular and the subocular supralabial and the seven-striped dorsal pattern (versus five dark stripes in strongly marked *C. taeniatus* and *C. euclae*).

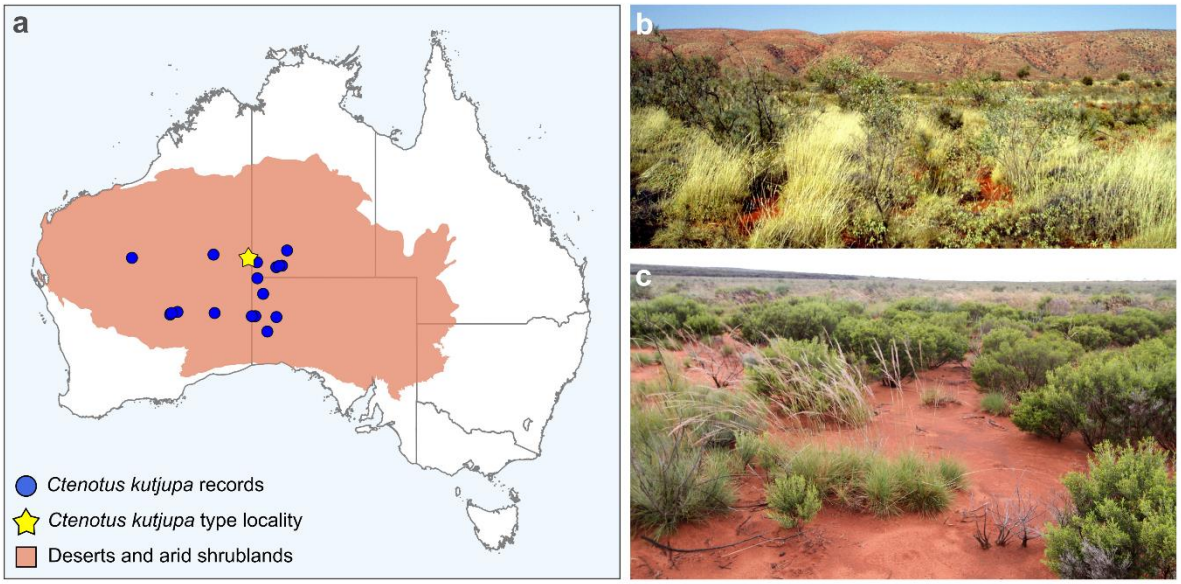
Storr (1971) suggested that the *C. brooksi* complex and *C. schomburgkii* differed in having a series of lamella-like keeled and spiny scales that extended onto the plantar surface from the base of the fourth toe. Our examinations suggest that this character is unreliable, with some *C. brooksi* complex specimens lacking it and some *C. schomburgkii* having it. The enlarged scales are also present in some *C. kutjupa*, but most lack them.

Figure 4. Coloration in life. a) Paratype of *Ctenotus kutjupa* sp. nov. (WAM R175023) from around Beyondie Lakes, Western Australia (WA). Picture by Ryan Ellis. b) *Ctenotus kutjupa* from the Tjirrkarli Aboriginal Lands, Gibson Desert, WA. Picture by John Harris. c) *Ctenotus schomburgkii* from Goongarrie National Park, WA. Picture by Daniel L. Rabosky. d) *Ctenotus schomburgkii* from Gluepot, South Australia. Picture by Kym Nicolson. e) Schematic representation of dorsal color pattern in *C. kutjupa* and *C. schomburgkii* in localities where they co-occur (see text). Note the continuous dark line down the middle of the scale row 1 in *C. kutjupa* (arrows).



Distribution: Central Australia, from about 120° E, in the vicinity of Beyondie Lakes and the Carnarvon Range, WA, east to about 132° E along a line from Watarrka (King's Canyon), NT, south through Uluru to Wyola Lake and Vokes Hill, SA. These sites are centered on the Great Victoria Desert and Central Ranges bioregions and include the southern portions of the Little Sandy Desert and Gibson Desert bioregions. The habitat at most of the localities that have yielded specimens is well-vegetated sand dunes. (Fig. 5).

Figure 5. *Ctenotus kutjupa* sp. nov. distribution and habitat. a) Localities where *C. kutjupa* is presently known to occur. Distribution of deserts and xeric shrublands (i.e., the arid zone's driest portion) as per Olson et al. (2001). b) Habitat east of the Pungkulpirri Waterhole, Western Australia (WA), collection site of holotype WAM R166437. Picture by Helen Vonow. c) Habitat in the vicinity of the Beyondie Lakes, WA, collection site of *C. kutjupa* paratype WAM R175023. Picture by Ryan Ellis.



Discussion

A new species of arid zone lizard

Through analyses of morphological, mitochondrial, and genome-wide nuclear data, we identified and described *Ctenotus kutjupa*, a wide-ranging species of Australian arid zone lizard. *Ctenotus kutjupa* was previously confused with *C. schomburgkii*, but our morphological examinations informed by the genetic patterns confirmed that these two taxa differ consistently in coloration and scalation. Further supporting their distinctiveness and independence, *C. kutjupa* and *C. schomburgkii* co-occur over a large area encompassing the Great Victoria Desert, Central Ranges, Little Sandy Desert, and Gibson Desert bioregions. In three cases, specimens of both taxa have identical associated locality information, namely in two sites northwest of Laverton, WA (Big Shot Bore, Point Salvation) and one on the Ann Beadell Highway at the border between the states of Western Australia and South Australia. More broadly, at most other localities where *C. kutjupa* was found, *C. schomburgkii* was collected within only 5–10 km. While widely sympatric, the two taxa seem to have somewhat different habitat associations. *Ctenotus kutjupa* appears locally uncommon and associated with well-vegetated dunes and soft sand. In turn, *C. schomburgkii* is an abundant generalist that occupies a wide range of desert habitats (Pianka 1969; Robinson et al. 2003; Rabosky et al. 2011).

The discovery of specimens of *C. kutjupa* among collections of *C. schomburgkii* leads to no significant change to the current diagnosis of the latter taxon. The only morphological feature we introduce is the patterning around the facial region, with a strong white labial stripe and dark posterior orbital margin in *C. schomburgkii*, compared to the pale postocular crescent and pale and dark postocular patches of *C. kutjupa*. The seven dark dorsal lines and strongly contacting nasals and prefrontals that differentiate *C. kutjupa* from *C. schomburgkii* would have done so even based on the original definition of *C. schomburgkii* by Storr (Storr 1969, 1970). However, at that time, none of the specimens of *C. kutjupa* we have identified had yet been acquired by the WAM, MAGNT, or SAMA collections.

436
437 *Standing knowledge gaps on Australia's arid zone biotas*
438 Besides the new taxon, this study highlights additional issues related to species
439 boundaries, diversity, and distributions in *Ctenotus*. Our survey of genetic variation within *C.*
440 *schomburgkii* suggests that, beyond the lineage corresponding to *C. kutjupa*, the name *C.*
441 *schomburgkii* comprises at least three additional lineages that come into contact in southern
442 and northwestern Australia. Notably, two of them are potentially sympatric along the eastern
443 Nullarbor Plain and Great Victoria Desert (subclades 1 and 3 in Fig. 1). Furthermore, two
444 subclades (subclades 2 and 3) appear to come into contact across the Pilbara region in
445 Australia's northwest and may be broadly sympatric or parapatric across the Great Sandy and
446 Tanami bioregions. These three subclades presently assigned to *C. schomburgkii* may
447 correspond to additional species, as the maintenance of divergent lineages in sympatry would
448 necessitate them to be reproductively isolated.
449 On the other hand, morphological support for additional species is currently lacking.
450 Our examinations confirmed that *C. schomburgkii* shows extensive variation in dorsal
451 coloration (Storr et al. 1999). However, at least part of this variation represents local
452 polymorphism, as individuals from the same site can show varying degrees of fusion and
453 fading of dorsal stripes and spots. This pattern makes it difficult to assess the extent to which
454 this variation is geographically structured, confounding our ability to identify characters that
455 may identify each of the three *C. schomburgkii* molecular clades. Previous assessments
456 suggested that some regionally distinctive coloration variation occurs in Australia's
457 southwest and northwest (Storr 1969, Storr et al. 1999), but we had access to few genetic
458 samples from these regions. Additional sampling will be needed to determine what
459 proportion of the observed trait variation is geographic versus polymorphic, particularly in
460 undersampled and remote desert locations. This improved sampling may reveal additional
461 unrecognized species in these ubiquitous yet poorly known desert lizards.
462
463 *Phylogenetic relationships of* C. schomburgkii *and related taxa*
464 In assessing the phylogenetic relationships of taxa morphologically allied to *C.*
465 *schomburgkii*, we not only confirm the genetic distinction of *C. kutjupa* but also provide a
466 test of whether Storr's phenetic groups, erected as aides to species identification, also capture
467 evolutionary relationships. Our results did not support as monophyletic each of the two
468 species groups of *Ctenotus* that contain our focal taxa, namely the *C. schomburgkii* and *C.*
469 *colletti* groups. Nevertheless, we found most taxa assigned to each of these groups to be each
470 other's closest relatives. These results agree with previous molecular analyses of *Ctenotus*
471 (Rabosky et al. 2014a; Singhal et al. 2017; Prates et al. 2021).
472 Our findings suggest that the coloration characters used to delineate species groups in
473 *Ctenotus* are evolutionarily labile and hence imperfect predictors of evolutionary
474 relationships. Both the *C. schomburgkii* and *C. colletti* groups are diagnosed from other
475 species groups by their small size (mean SVL mostly under 50 mm) and spinose subdigital
476 lamellae. The two groups are diagnosed from one another solely by a lateral color pattern of
477 spots or stripes, respectively (Storr et al. 1999). Among those three characters, we found that
478 only the small size occurs in all taxa within the two molecular clades that (roughly)
479 correspond to the *C. schomburgkii* and *C. colletti* morphological groups. Strongly

compressed spinose subdigital lamellae, thought to differentiate both groups from other *Ctenotus*, are missing in *C. rosarium* (*C. schomburgkii* clade) and *C. halysis* (*C. colletti* clade). The spotted versus striped lateral patterns proposed to distinguish the *C. schomburgkii* and *C. colletti* groups emerge as unreliable, with the striped *C. calurus* and *C. zebrilla* branching within the *C. schomburgkii* clade, and the spotted *C. ehmanni*, *C. mesotes*, *C. halysis*, and *C. tantillus* in the *C. colletti* clade. As a result, morphological corroboration for the two clades is currently lacking.

A pattern of diverging traits in closely related species, as well as of trait convergence across distantly related species, can result from the action of selection. Previous studies have documented evidence for selection-driven phenotypic and phylogenetic overdispersion in communities of *Ctenotus* lizards (Rabosky et al. 2007, 2011). These patterns of trait divergence challenge the view that *Ctenotus* merely represents a continental-scale *non-adaptive radiation*, whereby morphological and ecological attributes are conserved despite the formation of new species (Gittenberger 1991; Rundell and Price 2009). Our phylogenetic results suggest that the adaptive significance of variation in dorsal coloration and foot scalation in *Ctenotus* may be a productive avenue of future research.

Concluding remarks

Our combined analyses of morphological and genetic variation support the recognition of *C. kutjupa*, a new species of wide-ranging Australian arid zone lizard. Moreover, we find evidence of multiple genetic lineages within the morphologically variable *C. schomburgkii*, which may prove to correspond to additional species. However, limited genetic sampling in key geographic regions, combined with high morphological variability within each clade, limits our ability to assess their separation and distinction at this time. Lastly, our results contradict the presumed affinities of several taxa, indicating that certain morphological characters used to inform the taxonomy of *Ctenotus* can vary among closely related species and thus show greater evolutionary lability than generally assumed (see also Rabosky et al. 2014b).

The results of this investigation indicate that our understanding of species diversity and evolutionary relationships in the lizard-rich Australian deserts remains incomplete. These knowledge gaps likely bias inferences of regional biogeography and ecology and might lead to the development of incomplete or inadequate conservation measures. As illustrated by *C. kutjupa*, collecting voucher specimens through field sampling in understudied regions, coupled with population genetic screening of lineage diversity, will be crucial to properly characterizing the composition and assembly of Australia's diverse vertebrate communities.

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Data Availability

Newly generated mitochondrial data were uploaded in GenBank (accession numbers OM966724–OM966802); see Table S1 for the accession numbers of sequences generated by previous studies. Nuclear ddRAD data used in this investigation are available in the Sequence Read Archive (BioProjects PRJNA755251 and PRJNA382545). This publication and associated nomenclatural acts were registered in the ZooBank (Life Science Identifier urn:lsid:zoobank.org:pub:83DE5310-1F10-4CA4-91D8-AF8BF4C9D24D).

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686

687 **Figure Legends**

688

689 **Figure 1.** Evolutionary relationships between samples assigned to *Ctenotus schomburgkii*
690 based on the *cytochrome B* mitochondrial marker (a) and the nuclear ddRAD data (b). For
691 clarity, a maximum of two samples per locality per taxon is shown on trees, and other taxa in
692 the *C. schomburgkii* species group are shown as a single terminal. Nodal bootstrap support
693 values > 95 are indicated with an asterisk, and support values within major subclades are
694 omitted. Maps indicate the geographic distributions of mitochondrial (c) and nuclear (d)
695 subclades.
696

697 **Figure 2.** Evolutionary relationships of taxa assigned to the *Ctenotus schomburgkii*
698 morphological species group based on 468,161 base pairs across 3,321 restriction-site
699 associated DNA loci. Purple triangles on tips indicate taxa traditionally assigned to the *C.*
700 *schomburgkii* group based on morphology; orange circles indicate taxa previously assigned to
701 the *C. colletti* group. For clarity, a maximum of 15 samples per taxon is shown. Nodal
702 bootstrap support values > 95 are indicated with an asterisk, and within-taxon support values
703 are omitted.
704

705 **Figure 3.** a) Holotype of *Ctenotus kutjupa* sp. nov., WAM R166437. Picture by Paul
706 Doughty. b) Head scales of paratype SAMA R62233, showing the diagnostic scalation
707 combination of the prefrontals (prf) and nasals (nas) in medial contact and the presence of a
708 presubocular scale (pso).
709

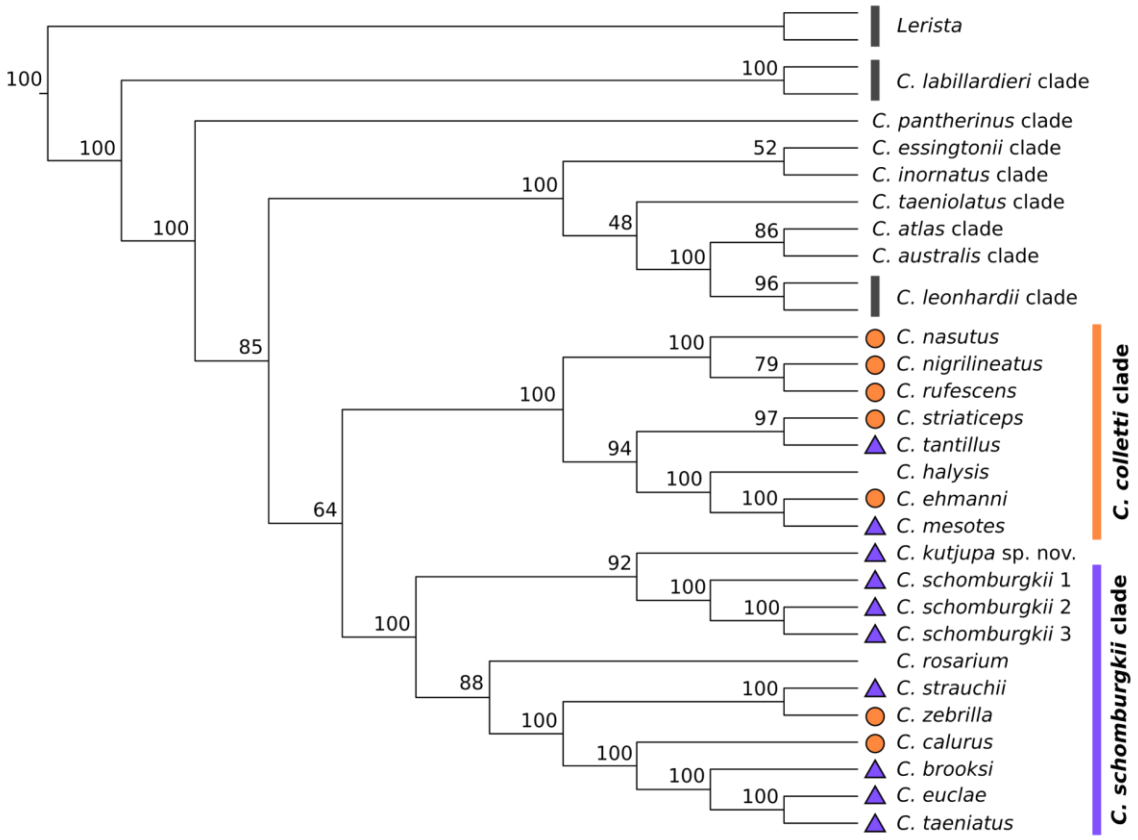
710 **Figure 4.** Coloration in life. a) Paratype of *Ctenotus kutjupa* sp. nov. (WAM R175023) from
711 around Beyondie Lakes, Western Australia (WA). Picture by Ryan Ellis. b) *Ctenotus kutjupa*
712 from the Tjirrkarli Aboriginal Lands, Gibson Desert, WA. Picture by John Harris. c) *Ctenotus*
713 *schomburgkii* from Goongarrie National Park, WA. Picture by Daniel L. Rabosky. d)
714 *Ctenotus schomburgkii* from Gluepot, South Australia. Picture by Kym Nicolson. e)
715 Schematic representation of dorsal color pattern in *C. kutjupa* and *C. schomburgkii* in

localities where they co-occur (see text). Note the continuous dark line down the middle of the scale row 1 in *C. kutjupa* (arrows).

Figure 5. *Ctenotus kutjupa* sp. nov. distribution and habitat. a) Localities where *C. kutjupa* is presently known to occur. Distribution of deserts and xeric shrublands (i.e., the arid zone's driest portion) as per Olson et al. (2001). b) Habitat east of the Pungkulpirri Waterhole, Western Australia (WA), collection site of holotype WAM R166437. Picture by Helen Vonow. c) Habitat in the vicinity of the Beyondie Lakes, WA, collection site of *C. kutjupa* paratype WAM R175023. Picture by Ryan Ellis.

Supplementary Figure and Legends

Figure S1. Phylogenetic relationships between taxa in the *Ctenotus schomburgkii* and *C. colletti* species groups, inferred using the SVD Quartets species-tree method (see text for details). Purple triangles on tips indicate taxa traditionally assigned to the *C. schomburgkii* group based on morphology; orange circles indicate taxa previously assigned to the *C. colletti* group.



Supplementary Table Legends

737

738 **Table S1.** Specimen information. Includes GenBank and Sequence Read Archive accession
739 numbers and museum vouchers examined.

740