1   **Notes from the taxonomic disaster zone: Evolutionary drivers of intractable species**

2   **boundaries in an Australian lizard clade (Scincidae: *Ctenotus*)**

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21

Abstract (250 words max)

Genomic-scale datasets, sophisticated analytical techniques, and conceptual advances have disproportionately failed to resolve species boundaries in some groups relative to others. To understand the processes that underlie taxonomic intractability, we dissect the speciation history of an Australian lizard clade that arguably represents a "worst-case" scenario for species delimitation within vertebrates: the *Ctenotus inornatus* species group, a clade beset with decoupled genetic and phenotypic breaks, uncertain geographic ranges, and parallelism in purportedly diagnostic morphological characters. We sample hundreds of localities to generate a genomic perspective on population divergence, structure, and admixture. The results reveal rampant paraphyly of nominate taxa in the group, with lineages that are either morphologically cryptic or polytypic. Isolation-by-distance patterns uncover spatially continuous differentiation among certain pairs of putative species, yet genetic and geographic distances are decoupled in other pairs. Comparisons of mitochondrial and nuclear gene trees, tests of nuclear introgression, and historical demographic modeling identify gene flow between divergent candidate species. Levels of admixture are decoupled from phylogenetic relatedness; gene flow is often higher between sympatric species than between parapatric populations of the same species. Such idiosyncratic patterns of introgression contribute to species boundaries that are fuzzy while also varying in fuzziness. Our results suggest that "taxonomic disaster zones" like the *C. inornatus* species group result from spatial variation in the porosity of species boundaries and the resulting patterns of genetic and phenotypic variation. This study raises questions about the origin and persistence of hybridizing species and highlights the unique insights provided by taxa that have long eluded straightforward taxonomic categorization.

Introduction

Species delimitation has long relied on the phenotypic attributes of organisms under the premise that character differences reflect evolutionary divergence (de Queiroz, 1998, 2007; Hennig, 1966). Molecular approaches revolutionized taxonomy by revealing that species boundaries inferred from morphological characters can be inconsistent with patterns of evolutionary divergence, sometimes supporting the refinement of morphological diagnoses (Prates, Hutchinson, et al., 2022; Sites & Marshall, 2004; Teixeira et al., 2016). Observations of uncoupled genetic and morphological differentiation have now become ubiquitous, as illustrated by increasing reports of polymorphic and cryptic species (Vacher et al., 2020; Veijalainen et al., 2012; Zamudio et al., 2016). Over the last decades, a suite of methods has been proposed to infer species boundaries based primarily on patterns of genetic variation (Carstens et al., 2013). These methods include genotypic clustering algorithms (Pritchard et al., 2000), metrics of genealogical discordance (Cummings et al., 2008), tests of alternative species schemes under the multispecies coalescent model (Yang & Rannala, 2010), estimates of population gene flow (Smith & Carstens, 2020), and genetic-based estimates of reproductive isolation (Singhal et al., 2018). With the promise of providing process-based and objective inference of species boundaries (Fujita et al., 2012), molecular delimitation approaches have become a standard component of systematic and evolutionary investigations.

Many clades regarded as taxonomically challenging have now been scrutinized using large genetic datasets and sophisticated delimitation methods (e.g., Leaché et al., 2018; Pyron et al., 2020; Rivera et al., 2021; Wagner et al., 2013). Typically, multiple analytical approaches are combined in an integrative assessment of whether populations are on separate evolutionary trajectories. However, different approaches can yield incongruent inferences of species

68   boundaries, leading some authors to advocate for consensus species partitions across multiple

69   analytical frameworks (Carstens et al., 2013; Shaik et al., 2021). Still, objectively deriving a

70   single scheme based on conflicting or inconclusive results can be difficult to impossible (e.g.,

71   Firneno et al., 2021; McKay et al., 2013; Tilley et al., 2013; Willis, 2017). Even in the face of the

72   same patterns, different authors often disagree on the number of species involved (Burbrink &

73   Ruane, 2021; de Queiroz, 2020; Hillis et al., 2021; Zachos et al., 2020). Moreover, the units

74   emerging from delimitation algorithms might represent distinct populations rather than species

75   (Sukumaran & Knowles, 2017). Finally, despite the increasing consensus around the definition

76   of species as separately evolving metapopulation lineages (de Queiroz, 1998), translating

77   conceptual definitions of species into empirical delimitation is not trivial (de Queiroz, 2007;

78   Prates, Doughty, et al., 2022). Speciation is an extended and often non-linear process, whereby

79   species may constitute historical and geographic continuums that can be difficult to partition

80   (Bouzid et al., 2022). Therefore, modern datasets and analytical techniques, integrative

81   approaches, and conceptual advances have not always made species delimitation more accurate

82   or objective (Barley et al., 2018; Garnett & Christidis, 2017; Hillis, 2019; Sites & Marshall,

83   2004; Sukumaran & Knowles, 2017).

84   Many existing taxonomies are based on morphological data, and limitations of these data

85   might in part explain inconsistent or unclear species boundaries (Cadena & Zapata, 2021;

86   Winker, 2009). However, such inconsistencies might also originate from patterns of genetic

87   variation that map poorly onto how populations are represented in inferential frameworks. This

88   poor mapping might result from biological processes. For instance, inferences of genetic

89   structure and phylogenetic relationships can overestimate population differentiation in the

90   presence of isolation-by-distance (Barley et al., 2018; Bradburd et al., 2018; Irwin, 2002),

91   particularly if substantial sampling gaps exist (Battey et al., 2020). Moreover, genetic

92   introgression can lead to oversplitting of species. Admixed populations can be mistaken as

93   cryptic species due to unique allele combinations, particularly when admixture is geographically

94   structured (Chan et al., 2020, 2021). Introgressive hybridization and incomplete allele sorting can

95   also lead to heterogeneous divergence among genome regions. This heterogeneity hampers

96   inferences of species boundaries and relationships, as reported for groups ranging from lizards to

97   cichlid fishes and oak trees (Camargo et al., 2012; McVay et al., 2017; Turner et al., 2001). In

98   addition, unclear species boundaries might result from processes that shape patterns of

99   phenotypic variation. For instance, geographically segregated phenotypes (i.e., polytypism)

100   owing to phenotypic plasticity or local adaptation can be interpreted as evidence of population

101   separation, even when gene flow is high (Mayr, 1963; Zamudio et al., 2016). Conversely,

102   morphological crypsis owing to developmental or ecological constraints can conceal genetically

103   divergent species (Struck et al., 2018; Zamudio et al., 2016). These findings suggest that

104   logistical challenges to species delimitation might emerge from particular evolutionary

105   processes. Uncovering these processes might allow us to understand why certain clades have

106   eluded, and perhaps are not amenable to, taxonomic categorization (Willis, 2017).

107   Here, we seek to understand the processes that might underlie "taxonomic disaster zones"

108   — i.e., clades within which phenotypic and genotypic species boundaries are frequently unclear,

109   inconsistent, or conflicting. To this goal, we focus on one of the most challenging Australian

110   vertebrate clades: the *inornatus* group of the lizard genus *Ctenotus* (Scincidae). In this group,

111   identifying species has long been regarded as difficult owing to ambiguous morphological

112   diagnoses based on few or poorly understood characters, which, in turn, renders geographic

113   ranges uncertain (Bush et al., 2007). Species in the *C. inornatus* group are a conspicuous and

114 often dominant component of Australian arid zone lizard assemblages (James & Shine, 2000;

115 Pianka, 1969, 1972, 1986; Rabosky et al., 2007, 2011). The 16 or so species-level taxa

116 commonly recognized in this group have been defined primarily based on dorsal coloration

117 patterns, scalation, and body proportions (Horner & King, 1985; Storr, 1969, 1970, 1971, 1978;

118 Storr et al., 1999). More recently, studies incorporating genetic data have suggested that

119 variation in these traits does not necessarily reflect evolutionary divergence. For instance, a

120 combined analysis of morphological characters, one mitochondrial DNA marker, and one

121 nuclear DNA locus from more than 350 specimens inferred rampant taxon paraphyly, within-

122 species polymorphism, and among-species sharing of characters previously thought to diagnose

123 species (Rabosky et al., 2014). This scenario suggests that several traditionally recognized taxa

124 in the *C. inornatus* species group lack genetic coherence or distinctiveness.

125 In principle, the unexpected lack of genetic support for several taxa in the *C. inornatus*

126 species group might reflect an over-reliance on mitochondrial DNA and limited geographic

127 sampling (Rabosky et al., 2014). Here, we revisit species limits with improved sampling of

128 populations and sequencing of thousands of nuclear loci. Our approach comprises six steps. We

129 start with analyses performed without reference to taxonomic assignments, namely (i) inferring

130 phylogenetic relationships among hundreds of samples, and (ii) characterizing population genetic

131 structure using genotypic clustering. We then (iii) compared the results with a mitochondrial

132 analysis that incorporated additional samples beyond what was used in previous studies. Given

133 evidence for multiple genetic units within particular widespread nominal taxa, we then (iv) tested

134 whether inferred genetic breaks are explained by simple models of isolation-by-distance (Fig. 1).

135 This simple assessment is rarely included in delimitation studies but provides critical information

136 on the extent to which genetic groups interpreted as putative species might emerge from spatial

137 heterogeneity in sampling alone (Battey et al., 2020). Based on evidence of incomplete lineage

138 separation and mitochondrial capture, we (v) test for introgression by determining if patterns of

139 nuclear allele sharing exceed expectations from simple stochastic allele sorting. Finally, we (vi)

140 estimate migration rates between pairs of putative species or geographically defined populations

141 using historical demographic modeling. Based on the results, we discuss the biological

142 underpinnings of fuzzy species boundaries in this and other challenging clades.

143

144 **Material and Methods**

145

146 *Terminology and species criteria*

147   This study uses the terms "taxa" and "taxon" to refer to the nominal entities currently

148 recognized in *Ctenotus* taxonomy. "Traditional" and "traditionally recognized" taxa refer to

149 definitions proposed based on morphological attributes (Horner & King, 1985; Storr, 1969, 1970,

150 1971, 1978). Six traditional taxa were deemed invalid by the combined genetic-morphological

151 analysis of Rabosky et al. (2014). These taxa are indicated between quotes: *C.* "*borealis*", *C.*

152 "*brachyonyx*", *C.* "*fallens*", *C.* "*helenae*", *C.* "*saxatilis*", and *C.* "*severus*".

153   We consider "taxa" as operational classification devices that may or not correspond to

154 "species" in an evolutionary sense. By "species", we specifically refer to a conceptual category

155 corresponding to separately evolving metapopulation lineages (De Queiroz 1998, 2007).

156 Applying this concept to species delimitation practice is often not trivial. Our approach employs

157 operational taxonomic units (OTUs), which we delimit based on attributes widely proposed as

158 *species criteria* (Mayr 1963; Cracraft 1987; De Queiroz 1998; Mallet 2013, 2020; Singhal et al.,

159 2018). These criteria include: 1) conspecific individuals tend to cluster phylogenetically owing to

160   shared derived genetic variants (i.e., branching patterns reflect species cohesiveness and

161   distinctiveness); 2) conspecifics comprise a cohesive genotypic pool, sharing strongly correlated

162   genome-wide allele frequency patterns; 3) conspecifics span a coherent and mostly continuous

163   geographic area, allowing gene flow between populations; and 4) regional differences in allele

164   frequencies across a species' range follow a continuous isolation-by-distance pattern, without

165   sharp breaks that might arise from geographic or reproductive barriers. We consider the OTUs

166   (or "units") delimited based on these criteria as candidate species.

167   We found multiple OTUs within four taxa: *C. inornatus*, *C. robustus*, *C. spaldingi*, and

168   *C. superciliaris*. We refer to these intra-taxon OTUs using labels that refer to their geographic

169   ranges, such as *inornatus*-N, *inornatus*-S, and *superciliaris*-W. Other taxa corresponded to a

170   single OTU each; we refer to them using their corresponding taxon name: *burbidgei*, *eutaenius*,

171   *lateralis*, *mastigura*, and *rimacola*.

172

173   *Motivating case studies of taxonomic discord in the* Ctenotus inornatus *species group*

174   Below, we briefly introduce four taxonomic issues to illustrate the confusion surrounding

175   the *C. inornatus* species group and set up the structure of our analyses. Recently, the Australian

176   Society of Herpetologists (ASH, 2022) recommended broad spatial and genomic sampling to

177   properly address these issues.

178

179   *What is* Ctenotus robustus*?*

180   *Ctenotus robustus* Storr, 1970 and *Ctenotus spaldingi* (Macleay, 1877) are

181   morphologically similar taxa whose definitions and geographic ranges have been transformed by

182   genetic evidence (Rabosky et al., 2014). *Ctenotus robustus* had been thought to encompass a

183   broad arc from the Flinders Ranges (South Australia) to the Pilbara region (Western Australia),

184   while *C. spaldingi* had been restricted to Australia's northeast (Queensland, Northern Territory)

185   (Fig. 2). However, a genetic analysis found nominal populations of *C. robustus* to be nested in a

186   northwestern lineage, while nominal *C. spaldingi* occurs from northern (Cape York) to southern

187   (Victoria) eastern Australia (Rabosky et al., 2014). Consequently, all eastern and southeastern

188   populations of *C. robustus* were assigned to *C. spaldingi*. Adding to confusion, the presumed

189   type locality of *C. robustus* lies outside this taxon's traditional range (Fig. 2). We revisit the

190   genetic limits between *C. robustus* and *C. spaldingi* and reassess recently proposed changes in

191   their geographic distributions.

192

193   *Is* Ctenotus borealis *just an atypical* C. robustus*?*

194   *Ctenotus* "*borealis*" Horner and King, 1985 is a taxon from Australia's Top End that is

195   morphologically similar to *C. robustus* but shows subtle coloration differences (Fig. 2). This

196   taxon was recently synonymized to *C. robustus* based on lack of differentiation in two loci

197   (Rabosky et al., 2014), but this recommendation remains controversial (ASH, 2022). We employ

198   genome-wide data to assess whether these two names correspond to distinct lineages.

199

200   Ctenotus superciliaris *and* C. "saxatilis"*: Same or different?*

201   *Ctenotus* "*saxatilis*" Storr, 1970 is a widespread taxon (Fig. 2) characterized by strongly

202   marked dorsolateral stripes and spots. However, mitochondrial data suggest that this phenotype

203   is shared by multiple divergent lineages in northern Australia (Fig. 3 in Rabosky et al., 2014).

204   One of these lineages is distinguished by its supraciliary scale configuration and was thus

205   described as a new taxon, *Ctenotus superciliaris* Rabosky, Hutchinson, Donnellan, Talaba and

206   Lovette, 2014. However, Storr et al. (1999) claimed that this same character diagnoses *C.*

207   "*saxatilis*", albeit not mentioning it in this taxon's formal description (Storr, 1970). Rabosky et

208   al. (2014) examined the holotype of *C.* "*saxatilis*" and found it to have the scalation pattern

209   typical of *Ctenotus inornatus* (Gray, 1845) rather than that of *C. superciliaris,* thus

210   synonymizing *C.* "*saxatilis*". Increasing confusion, the name *C.* "*saxatilis*" remains widely used

211   (ASH, 2022; Uetz et al., 2022). We employ genome-wide data to test if *C. superciliaris* and *C.*

212   "*saxatilis*" are genetically coherent and distinctive from *C. inornatus*.

213

214   *Arid zone members of the* C. inornatus *complex: One species or five?*

215   Four additional taxa were synonymized with *C. inornatus* (Rabosky et al., 2014):

216   *Ctenotus* "*brachyonyx*" Storr, 1971, a striped taxon from the southeastern deserts; *Ctenotus*

217   "*fallens*" Storr, 1973, with dorsal stripes and lateral spotting, from the western coast; *Ctenotus*

218   "*helenae*" Storr, 1969, a weakly patterned taxon from the central deserts; and *Ctenotus* "*severus*"

219   Storr, 1969, with lateral elements but no vertebral or paravertebral stripes, from the western

220   deserts (Fig. 2). Individuals with the traits presumed to diagnose each of these taxa did not

221   cluster in a mitochondrial tree, suggesting that the group's taxonomy might be confounded by

222   evolutionary lability in coloration (e.g., Fig. 7 in Rabosky et al., 2014). However, these names

223   remain broadly recognized (ASH, 2022; Uetz et al., 2022). We investigate whether each of them

224   comprises a coherent and divergent genetic pool.

225

226   *Genetic sampling*

227   Our nuclear dataset included 13 ingroup taxa under the traditional morphological

228   taxonomy (Storr et al., 1999), corresponding to seven taxa following Rabosky et al. (2014). The

229   original identification of specimens in our molecular dataset (as made by field collectors and

230   museum staff, including ourselves) largely applied that traditional scheme, as follows (numbers

231   in parentheses indicate the number of samples originally assigned to each taxon): *C. "borealis"*

232   (four specimens), *C. "brachyonyx"* (five), *C. eutaenius* (three), *C. "fallens"* (three), *C. "helenae"*

233   (52), *C. inornatus* (28), *C. lateralis* (15), *C. mastigura* (one), *C. rimacola* (two), *C. robustus*

234   (55), *C. "saxatilis"* (31), *C. "severus"* (three), and *C. spaldingi* (16). As outgroups, we included

235   representatives of other *Ctenotus* species groups and the closely related genus *Lerista*: *C. atlas*

236   (two), *C. australis* (four), *C. essingtonii* (two), *C. leonhardii* (two), *C. nigrilineatus* (two), *C.*

237   *pantherinus* (two), *C. schomburgkii* (two)*, C. taeniolatus* (two), *L. bipes* (two), and *L. ips* (two)

238   (Table S1 provides nuclear DNA sample information).

239   Our analyses incorporated a double-digest restriction site-associated (ddRAD) dataset

240   generated by comprehensive analyses of sphenomorphine skinks (Prates, Singhal, et al., 2022;

241   Singhal et al., 2017) and available in the Sequence Read Archive (BioProjects PRJNA755251

242   and PRJNA382545). Briefly, DNA was digested with the restriction enzymes EcoRI and MspI,

243   tagged with individual barcodes, size-selected (150–250 bp), PCR-amplified, multiplexed, and

244   sequenced on an Illumina platform. We used *ipyrad* v. 0.9.84 (Eaton & Overcast, 2020) to

245   demultiplex reads (allowing no mismatches from individual barcodes), perform *de novo*

246   assembly (minimum clustering similarity = 0.90), align loci, and call single nucleotide

247   polymorphisms (SNPs). We enforced a minimum Phred quality score (= 33), sequence coverage

248   (= 6x), and read length (= 35 bp); and a maximum proportion of heterozygous sites per locus (=

249   0.5) and number of alleles per nucleotide site within an individual (= 2, i.e., a diploid genome).

250   After these steps, we generated a final dataset for phylogenetic inference by retaining loci

251   present in at least 30% of the sampled individuals. The final phylogenetic dataset included

252 524,324 base pairs from 3,694 ddRAD loci. Moreover, to reduce missing data in population

253 genetic analyses, we generated three datasets corresponding to each of three major inferred

254 clades ("species complexes"; see Results), retaining loci present in at least 50% of the sampled

255 individuals. The final population genetic datasets included 6,287 (*spaldingi* complex), 7,447

256 (*superciliaris* complex), and 3,534 (*inornatus* complex) unlinked SNPs.

257   This nuclear phylogenetic dataset included a total of 242 specimens. To further expand

258 our sampling, we also incorporated a mitochondrial dataset comprising 485 samples (see Table

259 S2 for sample information; see Fig. S1 and Fig. S2 for the geographic localities of samples

260 partitioned by delimited OTU and proposed taxon respectively). Of those, 348 samples were

261 sequenced by previous efforts (Rabosky et al., 2011, 2014). Besides broader geographic

262 sampling for each taxon, this dataset included *Ctenotus burbidgei* Storr 1975, for which nuclear

263 data were not available. We amplified, sequenced, edited, and aligned a 1,143 base pair fragment

264 of the cytochrome B gene following standard protocols (Rabosky et al., 2009). Newly generated

265 mitochondrial sequences were uploaded to GenBank (accessions numbers OQ091785–

266 OQ091921; Table S2).

267

268 *Inferring phylogenetic relationships*

269   We inferred evolutionary relationships based on the nuclear and mitochondrial datasets

270 separately. Phylogenetic inference incorporated both variant and invariant sites under maximum

271 likelihood using RAxML-HPC v. 8.2.12 (Stamatakis, 2014) and employing the GTRCAT model

272 of nucleotide evolution. For this analysis, loci were concatenated, and polymorphic sites were

273 coded as ambiguities. We also inferred a nuclear SNP-based tree under the multispecies

274 coalescent framework using SVD Quartets (Chifman & Kubatko, 2014) as implemented in the

275   command line version of PAUP v. 4 (Swofford, 2002). This analysis incorporated one SNP from

276   each locus for a total of 3,692 SNPs and sampled all possible quartets. In both phylogenetic

277   analyses, we estimated node support based on 1,000 bootstraps.

278

279   *Inferring genetic structure*

280   We estimated patterns of nuclear admixture and allele sharing using sNMF, a genotypic

281   clustering method that does not assume Hardy-Weinberg equilibrium to identify clusters (Frichot

282   et al., 2014). To avoid the spurious grouping of densely sampled localities (Lawson et al., 2018;

283   Puechmaille, 2016), we limited the maximum number of samples per collecting site to five. In

284   these analyses, we dropped four highly divergent lineages; although we consider these lineages

285   as OTUs (see Results), they are represented by < 4 samples, and small sample sizes can lead to

286   spurious grouping of samples in genotypic clustering analyses (Puechmaille, 2016; Lawson et

287   al., 2018). We removed SNPs with a minimum allele frequency < 0.05 (within each of the three

288   complexes) to improve the inference of population structure (Linck & Battey, 2019) and

289   minimize spurious SNPs from sequencing errors (Ahrens et al., 2018) using VCFtools v. 0.1.16

290   (Danecek et al., 2011). After extracting a single SNP per locus, individuals that had data for less

291   than 50% of the final SNPs were excluded. Outgroups were not included in these analyses. We

292   ran sNMF using the *LEA* R package (Frichot & François, 2015). Preliminary analyses supported

293   that the number of inferred clusters is robust to the regularization parameter in sNMF; this

294   parameter was set to 500 in the final analyses. The tolerance parameter was set to the default

295   value (0.00001). To infer the best-fitting number of genotypic clusters (K), we compared K = 1–

296   10 with 20 replicates for each K. The K value that yielded the lowest cross-entropy value across

297   replicates was considered to be the best-fit K.

298   We then confirmed whether samples inferred in the same genotypic cluster also group in

299   genotypic space. For that purpose, we performed a Principal Component Analysis on the

300   unlinked SNP data using the *LEA* R package and inspected biplots of principal components.

301

302   *Testing the robustness of results to isolation-by-distance*

303   Phylogenetic and population genetic structure are widely employed as indicative of

304   species divergence and boundaries. However, genetic breaks can emerge over the range of a

305   continuously distributed species due to isolation-by-distance (IBD), despite high gene flow

306   connecting adjacent locations (Irwin, 2002; Wright, 1943). Thus, we investigated whether IBD

307   alone can account for the population structure emerging from our genetic analyses.

308   Methods are available that can account for IBD while performing genotypic clustering

309   (Bradburd et al., 2018), but these approaches were not computationally tractable for our large

310   dataset. Thus, we assessed IBD patterns based on pairwise F_{ST} (Weir & Cockerham, 1984; Weir

311   & Hill, 2002), applying the following reasoning. Isolation-by-distance is the relationship

312   described by genetic (here, F_{ST}) versus geographic distances (Irwin, 2002; Wright, 1943) (Fig.

313   1a). Under simplifying assumptions (e.g., equilibrium demography, landscape homogeneity), we

314   expect the IBD relationship between populations of the same species to follow a simple linear

315   relationship (Fig. 1b), whose slope is a function of population density and effective gene

316   dispersal (Rousset, 1997). In this case, geographic separation alone can explain genetic

317   differentiation, reflecting the continuous decay of population connectivity over a species' range.

318   By contrast, when a single analysis includes populations from distinct species, we may expect

319   the IBD relationship within and between populations to be discontinuous (i.e., to be described by

320   multiple curves) (Fig. 1c). In some cases, populations could be highly differentiated with

321 minimal effects of geographic separation on levels of genetic differentiation (Fig. 1d). This

322 situation points to mechanisms that restrict gene flow in parapatry, such as reproductive

323 isolation, and, as such, supports delimitation of species. To determine which patterns best match

324 our taxa, we estimated pairwise individual F_{ST} based on the SNP data using the *BEDASSLE* R

325 package (Bradburd et al., 2013). To calculate a matrix of geographic distances, we used the R

326 package *fossil* (Vavrek, 2011).

327

328 *Modeling demographic history*

329   To characterize gene flow among populations, we performed historical demographic

330 inference using G-PhoCS v. 1.3 (Gronau et al., 2011), implementing an isolation-with-migration

331 model (Nielsen & Wakeley, 2001; Pinho & Hey, 2010). We estimated gene flow between three

332 classes of population pairs (see Fig. S3 for their geographic locations):

333   1) Pairs of populations corresponding to the OTUs resulting from the phylogenetic and

334 genotypic clustering analyses. This set often included pairs of populations that are sympatric and

335 divergent, thus providing estimates of gene flow across candidate species.

336   2) Pairs including populations corresponding to morphology-defined taxa that were

337 recently synonymized based on multi-locus data (Rabosky et al., 2014). This set aims to assess

338 the degree of separation of populations assigned to *C. "borealis"*, *C. "brachyonyx"*, *C. "fallens"*,

339 and *C. "severus"* relative to closely related samples from adjacent geographic regions but

340 assigned to another taxon. We defined four such adjacent populations: Pilbara, central deserts,

341 western shrublands, and northern Australia.

342   3) Pairs corresponding to a single population randomly split into two. This set aims to

343   provide a reference of panmixia by artificially separating samples from the same lineage and

344   geographic region.

345   G-PhoCS imposes prior distributions (given by shape, α, and rate, β) on three classes of

346   population genetic parameters: θ (= 4Nμ, where N corresponds to the effective population size

347   and μ to the per-generation mutation rate); the splitting time parameter τ (= Tμ, where T

348   corresponds to the tree height in number of generations); and m (= M/μ, where M corresponds to

349   the proportion of individuals in one population that originated from another population in each

350   generation (note that these migration parameter definitions follow the developers of G-PhoCS;

351   see supplementary material in Gronau et al., 2011). For θ and τ, we used gamma (shape = 1, rate

352   = 100) prior densities. For m, we used a migration band in each direction under a gamma (shape

353   = 0.001, rate = 0.00001) prior. Prior distributions were chosen to encompass a wide range of

354   biologically plausible population histories (i.e., effective population sizes ranging from tens of

355   thousands to millions, migration levels ranging from zero to high enough to constrain population

356   divergence; an R script used to help define prior distributions is provided in GitHub). We then

357   simulated posterior parameter distributions using Markov chain Monte Carlo (MCMC). We ran

358   two MCMC simulations per pair, each consisting of 300,000 steps, sampling every 100 steps,

359   and discarding the first 25% of the steps as burn-in. Chain stationarity and convergence were

360   confirmed by plotting parameter traces in R. Fine-tuning parameters controlling MCMC

361   acceptance rates were defined automatically. Owing to computational times, G-PhoCS analyses

362   used a maximum of 12 samples per population and of 4,500 loci (which included both invariant

363   and variant sites).

364   To convert mutation rate-scaled estimates of population genetic parameters into absolute

365   estimates, we assumed 7.6×10^{-9} substitutions per site per year in lizards (Gottscho et al., 2017)

366   and a generation time of two years based on an age at maturity of 22 months in *C. "helenae"*

367   (James, 1991a, 1991b). Based on posterior parameter estimates, we calculated the effective

368   number of gene migrations received by a population per generation, 2NM, also known as the

369   population migration rate. Theory predicts that, when 2NM is greater than 1, divergence between

370   constituent subpopulations will be constrained (Nielsen & Slatkin, 2013; Pinho & Hey, 2010;

371   Wright, 1931). Given caveats pertaining to 2NM estimates (Whitlock & McCauley, 1999), we

372   interpret the results carefully within the broader scenario of introgression emerging from the

373   phylogenetic, genotypic clustering, IBD, and ABBA-BABA analyses.

374

375   *Estimating excess allele sharing from introgression*

376   Finally, to further test nuclear introgression among candidate species, we estimated two

377   ABBA-BABA-class statistics (Green et al., 2010): Patterson's D (Patterson et al., 2012) and f-

378   branch (Malinsky et al., 2018). These approaches employ a four-population tree with structure

379   (((P2, P1), P3), O), where O is an outgroup. Typically, many ancestral (A) and derived (B)

380   alleles show a BBAA structure (with allele ordering following the tree given above). However,

381   incomplete lineage sorting leads to ABBA and BABA patterns, which should occur in equal

382   frequencies. Introgression between P3 and P1 or P2 leads to an excess of ABBA or BABA

383   patterns, which is captured by D and related statistics. One limitation of this approach is that

384   multiple closely related populations can appear introgressed owing to a single introgression event

385   involving a common ancestor. To minimize this issue, the f-branch metric accounts for

386   correlated allele frequencies among populations. This approach allows identifying gene flow

387 events involving the internal branches of a tree, which correspond to the ancestors of sampled

388 populations (Malinsky et al., 2018). We estimated D and f-branch metrics based on the unlinked

389 SNP data (i.e., one SNP per locus) in Dsuite (Malinsky et al., 2021), using the Benjamini-

390 Hochberg correction to control for false-discovery rates (Benjamini & Hochberg, 1995) and

391 estimating significance using a block-jackknifing approach (Durand et al., 2011; Green et al.,

392 2010). We considered all combinations of candidate species given constraints from the estimated

393 coalescent-based phylogenetic tree and used *C. essingtonii* as an outgroup.

394

395 **Results**

396

397 *Overall phylogenetic patterns*

398   Phylogenetic trees of the *C. inornatus* species group based on nuclear DNA under

399 maximum likelihood on the concatenated loci (Fig. 3) or using a coalescent-based approach on

400 unlinked SNPs (Fig. S4) identified three major clades, which we informally refer to as the

401 *inornatus*, *robustus*, and *superciliaris* complexes (Fig. 3). We comment on patterns of genetic

402 structure and admixture within each of these complexes separately.

403

404 *Lineage delimitation in the* robustus *complex*

405   The *robustus* complex contains six deeply divergent and well-supported lineages that we

406 consider operational taxonomic units (OTUs) (Fig. 3). Three of these lineages were well-sampled

407 and included in genotypic clustering analyses; each corresponded to a cluster (Fig. 4). One such

408 cluster spans a large latitudinal range in eastern Australia (northern Queensland to Victoria); we

409 refer to it as the OTU *spaldingi*-S (dark orange in Fig. 4). Another OTU, *spaldingi*-NE (light

410   orange in Fig. 4), occurs in northern and northeastern Australia (Northern Territory,

411   Queensland). A third OTU, *robustus*-NW (maroon in Fig. 4), occurs along northern and

412   northwestern Australia reaching the Pilbara region. PCA analyses on the unlinked SNP data

413   confirmed that samples inferred in the same cluster grouped together in genotypic space.

414   Moreover, each inferred cluster occupied a distinct portion of genotypic space (Fig. S5).

415   To avoid spurious grouping of sparsely sampled but highly divergent lineages, we

416   removed three highly divergent lineages from our clustering analysis. However, our phylogenetic

417   analyses of both mtDNA and nuclear SNPs support these lineages as distinct OTUs (Fig. 4 and

418   5). Among them is a lineage composed of northern Australian specimens morphologically

419   assigned to *Ctenotus rimacola* Horner and Fisher, 1998. Another such lineage is the sister of

420   *robustus*-NW, which we refer to as *robustus*-TE. This OTU is sympatric with *robustus*-NW in

421   the Top End region and thus likely represents a separate species. OTUs *robustus*-NW and

422   *robustus*-TE composed coherent but non-sister mitochondrial lineages (Fig. 5; Fig. S6). Another

423   highly divergent lineage occurs in the Cape York Peninsula; we refer to it as *spaldingi*-CY.

424   Mitochondrial results further support that this OTU is highly divergent. We note that the relative

425   position of *spaldingi*-CY was the only difference between the concatenated and coalescent-based

426   nuclear trees; this OTU was inferred as sister to *spaldingi*-S under concatenation, but as sister to

427   *spaldingi*-NE under a coalescence-based framework (Fig. S4).

428   To assess whether evidence of distinct genetic pools across regions might simply reflect

429   isolation-by-distance (IBD), we estimated genetic distances across space within and between the

430   delimited OTUs. The results further support that OTUs in the *robustus* complex correspond to

431   separate species. For instance, the relationship between genetic and geographic distances (i.e.,

432   the IBD pattern) within and between *robustus*-NW and *robustus*-TE, which are sympatric in

433   northern Australia, was largely discontinuous (Fig. 6). This pattern of genetic differentiation

434   decoupled from levels of geographic separation points to mechanisms limiting gene flow across

435   distinct species (Fig. 1). A similar pattern was observed between *spaldingi*-CY, *spaldingi*-NE,

436   and *spaldingi*-S, which, based on currently sampling, appear parapatric in northeastern Australia

437   (Fig. 6). These results further support that these OTUs correspond to separate species in an

438   evolutionary sense, even though we conservatively treat them as intra-taxon units (Fig. 3).

439   Consistent with the IBD patterns, historical demographic modeling suggests low gene

440   flow across candidate species in the *robustus* complex (Fig. 7). Population migration rates

441   (2NM) approached zero in both directions between all OTUs in this complex (median value

442   range = 0.01–0.07), including sympatric pairs. This was the case, for instance, of the pairs

443   comprising *robustus*-NW, *robustus*-TE, and *spaldingi*-NE, all of which co-occur in the Top End

444   (Table S3).

445

446   *Support for traditional taxa in the* robustus *complex*

447   We also assessed whether traditionally recognized taxa are consistent with the genetic

448   patterns. Fig. 4 (left) shows the morphology-based assignment of individuals to taxa relative to

449   the inferred tree. The results support that the traditional (i.e., morphology-based) taxa are broadly

450   paraphyletic. For instance, specimens assigned to *C. robustus* (white circles in Fig. 4) were found

451   nested in two non-sister lineages, corresponding to *robustus*-NW and *spaldingi*-S. Both of these

452   OTUs occur far from the presumed type locality of *C. robustus* (Fig. 2), previously associated

453   with the northern lineage (Rabosky et al., 2014). Similarly, samples morphologically assigned to

454   *C. spaldingi* (black circles in Fig. 4) formed two non-sister lineages, corresponding to *spaldingi*-

455   CY and *spaldingi*-N. Likewise, samples identified as *C.* "*borealis*" (dark gray in Fig. 4) were

456 nested among geographically adjacent samples identified as *C. robustus*, both corresponding to

457 *robustus*-NW. Historical demographic analyses inferred high gene flow between populations

458 assigned to *C. "borealis"* and *C. robustus* (Fig. 7), with relatively high 2NM between them in

459 both directions (0.46 and 0.82).

460   Pending analyses of morphological variation and additional geographic sampling to

461 formally describe new taxa based on the inferred OTUs, we provisionally assign the OTUs to

462 existing taxon names (Fig. 3). In doing so, we aim to provide labels that reflect evolutionary

463 relationships while minimizing change relative to the most recent assessment (Rabosky et al.,

464 2014). We refer the populations corresponding to *spaldingi*-CY, *spaldingi*-S, and *spaldingi*-N to

465 the taxon *C. spaldingi*. OTUs *robustus*-NW and *robustus*-TE are referred to *C. robustus*. This

466 definition of *C. robustus* excludes populations from eastern and southern Australia traditionally

467 assigned to this name (Fig. 2) but found to correspond to the *C. spaldingi* lineage (Fig. 4),

468 corroborating the findings of Rabosky et al. (2014).

469

470 *Lineage delimitation in the* superciliaris *complex*

471   The *superciliaris* complex contains three well-supported lineages that we treat as OTUs

472 (Fig. 3); each corresponds to a genotypic cluster (Fig. 8). One such OTU spans Australia's

473 northwestern coast into the northern interior (Western Australia, Northern Territory); we refer to

474 it as *superciliaris*-W (green in Fig. 8). This OTU is sister to *superciliaris*-E, which occurs from

475 the Top End into the central deserts (Northern Territory) (cyan in Fig. 8). The third OTU occurs

476 in the Kimberley, a western Australian region scarcely represented in previous analyses (dark

477 blue in Fig. 8). This OTU appears to correspond to the taxon *Ctenotus mastigura* Storr 1975. Our

478 mitochondrial analysis inferred this putative *C. mastigura* to be the sister of *C. burbidgei* (Fig. 5;

479   Fig. S6), a taxon also from the Kimberley not represented in the nuclear dataset, in agreement

480   with their presumed close relationships based on morphology (Storr, 1975).

481   Similar to the *robustus* complex, IBD analyses suggest that delimited OTUs in the

482   *superciliaris* complex correspond to separate species. The relationship between genetic and

483   geographic distances within and between OTUs was largely discontinuous, with genetic

484   differentiation largely independent from geographic separation (Fig. 6). IBD patterns also

485   suggest that *superciliaris*-E might correspond to two separate units, one restricted to the Top End

486   and another further south (see Fig. S7). Consistent with this scenario, the mitochondrial analysis

487   inferred samples corresponding to each of these two regions as nested in divergent lineages (Fig.

488   5; Fig. S6). Population migration rate estimates (2NM) suggest relatively low gene flow among

489   OTUs in the *superciliaris* complex (0.08–0.21), consistent with multiple separate species.

490

491   *Support for traditional taxa in the* superciliaris *complex*

492   As in the *robustus* complex, we find paraphyly of traditionally recognized taxa in the

493   *superciliaris* complex (Fig. 8). The original morphological identification of specimens mostly

494   corresponded to *C. inornatus* and *C. "saxatilis"*. These two taxa were inferred as broadly

495   paraphyletic, with samples scattered throughout the *superciliaris* complex and also the *inornatus*

496   complex (Fig. 9). Previous morphological examinations established that the name-bearing types

497   of *C. inornatus* and *C. "saxatilis"* are *inornatus* complex specimens (Rabosky et al., 2014).

498   Pending morphological assessments to support the description of new taxa from the OTUs, we

499   refer the populations corresponding to *superciliaris*-W and *superciliaris*-E to the taxon *C.*

500   *superciliaris*.

501

502   *Lineage delimitation in the* inornatus *complex*

503   The *inornatus* complex contains four well-supported lineages, here treated as OTUs (Fig.

504   3). Three of them were well-sampled and included in the genotypic clustering analyses; each

505   corresponded to a cluster (Fig. 9). One such OTU occurs in Australia's northeast (Queensland),

506   corresponding to the taxon *Ctenotus lateralis* Storr, 1978 (red in Fig. 9). This lineage is sister to

507   a highly divergent lineage from eastern Queensland that appears to *Ctenotus eutaenius* Storr,

508   1981. A third lineage occurs in the Top End and Kimberley regions (Western Australia, Northern

509   Territory); we refer to it as *inornatus*-N (pink in Fig. 9). This OTU is sister to a lineage spanning

510   most of Australia's arid zone, which we refer to as *inornatus*-S (purple in Fig. 9).

511   Contrasting with patterns in the *robustus* and *superciliaris* complexes, IBD relationships

512   within and between groups were highly overlapping and nearly continuous across certain OTUs

513   in the *inornatus* complex (Fig. 6). Geographic separation alone appears to account for most of

514   the genetic differentiation between *inornatus*-N and *inornatus*-S, consistent with a gradient of

515   population connectivity over the range of a single species (Fig. 1). In agreement with this

516   finding, we estimated substantial gene flow between *inornatus*-N and *inornatus*-S, with the

517   highest population migration rates (2NM) relative to all other OTU pairs (median values in each

518   direction = 0.51 and 0.58). Gene flow between *inornatus*-N and *inornatus*-S was higher than that

519   between other parapatric OTUs in the *inornatus* complex, such as the pairs *C. eutaenius* and *C.*

520   *lateralis* or *inornatus*-S and *C. lateralis* (Fig. 7). These results support that *inornatus*-N and

521   *inornatus*-S correspond to the same evolutionary species, albeit one with detectable population

522   structure.

523

524   *Support for traditional taxa in the* inornatus *complex*

525   As in the *robustus* and *superciliaris* complexes, traditionally recognized taxa in the

526   *inornatus* complex showed widespread paraphyly. This complex includes many samples

527   morphologically assigned to *C.* "*saxatilis*" and *C. inornatus*, two taxa scattered throughout this

528   (Fig. 9) and the other (Fig. 4, 8) complexes. We also inferred polyphyly among several taxa

529   corresponding to regional coloration phenotypes. Namely, *C.* "*helenae*" was interspersed among

530   samples of *C. inornatus*, *C.* "*fallens*", and *C.* "*severus*". Among them, *C.* "*fallens*" and *C.*

531   "*severus*" grouped in their own lineages, yet nested among *C.* "*helenae*" and *C.* "*saxatilis*". In

532   turn, *C.* "*brachyonyx*" formed a paraphyletic assemblage with *C.* "*saxatilis*". We note that

533   genotypic clustering grouped *C.* "*brachyonyx*", *C.* "*helenae*", *C. inornatus*, *C.* "*saxatilis*", and *C.*

534   "*severus*" into a single cluster, corresponding to OTU *inornatus*-S (Fig. 9). This finding suggests

535   extensive allele sharing across the arid zone despite regional coloration variation, broadly

536   agreeing with findings based on few loci (Rabosky et al., 2014).

537   To further assess the coherence and distinction of those recently disputed taxa, we

538   estimated population migration rates between them and samples from adjacent geographic

539   regions. We considered four such regions: western shrublands, Pilbara, central deserts (all

540   corresponding to *inornatus*-S), and a northern population (corresponding to *inornatus*-N).

541   Samples from these regions appear to correspond to the same species (see above), providing a

542   reference of intraspecific gene flow levels. This exercise revealed relatively high gene flow

543   between *C.* "*severus*" and samples from the adjacent western shrublands and Pilbara (Fig. 7); in

544   both cases, estimates were asymmetrical, with higher numbers of migrant genes out of *C.*

545   "*severus*" (0.34–1.23) than into it (0–0.03). These estimates were comparable to those among

546   localities presumed to correspond to the same species, such as the pair including the western

547   shrublands and Pilbara samples (0.23 and 0.93) or that including the northern and Pilbara

548   samples (0.15 and 0.22) (Fig. 7; Table S3).

549   By contrast, numbers of gene migrations were relatively lower between other taxa and

550   their adjacent populations. This was the case of *C. "brachyonyx"* relative to both the central

551   deserts and western shrublands. Gene flow was also asymmetrical in this case, higher into *C.*

552   *"brachyonyx"* (0.15–0.21) than out of it (0–0.01). Similarly, we inferred higher gene migrations

553   into *C. "fallens"* (0.10–0.16) than out of it with both the Pilbara and western shrublands samples

554   (0.08–0.11).

555   Random partitions of single populations yielded 2NM higher than 1 (1.57–2.89) (Table

556   S3), consistent with theoretical expectations of populations whose divergence is constrained by

557   high gene flow (Nielsen & Slatkin, 2013; Pinho & Hey, 2010; Wright, 1931).

558

559   *Evidence of introgression across distantly related lineages*

560   Our phylogenetic and genetic clustering results revealed evidence of admixture between

561   species from distinct complexes. For instance, we found highly similar or shared mitochondrial

562   haplotypes between distantly related nuclear OTUs (Fig. 5; Fig. S6), which might indicate allele

563   capture across species boundaries. An extreme case was that of a mitochondrial lineage grouping

564   samples from four nuclear OTUs representing two complexes: *inornatus*-N, *inornatus*-S,

565   *superciliaris*-E, and *superciliaris*-W (uppermost lineage in Fig. 5). Notably, these four OTUs co-

566   occur in northern Australia.

567   Gene flow estimates based on the nuclear loci further supported that certain distantly

568   related OTUs are mutually introgressed (Fig. 7). For instance, we inferred some gene flow

569   between *inornatus*-N (*inornatus* complex) and putative *C. mastigura* (*superciliaris* complex)

570 (0.13 and 0.16 in each direction) (Table S3). These analyses also inferred some gene flow

571 between *inornatus*-N and *superciliaris*-W (0.09 and 0.13), as well as between *inornatus*-S and

572 *superciliaris*-W (0.06 and 0.11).

573   In agreement with these results, ABBA-BABA allele patterns support substantial nuclear

574 introgression between OTUs from distinct complexes (Fig. 10). The proportion of loci inferred to

575 have experienced introgression across delimited species were often high, up to 15–25%. This

576 was the case for *inornatus*-N and each of *superciliaris*-E, *superciliaris*-W, and *C. mastigura*.

577 Estimates of Patterson's D, a metric related to f-branch, were broadly consistent with these

578 results (Fig. S8). ABBA-BABA patterns also suggest that admixture events might have involved

579 the ancestors of living species, as revealed by f-branch estimates corresponding to internal

580 branches of the tree (Fig. 10). This was the case of the branch connecting *inornatus*-N and

581 *inornatus*-S, found to have introgressed with each of the three *superciliaris* complex OTUs.

582 Likewise, the presumed ancestor of *superciliaris*-E and *superciliaris*-W was inferred to have

583 introgressed with both *inornatus*-S and *C. lateralis*. These introgressed OTUs often showed high

584 levels of cytonuclear discordance (Fig. 5; Fig. S6).

585

586 **Discussion**

587   This study inferred population structure and history based on broad geographic and

588 genetic sampling of lizards long considered to be challenging taxonomically. Beyond attempting

589 to delimit species, we sought to identify the evolutionary processes that might underlie

590 ambiguous genotypic and phenotypic species boundaries. Our results revealed a perfect storm of

591 rampant taxon paraphyly, both morphologically cryptic and polytypic lineages, and a wide range

592 of gene flow levels between candidate species. In addition, we found evidence of mitochondrial

593   capture and heterogeneous patterns of nuclear introgression across divergent OTUs. Mutually

594   introgressed OTUs have partially overlapping ranges, particularly in northern Australia. These

595   findings suggest that taxonomic uncertainty may result from spatial variation in the porosity of

596   species boundaries and the resulting geographic patterns of genetic and phenotypic variation.

597   Below, we discuss the implications of our findings to inferences of speciation and the

598   systematics of challenging clades.

599

600   *Consequences of widespread admixture for inference of species boundaries*

601   This study demonstrates that extensive sampling might be necessary to uncover instances

602   of geographically widespread genetic admixture (Oliver et al., 2020; Singhal et al., 2018), which

603   might otherwise lead to overestimation of population separation. In particular, IBD analyses

604   support genetic intergradation across localities corresponding to *inornatus*-N and *inornatus*-S

605   (Fig. 6). This pattern suggests that these two OTUs comprise a genetic and geographic

606   continuum, consistent with substantial gene flow between them (Fig. 7). Still, these two OTUs

607   formed coherent and distinctive lineages and genotypic clusters (Fig. 9), thereby conforming to

608   species criteria that have been widely applied (Cracraft, 1987; de Queiroz, 1998; Mallet, 2013,

609   2020). This scenario supports that certain approaches can infer population separation, often

610   interpreted as evidence of multiple species, in the presence of spatially extensive gene flow, in

611   line with theoretical and empirical investigations (Barley et al., 2018; Battey et al., 2020;

612   Bradburd et al., 2018; Irwin, 2002). Such continuous genetic differentiation frequently coincides

613   with clinal phenotypic differentiation, further contributing to blurred species limits (e.g.,

614   Ahossou et al., 2020; Chambers & Hillis, 2020; Dickens et al., 2021; Pereira & Wake, 2009). In

615   the case of *inornatus*-N and *inornatus*-S, denser sampling along putative contact zones could

616 lead to increased resolution of genotypic transitions and admixture. More broadly, our findings

617 illustrate how characterizing the geographic extent of such transitions is crucial to avoid

618 imposing discrete taxonomic structures on continuous patterns of variation (Braby et al., 2012;

619 Chambers & Hillis, 2020; Mayr, 1963; Prates, Doughty, et al., 2022; Wilson & Brown, 1953).

620

621 *Phenotypic conservatism conceals evolutionary separation*

622 This study supports the idea that poor taxonomic resolution can also result from species

623 formation with little to no morphological change (Bickford et al., 2007; Camp & Wooten, 2016;

624 Fišer et al., 2018). Unlike the *inornatus* complex, IBD does not explain the differentiation of

625 OTUs in the *robustus* and *superciliaris* complexes. In these cases, genetic distances are primarily

626 decoupled from geographic distances (Fig. 6), matching expectations of evolutionary separation

627 (Fig. 1d). Accordingly, we inferred low gene flow between OTUs in both complexes (Fig. 7).

628 This scenario suggests that multiple morphologically cryptic species occur within single nominal

629 taxa, as observed here within each of *C. robustus*, *C. spaldingi*, and *C. superciliaris*. One of

630 these candidate species, *robustus*-TE, is sympatric with its sister, *robustus*-NW, passing what is

631 typically considered the strongest test for evolutionary separation: the maintenance of distinct

632 gene pools in sympatry (Mayr, 1963). Speciation without morphological change can result from

633 stabilizing selection, developmental constraints, or neutral divergence between populations in

634 similar, potentially constant environments (Zamudio et al., 2016). Moreover, diverging traits

635 across species can be cryptic to humans, as is the case of chemical signals or ecophysiological

636 tolerances (Zozaya et al., 2019; Cadena & Zapata, 2021). Future morphological examinations

637 informed by the genetic patterns might help identify characters that better reflect evolutionary

638 divergence (e.g., Prates, Hutchinson, et al., 2022; Teixeira et al., 2016).

639

Genetic introgression contributes to fuzzy species boundaries

Beyond IBD and cryptic divergence, we find that several unclear species boundaries in this lizard clade may result from genetic introgression. Our finding of conflicting genealogies between mitochondrial and nuclear DNA might originate from stochastic allele sorting among lineages (Singhal and Moritz 2012; Firneno et al. 2020). However, it seems unlikely that coherent nuclear units would retain unsorted mitochondrial alleles given the shorter coalescent times of mitochondrial relative to nuclear DNA (Palumbi et al. 2001). Alternatively, mitonuclear discordances can arise from mitochondrial capture through hybridization, as reported in many organisms (e.g., Currat et al., 2008; Good et al., 2008; Irwin et al., 2009). This process might explain the mitochondrial DNA paraphyly of several coherent nuclear DNA-based OTUs, such as *inornatus*-N, *inornatus*-S, *superciliaris*-E, and *superciliaris*-W. This propensity of mitochondrial genomes to introgress across species boundaries can limit their utility in species delimitation (Funk & Omland, 2003). To corroborate mitochondrial evidence of evolutionary separation (or lack thereof), investigators will continue to benefit from integrative analyses of morphological characters, behavioral traits, and multi-locus datasets (Cadena & Zapata, 2021; Padial et al., 2010; Schlick-Steiner et al., 2010).

In agreement with the mitochondrial patterns, analyses of nuclear loci support that excess allele sharing among species in the *C. inornatus* species group results from introgression. Remarkably, introgression levels appear largely decoupled from phylogenetic relatedness. ABBA-BABA analyses found that some of the highest levels of allele sharing occur among OTUs from distinct complexes (Fig. 10). Likewise, historical demographic modeling inferred higher gene flow between distantly related sympatric OTUs than between certain closely related

662   OTUs (Fig. 7). This decoupling between introgression levels and relatedness differs from

663   patterns reported in other clades (Barley et al., 2022; Hamlin et al., 2020; Peñalba et al., 2019;

664   Roux et al., 2016; Singhal & Bi, 2017; Winger, 2017). The proportions of loci inferred as

665   admixed in the *C. inornatus* group are similar to those of organisms thought to hybridize

666   extensively, such as the African cichlids and true toads (Malinsky et al., 2018; Rivera et al.,

667   2021; but see Dagilis et al., 2022). As in *Ctenotus* lizards, species delimitation in these groups is

668   challenging. Hybridization has long been attributed to blurred genotypic and phenotypic

669   boundaries in plants (Lotsy, 1925; McVay et al., 2017; Novaković et al., 2022; Robinson et al.,

670   2001; Shaw & Small, 2004), but our study contributes to a growing body of evidence that

671   hybridization is also a source of taxonomic uncertainty in animal clades (Gill, 2014; Pyron et al.,

672   2020).

673   Introgressive hybridization increases the variance in genealogical topologies and

674   coalescent times across genome regions, confounding molecular species delimitation. On one

675   hand, gene tree paraphyly owing to horizontal transfer can lead to spurious lumping of divergent

676   lineages (Gill, 2014). On the other hand, emergent allele combinations in admixed populations

677   can lead to spurious inference of separate lineages and genotypic clusters (Chan et al., 2020,

678   2021). Additionally, spatial phenotypic mosaics resulting from secondary contact can be

679   interpreted as polytypism within a single species (O'Connell et al., 2021). In the *C. inornatus*

680   species group, introgression might have contributed to phenotypic parallelisms that obscure

681   species boundaries. Specimens corresponding to *inornatus*-S and *superciliaris*-E (S) appear to

682   share the well-marked "*saxatilis*" coloration pattern in the central deserts; *inornatus*-N and

683   *superciliaris*-E (N) share the subdued "*inornatus*" pattern in the Top End; and *superciliaris*-W

684   shares both of these phenotypes with *inornatus* complex lineages along the northwestern coast

685   (Rabosky et al., 2014). Trait sharing among species might result from introgression in the genes

686   that underlie such traits (Smith & Kronforst, 2013; Svardal et al., 2020). However, this pattern

687   can also result from retained ancestral polymorphisms or parallel or convergent evolution (Mims

688   et al., 2010; Muir & Schlötterer, 2005; Zamudio et al., 2016; but see Edelman et al., 2019).

689   While the mechanisms underlying phenotypic parallelisms in *Ctenotus* are unknown, our results

690   confirm that certain characters broadly used to define scincid lizard taxa, such as coloration, do

691   not always reflect evolutionary divergence (e.g., Prates, Doughty, et al., 2022; Rabosky et al.,

692   2014; Rivera et al., 2020).

693   Lastly, our findings raise questions about the maintenance of species limits in this group.

694   For instance, migration rates between some combinations of sympatric species appear to be

695   higher than those inferred between parapatric species (Fig. 7). This is the case, for instance, of

696   the sympatric pair *inornatus*-N and putative *C. mastigura*, whose gene flow estimates, albeit low,

697   were higher than those between the closely related and parapatric *C. mastigura*, *superciliaris*-E

698   (N), and superciliaris-E (S). How do species remain cohesive in the face of opportunities for

699   hybridization? One possibility is that introgression from another species is restricted to a small

700   subset of a species' range or only part of their divergence history. Alternatively, specific barrier

701   loci might remain differentiated despite broad admixture across the genome (Baird, 1995;

702   Barton, 1983; Harrison & Larson, 2014). Another emerging question is why mitochondrial and

703   nuclear introgression in the *C. inornatus* group appear concentrated in northern Australia.

704   Studies of animals and plants have found introgressive hybridization in regions of high climatic

705   dynamism in the Quaternary (Dufresnes et al., 2020; Folk et al., 2018; Shu et al., 2022),

706   including the northern Australian monsoonal-arid zone interface and wet tropics (e.g., Catullo &

707   Keogh, 2014; Hoskin et al., 2011; Laver et al., 2018; Singhal & Moritz, 2012). Climate-driven

708   habitat changes may lead to introgression by promoting species range shifts and secondary

709   contact (Currat et al., 2008; Cutter & Gray, 2016; Folk et al., 2018; Garroway et al., 2010; Prates

710   et al., 2018). However, other groups from the monsoonal tropics show deep phylogeographic

711   structure, consistent with a history of range stability (e.g., Bowman et al., 2010; Moritz et al.,

712   2016; Potter et al., 2018; Rosauer et al., 2018), while some analyses have inferred dynamism also

713   in the arid zone (e.g., Byrne et al., 2008; Pepper & Keogh, 2021; Prideaux et al., 2007). We still

714   have a limited understanding of the historical processes that have structured the genomes of

715   *Ctenotus* lizards. Nevertheless, clades with elusive species boundaries, like the *C. inornatus*

716   species group, emerge as promising candidates for investigating the environmental drivers and

717   evolutionary consequences of introgressive hybridization.

718

719   *Taxonomic recommendations and outstanding issues*

720   Systematic uncertainty persists in our focal lizards, but our results provide clarity on

721   several issues. We found continuous spatial genetic differentiation between *inornatus* complex

722   populations from northern Australia (corresponding to *inornatus*-N) and the central arid zone

723   (*inornatus*-S). Some population genetic structure between these regions appears to align with a

724   physiographic transition from sandy desert to stony uplands and monsoonal woodlands in

725   northern Australia. Still, gene flow across this transition seems high enough to generate a pattern

726   consistent with IBD over the range of a single species. Populations corresponding to *inornatus*-N

727   and *inornatus*-S have been assigned to different taxa (Storr, 1969), which nonetheless cannot be

728   reliably identified based on current perceptions of their morphological variation (Rabosky et al.,

729   2014). Future morphological examinations informed by the genetic patterns might help clarify

730   whether some of the inferred OTUs are truly cryptic. For the time being, we recommend treating

731   the northern and southern populations as a single taxon, with the name *Ctenotus inornatus* (Gray,

732   1845) retaining priority to refer to both.

733   We found that some traditionally recognized taxa have limited coherence or divergence.

734   For instance, specimens assigned to "*C. saxatilis*" appear to correspond to multiple *inornatus* and

735   *superciliaris* complex lineages. Likewise, *C.* "*borealis*" and *C.* "*severus*" grouped respectively

736   with *C. robustus* and *C. inornatus*, with which they experience high gene flow. These findings

737   corroborate phylogenetic patterns inferred based on fewer loci (Rabosky et al., 2014). The case

738   of *C.* "*fallens*" and *C.* "*brachyonyx*" is less clear. Populations corresponding to these taxa were

739   genetically clustered within other taxa (*C.* "*helenae*", *C.* "*saxatilis*"), yet separated from other

740   samples by long branches. Relatively fewer alleles migrate between *C.* "*fallens*" or *C.*

741   "*brachyonyx*" and geographically adjacent populations, but these numbers appear to reflect lower

742   effective population sizes rather than lower proportions of migrants. For instance, their N

743   estimates were up to 20 times lower than those of other populations (Table S3). Such low N

744   contributed to low 2NM for *C.* "*fallens*" and *C.* "*brachyonyx*" despite high estimates of M, that

745   is, high proportions of individuals originating from other populations (Table S3). This scenario

746   suggests that 2NM, a composite metric designed to express absolute numbers of gene migrations

747   (Pinho & Hey, 2010), might conceal high relative gene flow when population sizes are small.

748   Overall, our findings suggest that several traditionally recognized taxa correspond to regional

749   forms within wide-ranging polytypic species, but broader sampling may be needed to resolve

750   specific cases.

751   Our analyses also revealed OTUs that might correspond to undescribed species. OTUs

752   *robustus*-NW and *robustus*-TE, both corresponding to the taxon *C. robustus*, are sympatric but

753   appear genetically isolated. Similarly, gene flow approached zero between *spaldingi*-CY and

754   *spaldingi*-NE, both referred to *C. spaldingi*. Finally, *superciliaris*-E (N), *superciliaris*-E (S), and

755   *superciliaris*-W, all within *C. superciliaris*, appear divergent despite their potential geographic

756   overlap. Further sampling in putative contact zones may be necessary to establish the degree of

757   separation between these populations. From a nomenclatural perspective, it is also important to

758   determine whether these OTUs correspond to valid yet poorly characterized taxa. For instance,

759   *spaldingi*-CY or *spaldingi*-S might correspond to the eastern taxa *C. capricorni* Storr, 1981 or *C.*

760   *nullum* Ingram and Czechura, 1990. Alternatively, they might represent names currently under

761   synonymy, such as *C. harringtonensis* (Wells and Wellington, 1985), *C. josephinae* (Wells and

762   Wellington, 1984), or *Ctenotus dorsale* (Boulenger, 1887). Lack of genetic data unambiguously

763   corresponding to these taxa prevents testing whether they are present in our sample. Given the

764   lack of DNA sequences for most name-bearing type specimens, assigning names to lineages

765   typically relies on morphological comparisons. It remains to be seen whether the emerging

766   genetic patterns will help identify morphological features that confidently assign unsequenced

767   specimens to lineages.

768   This investigation supports that resolving species boundaries in the *C. inornatus* species

769   group and other "taxonomic disaster zones" will require developing a broad understanding of

770   population history, spatial genetic variation, patterns of genomic introgression, and their effects

771   on phenotypic variation. This comprehensive understanding may, in turn, require renewed field-

772   based collecting and specimen vouchering in undersampled geographic regions. These

773   requirements illustrate the formidable challenges and opportunities brought by the increasing

774   conceptual and operational unification of taxonomic practice and speciation biology.

775

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788

789   **References**

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Data Accessibility Statement

Nuclear ddRAD data are available in the Sequence Read Archive (BioProjects PRJNA755251 and PRJNA382545); see Table S1 for ddRAD sample information. Newly generated mitochondrial data were uploaded to GenBank (accession numbers OQ091785–OQ091921); see Table S2 for the accession numbers of mitochondrial sequences, including those generated by previous studies. Computer scripts used to prepare the data and perform all analyses are available through GitHub (https://github.com/ivanprates/Ctenotus_inornatus_group). A copy of the supplementary material is available through GiHub.

Author contributions

IP, MNH, and DLR conceived and designed the study. SS, CM, and DLR acquired funding. MNH, CM, and DLR provided samples and resources. IP wrote computer scripts,

1261   processed the data, and prepared the visualization of results. IP, MNH, SS, CM, and DLR

1262   interpreted the results. IP and DLR drafted the manuscript with suggestions and edits by MNH,

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1271

1272   **Figure Legends**

1273

1274   **Fig. 1.** Expected relationships between genetic and geographic distances in the presence of one

1275   versus two species and implications for species delimitation. For dispersal-limited organisms,

1276   genetic differentiation between sampled localities should increase as a function of geographic

1277   separation, corresponding to Wright's (1943) isolation-by-distance (IBD) model (A). In the

1278   absence of geographic or reproductive barriers, we should observe continuous IBD among

1279   conspecific populations (B). In contrast, in the presence of additional factors restricting gene

1280   flow (e.g., unsuitable habitat), the relationship between genetic and geographic distances may

1281   become discontinuous (C). In this case, two IBD patterns emerge from the same analysis: one for

1282   pairs of populations with unrestricted gene flow (light gray ellipse), and a second, offset

1283   relationship for pairs of populations with restricted gene flow (dark gray ellipse). Note that these

1284   clusters emerge from the data, irrespective of *a priori* assignments to candidate species or taxa.

1285   Finally, population differentiation can become uncoupled from geographic separation when

1286   sampling encompasses fully reproductively isolated units, regardless of the underlying

1287   geography (D). This latter case would provide strong evidence for two separately evolving

1288   species.

1289

1290   **Fig. 2.** Location of geographic regions mentioned in this manuscript (upper left), presumed

1291   ranges of morphologically defined taxa traditionally recognized in the *Ctenotus inornatus*

1292   species group (smaller maps), and pictures of representative taxa (right). Maps of taxa not

1293   included in our analyses but otherwise presumed to belong to this species group (*C. capricorni*

1294   and *C. nullum*) were omitted. Dark polygons on taxon maps are expert-validated ranges derived

1295   for the IUCN Red List Australian squamate assessment as compiled by Roll et al. (2017). Yellow

1296   diamonds indicate the type locality of each taxon based on the original descriptions. Note that the

1297   type localities of *C. robustus* and *C. inornatus* lie outside the broadly accepted ranges of these

1298   taxa. All lizard pictures by Jules Farquhar.

1299

1300   **Fig. 3.** Overview of major inferred clades, operational taxonomic units (OTUs), and proposed

1301   provisional taxon scheme in the *Ctenotus inornatus* species group. Phylogenetic tree based on

1302   524,324 base pairs from 3,694 ddRAD loci (outgroups not shown). Asterisks indicate bootstrap

1303   nodal support > 95. For clarity, only support for the OTUs and deeper relationships are shown.

1304   OTUs shown resulted from our delimitation analyses and are considered candidate species (see

1305   text). Pending detailed analyses of morphological variation and additional geographic sampling

1306 of the OTUs, we outline a provisional taxonomic scheme with the goal of supporting field and

1307 museum workers.

1308

1309 **Fig. 4.** Genotypic clustering results (bars) and phylogenetic support for traditionally recognized

1310 taxa in the *robustus* complex. Nuclear phylogenetic relationships (left) as in Fig. 3 but pruned to

1311 this complex. Individuals shown in gray tip labels were not included in the clustering analysis

1312 due to high missing data or scarce sampling of the corresponding candidate species. Pie charts on

1313 maps indicate the average ancestry proportions corresponding to each cluster at each site.

1314 Clustering results support two clusters corresponding to the taxon *C. spaldingi*: *spaldingi*-S and

1315 *spaldingi*-NE, which might overlap in northeastern localities. Unit *robustus*-NW from northern-

1316 northwestern Australia corresponds to a subset of the taxon *C. robustus*. *Ctenotus rimacola*,

1317 *robustus-TE*, and *spaldingi*-CY are highly divergent from other units and considered candidate

1318 species, yet not included in clustering analyses owing to scarce sampling. Note that, despite a

1319 wide geographic sampling gap between northern and southern populations of *spaldingi*-S, our

1320 analyses did not support them as independent genetic groups.

1321

1322 **Fig. 5.** Phylogenetic relationships in the *Ctenotus inornatus* group based on the *cytochrome B*

1323 mitochondrial marker. The colors of tip symbols correspond to the operational taxonomic units

1324 (OTUs) delimited based on nuclear loci. For clarity, only samples with matching nuclear data are

1325 shown, except for individuals of *C. burbidgei* and the OTU *spaldingi*-CY, which had scarce or

1326 no nuclear data (for a complete mitochondrial tree, see Fig. S6). Asterisks indicate bootstrap

1327 nodal support > 95. For clarity, only support for the OTUs and deeper relationships are shown.

1328 There were notable instances of mitochondrial paraphyly of nuclear OTUs (lineages involved are

1329   indicated with red arrows). In particular, a mitochondrial lineage grouped samples of four

1330   nuclear OTUs from two distinct species complexes: *inornatus*-N, *inornatus*-S, *superciliaris*-E,

1331   and *superciliaris*-W. Note also that *superciliaris*-E was split into two distant mitochondrial

1332   lineages, each matching a divergent nuclear lineage (Fig. 8). Similarly, *robustus*-NW and

1333   *robustus*-TE, sisters in the nuclear tree (Fig. 4), formed distant mitochondrial lineages.

1334

1335   **Fig. 6.** Pairwise F_{ST} between individuals from the same (gray) or different (black) groups, as a

1336   function of the geographic distances between them. Groups are the operational taxonomic units

1337   (OTUs) delimited within currently recognized taxa, whose ranges are shown on maps. Genetic

1338   and geographic distances within and between groups should form a continuous relationship when

1339   these groups correspond to the same species (see Fig. 1). This appears to be the case for OTUs

1340   corresponding to the taxon *Ctenotus inornatus*. By contrast, the relationship between genetic and

1341   geographic distances within and between groups should be discontinuous in the presence of

1342   separate species (Fig. 1). This appears to be the case of OTUs within the taxa *C. spaldingi*, *C.*

1343   *robustus*, and *C. superciliaris*.

1344

1345   **Fig. 7.** Estimates of population migration rates (2NM), the effective number of gene migrations

1346   received by a population per generation. Each violin plot corresponds to a pair of populations,

1347   including (i) the operational taxonomic units (OTUs) inferred in this study, (ii) traditional taxa of

1348   disputed validity, and (iii) populations geographically adjacent to these taxa. In each pair, values

1349   are averages between the two directions of gene flow (see Table S3 for all estimates). To

1350   facilitate comparison, x-axes span the same 2NM range across plots. OTUs in the same complex

1351   showed relatively low (e.g., *spaldingi*-CY and *spaldingi*-S) to high (e.g., *inornatus*-N and

1352   *inornatus*-S) gene flow. We also find low (*C.* "*fallens*", *C.* "*brachyonyx*") to high (e.g., *C.*

1353   "*borealis*", *C.* "*severus*") absolute numbers of gene migrations among traditional taxa and their

1354   respective geographically adjacent populations. Note that gene flow levels between OTUs from

1355   different complexes (i.e., divergent species; dark blue violins) were often comparable or higher

1356   than intra-complex estimates.

1357

1358   **Fig. 8.** Genotypic clustering results (bars) and phylogenetic support for traditionally recognized

1359   taxa in the *superciliaris* complex. Nuclear phylogenetic relationships (left) as in Fig. 3 but

1360   pruned to this complex. Individuals shown in gray tip labels were not included in the clustering

1361   analysis due to high missing data. Pie charts on maps indicate the average ancestry proportions

1362   corresponding to each cluster at each site. Genotypic clustering identified three groups that may

1363   overlap geographically. Units *superciliaris*-W and *superciliaris*-E correspond to the taxon *C.*

1364   *superciliaris*.

1365

1366   **Fig. 9.** Genotypic clustering results (bars) and phylogenetic support for traditionally recognized

1367   taxa in the *inornatus* complex. Nuclear phylogenetic relationships (left) as shown in Fig. 3 but

1368   pruned to this complex. Individuals shown in gray tip labels were not included in the clustering

1369   analysis due to high missing data or scarce sampling of the corresponding candidate species. Pie

1370   charts on maps indicate the average ancestry proportions corresponding to each cluster at each

1371   site. Genotypic clustering identified two clusters corresponding to the taxon *C. inornatus* (sensu

1372   Rabosky et al. (2014)): *inornatus*-N and *inornatus*-S, which nonetheless establish substantial

1373   gene flow (see text). *Ctenotus eutaenius* is highly divergent from other units but not included in

1374   clustering analyses owing to scarce sampling. Note that unit *inornatus*-S, spanning most of

1375   Australia's central arid zone, grouped specimens traditionally assigned to several distinct taxa

1376   based on coloration patterns.

1377

1378   **Fig. 10.** Excess allele sharing among operational taxonomic units (OTUs) in the *Ctenotus*

1379   *inornatus* species group based on f-branch, an ABBA-BABA-class statistic. Colors indicate the

1380   proportion of an OTU's loci inferred to be introgressed from another OTU. Gray squares indicate

1381   comparisons not possible owing to tree topology constraints. The f-branch metric accounts for

1382   correlated allele frequencies among species to minimize cases where multiple species appear to

1383   be introgressed owing to a single introgression event that involved an ancestor (a limitation of

1384   the broadly used Patterson's D; see Fig. S8 for D results). Such ancestral events are indicated by

1385   the internal branches on the y-axis (blue dotted lines). Note substantial introgression across

1386   OTUs from distinct complexes, particularly *inornatus* and *superciliaris*, but also among non-

1387   sister OTUs within the three complexes.