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Molecular phylogeny and morphological revision of the *Ctenotus labillardieri* (Reptilia: Squamata: Scincidae) species group and a new species of immediate conservation concern in the southwestern Australian biodiversity hotspot

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Abstract

Ctenotus is the largest and most diverse genus of skinks in Australia with at least 97 described species. We generated large mitochondrial and nuclear DNA data sets for 70 individuals representing all available species in the *C. labillardieri* species-group to produce the first comprehensive phylogeny for this clade. The widespread *C. labillardieri* was sampled extensively to provide the first detailed phylogeographic data set for a reptile in the southwestern Australian biodiversity hotspot. We supplemented our molecular data with a comprehensive morphological dataset for the entire group, and together these data are used to revise the group and describe a new species. The morphologically highly variable species *C. labillardieri* comprises seven well-supported genetic clades that each occupy distinct geographic regions. The phylogeographic patterns observed in this taxon are consistent with studies of frogs, plants and invertebrates, adding strength to emerging biogeographic hypotheses in this iconic region. The species *C. catenifer*, *C. youngsoni*, and *C. gemmula* are well supported, and despite limited sampling both *C. catenifer* and *C. gemmula* show substantial genetic structure. The threatened *C. lancelini* from Lancelin Island and the adjacent mainland is the sister taxon to a new species from the Swan Coastal Plain, which we describe as *C. ora* **sp. nov.** This species is a habitat specialist, occurring primarily in sandy regions south of Perth that currently are under intense development. *Ctenotus ora* **sp. nov.** should be considered for conservation attention immediately.

Key words: cryptic species, lizard, skink, ND2, ATP, southwestern Australia, biodiversity hotspot, Swan Coastal Plain

Introduction

Southwestern Australia (SWA) is an iconic region, increasingly renowned for its high levels of biodiversity and endemism. It is recognized as one of the world's top 25 "biodiversity hotspots" (Cincotta *et al.* 2000; Myers *et al.* 2000), based largely on its highly diverse and endemic flora (Beard *et al.* 2000). More recent phylogeographic studies on SWA plants have demonstrated that plant genetic diversity is even more extreme than previously thought, with current taxonomy a gross underestimate of the true diversity (Byrne *et al.* 2003a,b; Hopper & Gioia 2004; Byrne & Hines 2004; Byrne 2007; Byrne & Hopper 2008;). A similar theme is emerging from genetic studies on SWA animals, including frogs (Driscoll 1998a,b; Berry 2001; Edwards 2007a,b; Edwards *et al.* 2007, 2008; Morgan *et al.* 2007), and invertebrates (Main 1996, 1999; Horwitz & Adams 2000; Gouws *et al.* 2006; Cooper *et al.* 2011; Rix & Harvey 2012).

Combining what is know about phylogeographic patterns of SWA taxa, it appears that major climatic and physical features occurring across SWA have been important in regional diversification. Climatic features, including strong rainfall and temperature gradients, have provided barriers to gene flow between Hopper's (1979) High Rainfall Zone (HRZ) and Transitional Rainfall Zone (TRZ) of SWA (Dodson & Macphail 2004; Hopper & Gioia 2004) (Figure 1). Furthermore, physical barriers including the subdued mountains of the Stirling Ranges and Darling Scarp, and granite outcrops, seem important for diversification (Wheeler & Byrne 2006; Yates *et al.* 2007; Byrne & Hopper 2008; Rix & Harvey 2012), especially in coastal areas subject to eustatic sea-level fluctuations (Rabosky *et al.* 2004; Edwards 2008). Southwestern Australia also has been divided into a number of biogeographic subregions (Interim Biogeographic Regionalisation of Australia [IBRA]) based on broad landscape distinc-

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tions including attributes of climate, geomorphology, landform and biota (Environment Australia 2004). In SWA, these subregions are characterised by narrow, coastal zones, including the Swan Coastal Plain, Warren, and two divisions of the Esperance Plains, while adjacent inland areas comprise more extensive Jarrah and Mallee subregions. Understanding the patterns, and the potential environmental drivers, of diversification in SWA is critical in the identification and conservation of genetic diversity within this biodiversity hotspot.

Ctenotus Storr 1964 is the largest and most diverse genus of skinks in Australia with at least 97 described species (Wilson & Swan 2008). A recently published molecular phylogeny for the genus identified a number of species groups within *Ctenotus* and highlighted their explosive diversification (Rabosky *et al.* 2007). *Ctenotus labillardieri* Duméril & Bibron is a divergent sister lineage to all other *Ctenotus* in their phylogeny. Detailed morphological work on specimens morphologically very similar to *Ctenotus labillardieri* resulted in the description of several species that together were considered part of the '*labillardieri* group' based on this similarity: *C. catenifer* Storr 1974, *C. delli* Storr 1974, *C. gemmula* Storr 1974, *C. labillardieri*, *C. lancelini* Ford 1969, and *C. youngsoni* Storr 1975 (Storr 1974; Storr, Smith, & Johnstone 1999). While Storr did not intend to necessarily imply monophyly of the species he included in this group, a close relationship is likely given the morphological similarity of distribution (and is supported in our study). While Rabosky *et al.* (2007) found *C. youngsoni* to group with more distantly related *Ctenotus*, recent genetic work suggests it is closely related to *C. labillardieri* and allies (D. Rabosky, pers. comm.).

We generated mitochondrial and nuclear DNA data for all available species in the *C. labillardieri* species group to generate the first comprehensive phylogeny for this clade. The widespread *C. labillardieri* was sampled extensively to provide the first detailed phylogeographic data set for a reptile in SWA. We supplemented our molecular data with a comprehensive morphological data set for the entire group and these data together are used to revise the group and describe a new species of immediate conservation concern.

Materials and methods

Taxonomic sampling. Tissue samples were obtained for a total of 70 individuals (Table 1). These samples included all members of the *C. labillardieri* species group except for *C. delli* for which there was no available tissue. We sampled *C. labillardieri* thoroughly (n=57) to cover the full range of the species. Despite extensive fieldwork, we were able to obtain only a few samples between Albany and Esperance, which appears to be a genuine gap in the range of this species (Figure 1). This result is corroborated by the very low numbers of museum samples, supporting the hypotheses that populations are naturally sparse or highly localized in this region (Bush *et al.* 2007; Wilson & Swan 2008).

Molecular data and analyses

Two molecular data sets were assembled to provide phylogenetically useful data at two hierarchical levels. Initially, a 1200 base-pair (bp) region of the mitochondrial genome was sequenced that included the entire mitochondrial NADH dehydrogenase subunit 2 (ND2) gene and part of the flanking tRNA-Methionine and tRNA-Asparagine regions. This region was sequenced in all 70 animals used in this study, and formed the basis for preliminary assessment of phylogenetic relationships of species in the *C. labillardieri* species group, as well as the detailed phylogeographic analysis for *C. labillardieri*. This gene has been used in a wide range of vertebrate taxa, including a number of Australian reptiles and amphibians at comparable taxonomic levels (i.e., Edwards 2007a; Edwards *et al*. 2007, 2008; Morgan *et al*. 2007; Pepper *et al*. 2006, 2008). For the same 70 individuals we then generated a nDNA data set comprised of 550bp of the gene ATP (Skinner 2007), a gene that has proven particularly useful in *Ctenotus* (Rabosky *et al.* 2007).

Amplification of the mitochondrial DNA fragments was conducted following the methods of Pepper *et al.* (2006). Amplification of nuclear genomic DNA was conducted using an activation step at 94ºC for 4 min, 14 cycles of denaturation at 94ºC for 30s, annealing at 60ºC for 25s (stepping down to 55ºC after 2 cycles and 50ºC for the last 10 cycles), followed by 30 cycles of denaturation at 94ºC for 30s, annealing at 46ºC for 25s and extension at 72ºC for 90s, with a final extension step at 72ºC for 5 min. Primer combinations used for PCR and sequencing are listed in Table 2. All PCR products were purified using an ammonium acetate clean-up method. Cycle-sequencing reactions were carried out following the methods of Pepper *et al.* (2006). Cleaned up sequence was run on an ABI 3100 auto-sequencer and edited sequences were aligned manually.

TABLE 1. Locality information for all individuals sampled in this study. Museum ID# refers to the voucher specimens held at the Western Australian Museum. Samples used in genetic (G) and morphological (M) analyses are noted.

Taxon	Museum ID#	Latitude (S)	Longitude (E)	Locality	Analyses
Ctenotus catenifer	68924	-30.40000	115.45000	Badgingarra National Park	M
	83015	-35.00000	117.93330	Emu Point, Albany	M
	83017	-35.00000	117.93330	Emu Point, Albany	M
	84505	-34.99020	117.98060	2.5km Ne Mount Martin	M
	84508	-34.99160	117.98330	Mount Martin	M, G
	90124	-34.30000	115.17920	2.5km Ne Augusta	M
	90365	-35.03160	116.85080	Walpole-Nornalup National Park	M
	91155	-34.40000	117.86670	4km E Talyuberlup Peak	M
	93732	-33.71660	115.33333	Ambergate Reserve	M
	117161	-35.00000	117.95000	Emu Point	M, G
	121359	-33.55000	115.05000	Dunsborough	M, G
	129085	-34.41660	115.68330	Lake Jasper	M, G
	132008	-33.82580	122.97360	Cape Arid National Park	M
	135711	-35.07300	117.62060	Kronkup Tip	M, G
	163142	-35.05130	117.76060	Albany Windfarm	M
	163143	-35.05130	117.76060	Albany Windfarm	M
Ctenotus delli	46191	-31.96660	116.16670	1 1/2 Miles S Mundaring Weir	M
	49284	-32.13330	116.30000	Dale Forest Mt Dale Area	M
	51035	-32.13330	116.30000	Mt Dale	M
	56861	-32.16660	116.25000	Mt Dale, Brookton Hwy	M
	57719	-31.90000	116.26670	7km E Of Sawyers Valley	M
	74977	-32.91660	116.21670	Se Dwellingup.	M
	75878	-32.91660	116.21670	Se Dwellingup	M
	96280	-32.05000	115.95000	South Canning Catchment	M
	103739	-32.51660	116.03330	North Dandalup	M
	104373	-32.53330	116.40000	7km Nw Of North Bannister	M
	113444	-32.51660	116.00000	North Dandalup Dam	M
	144173	-33.35630	116.22420	4km South Collie	M
	156643	-33.13910	116.42890	Collie Area	M
Ctenotus gemmula	59707	-31.70000	115.93330	Melaleuca Park	M
	77749	-32.72500	125.03330	Toolinna Rockhole	M, G
	91049	-34.43330	117.88330	7km Se Talyuberlup Peak	M
	94104	-32.72500	125.03330	6km Ne Toolinna Rockhole	M
	94475	-33.83330	119.83330	Fitzgerald River National Park	M
	94675	-34.13330	119.45000	Fitzgerald River National Park	M
	96257	-33.45000	121.73330	Scaddan	M
	100822	-33.81660	121.13330	Stokes National Park	M
	103345	-30.61660	115.41670	Cooljarloo	M
	116529	-33.65000	120.43330	5.5km E Bandalup Hill	M, G
	119462	-33.73970	118.97440	18km Ssw Ravensthorpe	M, G
	121402	-33.95000	120.45000	Mason Bay	M
	137202	-34.88330	118.60000	Stirling Range National Park	M
	140514	-30.57440	115.34170	Cataby	M, G
	143067	-33.61580	119.19940	Lake Magenta Nr	M
	144223	-33.57580	120.30750	Bandalup Hill	M, G
	150245	-33.34750	120.87170	Pyramid Lake Area	M
	156937	-34.54550	118.53080	Upper Kalgan	M

continued on next page

TABLE 1. (continued)

Taxon	Museum ID#	Latitude (S)	Longitude (E)	Locality	Analyses
Ctenotus labillardieri					
Clade 1	90231	-35.0333	117.4667	Koirchekup Hill	M, G
	97816	-35.05	118.0333	Michaelmas Island	M, G
	97817	-35.05	118.0333	Michaelmas Island	M, G
	115320	-34.3	116.15	Manjimup	M, G
	116249	-34.575	116.4	Mt Burnside	M, G
	116251	-34.575	116.4	Mt Burnside	M, G
	117354	-34.9333	116.45	Broke Inlet	M, G
	117357	-34.95	117.0333	Kent River Bridge	M, G
	117359	-34.9333	116.45	Broke Inlet	M, G
	129677	-34.85	117.35	Denmark	M, G
	136391	-35.0697	117.7814	Albany	M, G
	140763	-34.9636	116.6028	Walpole	M, G
	142046	-34.89039	118.40931	Cheyne Beach	M, G
	142938	-34.89039	118.40931	Cheyne Beach	M, G
	165587	-34.99	117.2819	Walpole	M, G
	165589	-34.99	117.2819	Walpole	M, G
Clade 2	134072	-33.74554	119.66578	Fitzgerald Road	G
	142908	-34.2166	119.4333	West Mt Barren	M, G
Clade 3	113336	-33.9833	115.1167	Margaret River	M, G
	113337	-33.9833	115.1167	Margaret River	M, G
	116229	-33.875	116.2833	Boyup Brook	M, G
	116236	-34.0166	115.7333	Nannup	M, G
	119037	-33.35	116	Wellington Dam	M, G
	127458	-34.3333	115.15	Augusta	M, G
	129644	-34.4013	115.6436	Lake Jasper	M, G
	132092	-34.3666	115.1333	Cape Leeuwin	M, G
	132093	-34.3666	115.1333	Cape Leeuwin	M, G
	135756	-34.1	115.0333	Cape Leeuwin	M, G
	140784	-34.3833	115.15	Augusta	M, G
	142327	-32.74642	116.15208	Dwellingup	M, G
	142382	-32.74642	116.15208	Dwellingup	M, G
	165590	-34.2386	115.6333	Karridale	M, G
Clade 4	129661	-33.8333	121.2833	Quagi Beach	M, G
	129662	-33.8333	121.2833	Quagi Beach	M, G
	134073	-33.92337	120.03042	East Mt Barren	G
Clade 5	142949	-34.36938	118.24863	Bluff Knoll	M, G
	DLE58	-34.3699	118.24982	Bluff Knoll	G
	DLE59	-34.3699	118.24982	Bluff Knoll	G
Clade 6	97507	-33.9	122.75	Ben Island	M, G
	97525	-34.0333	123.2167	Harlequin Island	M
	116228	-33.875	116.2833	Boyup Brook	M
	131903	-33.9972	122.1194	Mount Le Grand	M, G
	131904	-33.9972	122.1194	Mount Le Grand	M, G
	131908	-34.0041	122.1722	Hellfire Bay	M, G
	131914	-34.0041	122.1722	Hellfire Bay	M, G
	131916	-33.9472	122.5667	Mount Belches	M, G
	131919	-33.9472	122.5667	Mount Belches	M, G

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TABLE 1. (continued)

Taxon	Museum ID#	Latitude (S)	Longitude (E)	Locality	Analyses
	131924	-33.8166	122.2139	Mount Merivale	M, G
	131925	-33.8166	122.2139	Mount Merivale	M, G
	131926	-33.9722	122.1292	Mount Le Grand	M, G
	132164	-33.8166	123.0333	Thomas River	M, G
	135665	-34.1333	122.25	Mondrain Island	M, G
	135666	-34.1333	122.25	Mondrain Island	M, G
	135758	-34.1	115.0333	Cape Leeuwin	M
	140529	-34.0361	121.9917	Sandy Hook Island	M, G*
	140530	-34.0361	121.9917	Sandy Hook Island	M, G
	166084	-31.88139	116.17806	Stoneville	M, G
Clade 7	166085	-31.88139	116.17806	Stoneville	M, G
Ctenotus lancelini	18871	-31.00000	115.31670	Lancelin Island	M
	18873	-31.00000	115.31670	Lancelin Island	M
	18874	-31.00000	115.31670	Lancelin Island	M
	18875	-31.00000	115.31670	Lancelin Island	M
	50126	-31.00000	115.31670	Lancelin Island	M
	52098	-31.00000	115.31670	Lancelin Island	M
	52099	-31.00000	115.31670	Lancelin Island	M
	52100	-31.00000	115.31670	Lancelin Island	M
	121883	-31.01660	115.33330	Lancelin	M, G
	125723	-31.00000	115.31670	Lancelin Island	M, G
	125724	-31.00000	115.31670	Lancelin Island	M, G
Ctenotus youngsoni	54800	-26.38333	113.31667	False Entrance Well	M
	54825	-26.38333	113.31667	False Entrance Well	M
	57089	-26.13333	113.21667	Dirk Hartog Island	M
	64426	-26.90000	113.71667	19km South Tamala	M
	66208	-26.38333	113.31667	False Entrance Well	M
	66209	-26.38333	113.31667	False Entrance Well	M
	66293	-26.15000	113.16667	Steep Point	M
	81331	-26.43333	113.30000	Zuytdorp Point	M
	81332	-26.43333	113.30000	Zuytdorp Point	M
	82779	-26.40000	113.30000	Zuytdorp Point	M
	91692	-26.40000	113.30000	False Entrance Well	M
	103227	-26.55000	113.76667	Salutation Island	M
	103234	-26.55000	113.65000	Three Bays Island	M
	113648	-26.00000	113.20000	Dirk Hartog Island	G
	113664	-25.93333	113.16667	Dirk Hartog Island	G
	135504	-26.38333	113.31667	False Entrance Well	M, G
	135533	-26.38333	113.31667	False Entrance Well	G
Ctenotus ora **sp. nov.**	73591	-32.83330	115.65000	Yalgorup National Park	M
	81601	-33.35000	115.70000	Eaton	M
	119059	-32.66660	115.71670	Pinjarra	M, G
	131983	-33.53910	115.02030	Cape Naturaliste	M, G
	141244	-33.63330	115.03750	Yallingup Brook	M, G

Individual data sets were created for each gene, and we used unweighted heuristic parsimony and Bayesian approaches to analyze the data. Unweighted heuristic parsimony analyses were implemented with the computer program PAUP* (Swofford 2002). We used TBR branch swapping and ran the parsimony analysis five times from random starting points to make sure overall tree space was well searched. Bayesian analyses were implemented

with the computer program MrBayes (v3.0b4: Huelsenbeck & Ronquist 2001). We performed individual Bayesian analyses on each gene with the nDNA treated as a single unit and the mtDNA gene divided into four partitions: 1st, 2nd, 3rd codon positions for the coding region, and the tRNA. We allowed all parameters to be estimated from the data during the run. We used the default value of four Markov chains per run and also ran the full analysis two times to make sure overall tree-space was well sampled and to avoid getting trapped in local optima. We ran each analysis for a total of 4,000,000 generations and sampled the chain after every 100 generations, resulting in 40,000 sampled trees. Log-likelihood values reached a plateau after approximately 100,000 generations (1,000 sampled trees), so to make sure that we discarded the full burn-in phase, we discarded the first 10,000 trees and used the last 30,000 trees to estimate Bayesian posterior probabilities. *Ctenotus robustus* was used as the outgroup in phylogenetic analyses based on the results of Rabosky *et al.* (2007). We also created a concatenated data set using both genes and implemented a partitioned Bayesian analysis as above, but with five partitions: 1st, 2nd, 3rd codon positions for the coding region of ND2; tRNA, and nDNA. For each analysis we used the results of 10,000 unweighted parsimony bootstrap replicates and Bayesian posterior probabilities to assess branch support.

TABLE 2. Amplification and sequencing primers (5' to 3') used in this study. Modified (Mod.) primers are noted.

Gene	Name	Sequence	Source
tRNA-Asn	tRNA-Asn	5'-CTAAAATRTTRCGGGATCGAGGCC-3'	Read et al., 2001
tRNA-Met	L4437	5'-AAGCTTTCGGGGCCCATACC-3'	Macey et al., 1998
ND2	L4882	5'-CAACATGACAAAAAATCGCCCC-3'	Macey et al., 2000
ND2	AT4882	5'-CAACATGACAAAAATTRGCCCC-3'	Macey et al., 2000 (mod.)
ND2	CM4882	5'-CYACATGACAAAAAATTGCACC-3'	Macey et al., 2000 (mod.)
ATP	G613R	5'-TCTGTCCATAAACTAGCG-3'	Skinner, 2007 (mod.)
ATP	ATPSB1	5'-CGTGAGGGHAAYGATTTHTACCATGA-3'	Skinner, 2007 (mod.)

Morphological data analyses

We carried out a detailed assessment of morphological variation in the species group by examination of all available specimens housed in the Western Australian Museum that were included in the molecular phylogeny, as well as additional museum specimens where genetic sample sizes were low or where additional information was required (Table 1). Our morphological data set was based on the characters identified in the comprehensive morphological revision of the group by Storr (1974). Table 3 presents the morphological characters measured and their descriptions. In total 19 characters were measured in 133 specimens. Measurements were made with electronic callipers to the nearest 1mm, 0.5mm or 0.1mm as appropriate. We analyzed the 10 continuous charaters (see Table 3) with Principal Components Analysis (PCA), which does not identify groups *a priori*, to summarize inter-specific variation in these characters. We calculated standard principal components with variance-covariance and imputation of any missing data so we could include all individuals (although very little was missing).

Results

Molecular data. Full details of collection localities and voucher information for each individual are given in Table 1. Alignment of both the mtDNA and nDNA fragments was straightforward. The ND2 alignment comprised 1133 characters of which 353 (31%) were parsimony informative. The nuclear ATP gene alignment comprised 477 characters of which 33 (6.9%) were parsimony informative. Uncorrected "p" genetic distances in the mtDNA data set are summarized in Tables 4 and 5.

TABLE 3. Morphological characters and their abbreviations used in this study.

Character	Description
Continuous characters	
SVL	Snout-vent length
HeadL	Head length from tip of snout posterior of quadrate bone
HLL	Hind-limb length of fully extended limb, including claw
FLL	Fore-limb length of fully extended limb, including claw
ILL	Interlimb distance measured from posterior margin of forelimb to anterior margin of hindlimb
TailL	Tail length from vent to tip (original tails only)
LorW:H	The height to width ratio of the second loreal scale
MidB	Number of scales in the mid-body scale row in the middle of the body
Lam4Toe	Number of rows of lamellae under the 4th toe on one foot
VentS	Number of ventral scales counted from forelimb to vent
Categorical characters	
NasSS	Nasal scale separation scored as separated (S) or in contact (C)
PrefSS	Prefrontal scale separation scored as separated (S) or in contact (C)
SOS	Number of supraocular scales
SOSContact	Number of supraocular scales in contact with frontal scale (2, 3 or 4)
SupCil	Number of supracilaries, beginning with the scale adjoining the prefrontal and loreal, and ending with the scale still contacting cilaries and last supraocular (7, 8 or 9)
Palp	Number of palpebral scales (8, 9, 10, 11, 12 or 13)
UppLab	Number of upper labial scales (7, 8 or 9)
EarL	Number of ear lobule scales (3, 4 or 5)
EarLPos	The relative position of the largest ear lobule (1 - highest in ear, 2, 3, or 4 - lowest)

TABLE 4. Ranges of intra-clade uncorrected "p" genetic distances for the *C. labillardieri* species group.

Taxon	Uncorrected "p" Genetic Distance
Ctenotus catenifer	0.0080 – 0.0507
C. gemmula	0.0009 – 0.0630
C. labillardieri	0 – 0.0773
C. labillardieri Clade 1	0 – 0.0221
C. labillardieri Clade 2	0.0063
C. labillardieri Clade 3	0.0009 – 0.0257
C. labillardieri Clade 4	0.0044 – 0.0305
C. labillardieri Clade 5	0.0000 – 0.0090
C. labillardieri Clade 6	0.0000 – 0.0453
C. labillardieri Clade 7	0.0232
C. lancelini	0
C. youngsoni	0.0009 – 0.0063
C. ora **sp. nov.**	0 – 0.0098

Table 5. Ranges of inter-clade uncorrected "p" genetic distances for the *C. labillardieri* species group.

Taxon	*C. catenifer*	*C. ora* sp. nov.	*C. gemmula*	*C. labillardieri* Clade 1	*C. labillardieri* Clade 2	*C. labillardieri* Clade 3	*C. labillardieri* Clade 4	*C. labillardieri* Clade 5	*C. labillardieri* Clade 6	*C. labillardieri* Clade 7	*C. lancelini*
C. ora sp. nov.	0.1538 – 0.1679	-	-	-	-	-	-	-	-	-	-
C. gemmula	0.1412 – 0.1574	0.1252 – 0.1315	-	-	-	-	-	-	-	-	-
C. labillardieri Clade 1	0.1474 – 0.1600	0.1093 – 0.1199	0.1162 – 0.1364	-	-	-	-	-	-	-	-
C. labillardieri Clade 2	0.1528 – 0.1574	0.1110 – 0.1169	0.1215 – 0.1275	0.0177 – 0.0296	-	-	-	-	-	-	-
C. labillardieri Clade 3	0.1439 – 0.1573	0.1030 – 0.1164	0.1136 – 0.1242	0.0337 – 0.0471	0.0275 – 0.0350	-	-	-	-	-	-
C. labillardieri Clade 4	0.1463 – 0.1635	0.1094 – 0.1208	0.1194 – 0.1358	0.0386 – 0.0580	0.0368 – 0.0548	0.0458 – 0.0621	-	-	-	-	-
C. labillardieri Clade 5	0.1483 – 0.1536	0.1037 – 0.1103	0.1233 – 0.1278	0.0440 – 0.0541	0.0402 – 0.0431	0.0465 – 0.0532	0.0484 – 0.0574	-	-	-	-
C. labillardieri Clade 6	0.1475 – 0.1601	0.1164 – 0.1244	0.1296 – 0.1376	0.0568 – 0.0719	0.0549 – 0.0648	0.0613 – 0.0773	0.0601 – 0.0746	0.0610 – 0.0702	-	-	-
C. labillardieri Clade 7	0.1456 – 0.1552	0.1142 – 0.1238	0.1248 – 0.1433	0.0615 – 0.0727	0.0606 – 0.0682	0.0623 – 0.0694	0.0636 – 0.0739	0.0616 – 0.0672	0.0563 – 0.0687	-	-
C. lancelini	0.1610 – 0.1689	0.0426 – 0.0453	0.1288 – 0.1350	0.1057 – 0.1146	0.1078 – 0.1084	0.1021 – 0.1084	0.1112 – 0.1190	0.1049 – 0.1057	0.1111 – 0.1209	0.1169 – 0.1229	-
C. youngsoni	0.1677 – 0.1738	0.1721 – 0.1780	0.1622 – 0.1753	0.1572 – 0.1737	0.1601 – 0.1697	0.1601 – 0.1697	0.1537 – 0.1693	0.1572 – 0.1631	0.1686 – 0.1788	0.1561 – 0.1674	0.1693 – 0.1707

Table 6. Summary statistics for the morphological data collected on members of the *Ctenotus labillardieri* group (mean±SD and range). See Table 2 for character definitions and Materials and Methods for details of statistical tests.

Character	*C. catenifer*	*C. delli*	*C. gemmula*	*C. labillardieri*	*C. lancelini*	*C. ora* sp. nov.	*C. younsoni*
n	16	14	18	54	11	5	14
SVL	49.44 ± 2.44 (41-56)	49.36 ± 2.60 (38-59)	48.17 ± 2.30 (29-59)	58.70 ± 1.33 (29-75)	61.91 ± 2.94 (30-78)	47.40 ± 4.36 (33-60)	72.71 ± 2.61 (56-83)
HeadL	11.51 ± 0.38 (8.9-12.7)	11.81 ± 0.41 (8.9-13.4)	11.13 ± 0.36 (8.1-12.5)	12.79 ± 0.21 (8.3-18.3)	13.27 ± 0.46 (8.6-15.3)	11.88 ± 0.69 (9.0-14.4)	15.76 ± 0.41 (13.7-17.0)
HLL	18.94 ± 0.83 (16-21)	20.21 ± 0.89 (16-24)	20.22 ± 0.78 (15-29)	23.61 ± 0.45 (13-30)	24.64 ± 1.00 (14-30)	19.60 ± 1.49 (15-23)	30.07 ± 0.89 (25-34)
FLL	12.38 ± 0.53 (9-14)	12.64 ± 0.57 (9-16)	12.61 ± 0.50 (10-15)	14.94 ± 0.29 (8-18)	15.09 ± 0.64 (10-18)	13.60 ± 0.95 (11-18)	18.93 ± 0.57 (15-28)
ILL	27.89 ± 1.61 (22-35)	27.36 ± 1.72 (18-39)	27.25 ± 1.52 (15-35)	31.56 ± 0.88 (14-44)	40.10 ± 1.94 (17-52)	27.04 ± 2.88 (18-35)	40.79 ± 1.72 (30-53)
TailL	83.25 ± 5.89 (66-101)	92.00 ± 6.15 (63-141)	85.80 ± 5.27 (51-113)	98.14 ± 4.35 (45-130)	107.86 ± 7.71 (60-142)	78.67 ± 11.78 (65-88)	124.63 ± 7.21 (108-149)
LorH:W	0.72 ± 0.03 (0.41-1.00)	0.77 ± 0.03 (0.63-0.94)	0.73 ± 0.03 (0.44-0.91)	0.69 ± 0.02 (0.46-0.92)	0.76 ± 0.04 (0.63-1.00)	0.81 ± 0.05 (0.73-0.91)	0.68 ± 0.03 (0.59-0.83)
MidB	25.13 ± 0.36 (22-28)	26.86 ± 0.39 (24-30)	24.00 ± 0.34 (22-26)	26.04 ± 0.20 (23-29)	22.73 ± 0.44 (20-24)	22.80 ± 0.65 (21-24)	28.50 ± 0.39 (26-32)
Lam4Toe	22.67 ± 0.48 (19-24)	23.46 ± 0.51 (20-26)	23.11 ± 0.44 (20-26)	23.83 ± 0.26 (19-28)	23.11 ± 0.62 (22-25)	24.20 ± 0.83 (23-27)	21.14 ± 0.49 (18-23)
VentS	42.63 ± 0.74 (37-50)	43.43 ± 0.79 (39-49)	41.83 ± 0.70 (37-46)	45.34 ± 0.41 (40-54)	43.18 ± 0.89 (41-47)	43.40 ± 1.37 (41-48)	42.21 ± 0.79 (41-46)
NasSS	Y (100%)	N (14%) Y (86%)	Y (100%)	Y (100%)	Y (100%)	Y (100%)	N (100%)
PrefSS	Y (100%)	N (7%) Y (93%)	N (11%) Y (89%)	N (11%) Y (89%)	Y (100%)	Y (100%)	N (36%) Y (64%)
SOS	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)
SOSContact	2 (87%) 3 (6%) 4 (6%)	2 (79%) 3 (14%) 4 (7%)	2 (100%)	2 (92%) 3 (4%) 4 (4%)	2 (91%) 3 (9%)	2 (100%)	2 (100%)
SupCil	7 (88%) 8 (12%)	7 (58%) 8 (42%)	7 (76%) 8 (24%)	7 (49%) 8 (49%) 9 (2%)	7 (67%) 8 (33%)	7 (40%) 8 (60%)	7 (62%) 8 (38%)
Palp	8 (10%) 9 (20%) 11 (70%)	9 (14%) 10 (14%) 11 (43%) 12 (29%)	8 (8%) 9 (29%) 10 (14%) 11 (21%) 12 (21%) not scored (7%)	7 (2%) 8 (13%) 9 (15%) 10 (24%) 11 (33%) 12 (13%)	7 (20%) 10 (20%) 11 (60%)	11 (60%) 12 (20%) unscorable (20%)	11 (9%) 12 (64%) 13 (27%)
UppLab	7 (94%) 8 (6%)	7 (100%)	7 (16%) 8 (79%) 9 (5%)	7 (59%) 8 (41%)	8 (100%)	7 (60%) 8 (40%)	8 (100%)
EarL	3 (63%) 4 (31%) 5 (6%)	3 (25%) 4 (67%) 5 (8%)	3 (26%) 4 (63%) 5 (11%)	3 (30%) 4 (61%) 5 (5%) not scored (4%)	3 (73%) 4 (27%)	3 (80%) 4 (20%)	3 (86%) 4 (14%)
EarLPos	1 (40%) 2 (60%)	1 (8%) 2 (83%) 3 (9%)	1 (5%) 2 (84%) 3 (6%) 4 (5%)	1 (7%) 2 (70%) 3 (17%) 4 (2%) not scored (4%)	1 (27%) 2 (64%) 3 (9%)	1 (40%) 2 (60%)	1 (86%) 2 (14%)

The unweighted parsimony analyses and the Bayesian analyses of the mitochondrial data recovered a well-resolved topology with strong support for relationships among major clades (Figure 2a). As expected, analyses of the nDNA data set produced a much more conservative topology (Figure 2b), but one that is highly concordant with the mtDNA tree. Figure 3 shows the results of the partitioned Bayesian analysis of the combined multi-gene data set. Results of the un-partitioned analyses were virtually identical and are not shown. The combined multi-gene analysis produced a well-resolved and well-supported topology that is very similar to both individual gene trees. We focus description of the phylogeny and the discussion on results from the combined multi-gene data set.

The species *C. labillardieri* comprises seven well-supported clades (Figure 3) that occupy distinct geographic regions (Figure 1). There is relatively little support for the relationships between these *C. labillardieri* clades. The species *C. catenifer, C. youngsoni*, and *C. gemmula* are each very well supported with both *C. catenifer* and *C. gemmula* showing phylogeographic structure (Figure 3). The threatened species *C. lancelini* from Lancelin Island and the adjacent mainland is the sister taxon to three individuals that had been identified as *C. labillardieri*. Our molecular data demonstrate that these animals, from the Swan Coastal Plain, instead are more closely related to *C. lancelini*. We describe these animals, plus two additional specimens, as a new species below.

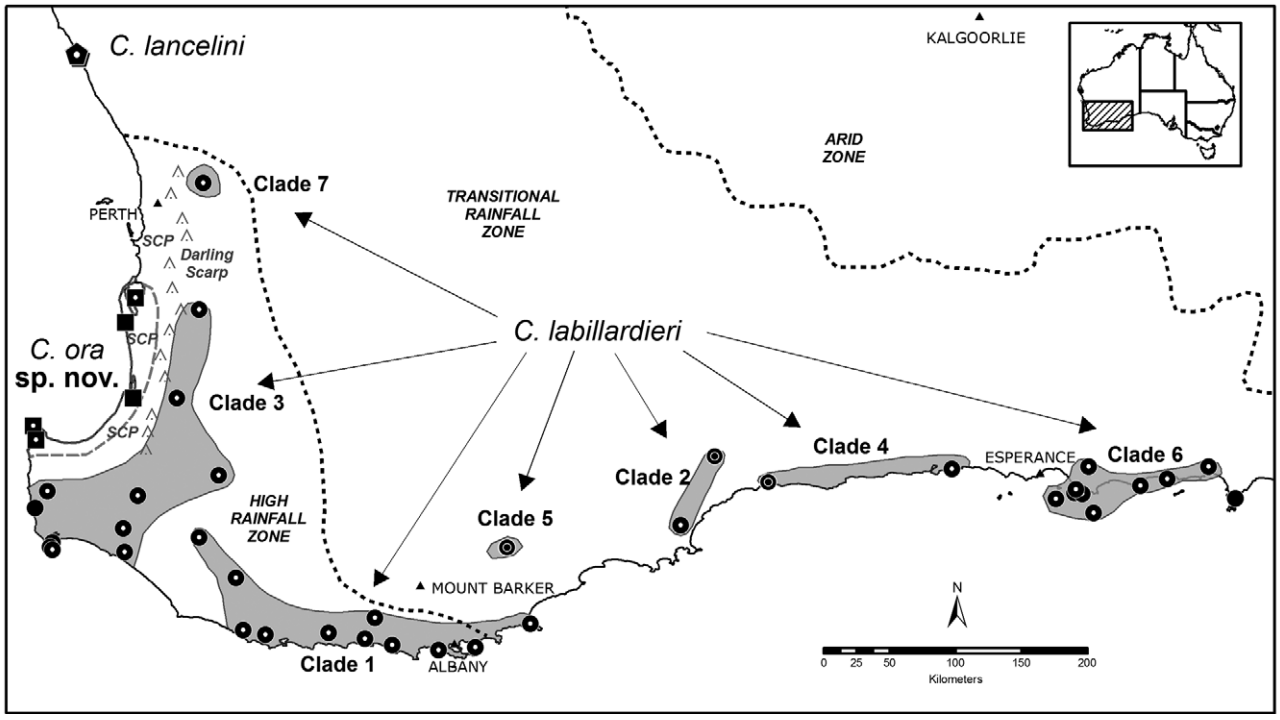

FIGURE 1. Distribution of the seven major *C. labillardieri* (circle) clades, the *C. lancelini* (pentagonal) clade and the new taxon, *C. ora* **sp. nov** (square). Specimens used in both the genetic and morphological analyses are denoted with a white center, and specimens for which we only had morphological data are solid. The few specimens of *C. labillardieri* for which we only had tissue samples are denoted with an inner white ring. SCP refers to the Swan Coastal Plain west of the Darling Scarp. Black dotted lines represent boundaries between rainfall zones, as defined by Hopper (1979). Grey dotted line demarks the known range for *C. ora* **sp. nov.**

Morphological data. Table 6 summarizes the morphological measurements. As expected, there is considerable overlap between species in most of the continuous and categorical variables measured but the species vary consistently in body size, shape and colouration. We summarize the results of our PCA analysis on the continuously distributed characters in Figure 4 where we show mean PC scores and standard deviations. PC1 explains 51.8% of the variation, PC2 explains 10.5% of the variation and the mean PC scores varied significantly among taxa (PC1: $F_{6,132} = 18.55$, $P < 0.0001$; PC2: $F_{6,132} = 11.70$, $P < 0.0001$). As expected, the first principal component was very highly correlated with snout-vent length and thus body size (SVL, $r^2 = 0.91$, $P < 0.001$) and the second principal component summarized shape and important scale differences among the species. Additional principal components explained negligible amounts of the variation. *C. younsoni* is the largest of the species, *C. labilardieri* and C. *lancelini* intermediate in size, and *C. catenifer, C. delli, C. gemmula* and *C. ora* **sp. nov.** (described below) the smallest (Figure 4, Table 6). *C. younsoni* is also the most divergent in terms of shape and meristics, *C. labilardieri* and *C. lancelini* are similar to each other in, and *C. catenifer, C. delli* and *C. gemmula* are extremely similar. *C. ora* **sp. nov.** (described below) is divergent from all the other species (Figure 4). Additional qualitative differences in coloration and color pattern are also important in distinguishing species.

Taxonomy. The genetic analysis provides a robust hypothesis of relationships among the species in the *C. labillardieri* species group and in addition demonstrates strong phylogeographic structure in *C. labillardieri*. Our detailed morphological analysis of all specimens included in our genetic work, and additional specimens, demonstrates that *C. labillardieri* is morphologically relatively homogeneous. Additional PCA of just *C. labillardieri*

specimens did not show any clear patterns of morphological variation relative to the seven identified genetic clades (not shown), therefore we represent *C. labilllardieri* as one entity in Figure 4. *Ctenotus labillardieri* does display geographic variation in back patterns (Figure 5), but this variation also does not correspond to the seven identified genetic clades. Therefore, we treat *C. labillardieri* a single species. The one exception to this is a clade previously identified as *C. labillardieri* based on morphology, but our genetic data show that it instead is most closely related to *C. lancelini*. Our morphological data show that these animals are morphologically divergent from both *C. labillardieri* and *C. lancelini* (Figure 4; Table 6) and we describe it as a new species below. We examined photographs of the type specimen of *Hinulia greyi* Gray 1845, a name now in synonymy of *C. labillardieri*, and confirmed that this specimen is not the new taxon we describe here.

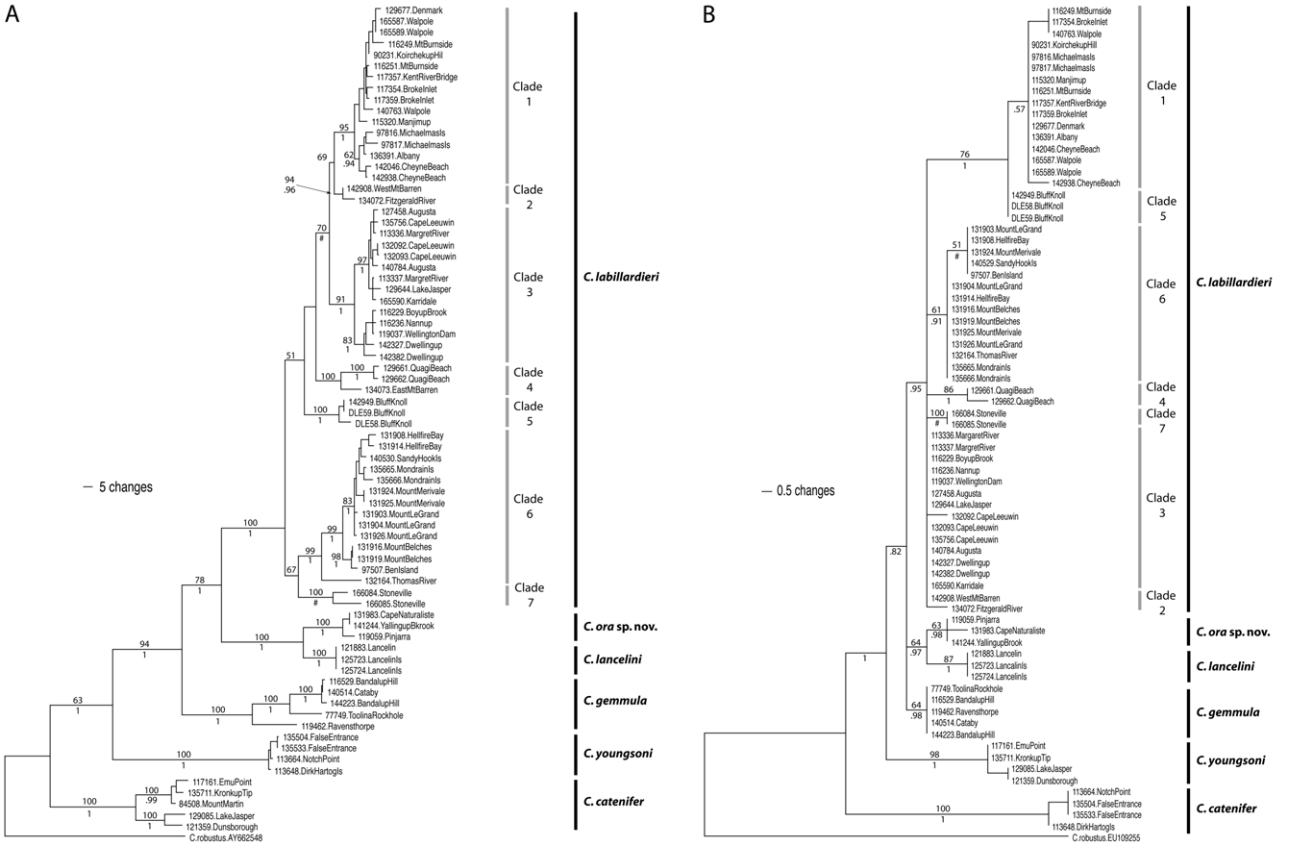

FIGURE 2. Individual gene trees for the two genes used in this study. A) Representative parsimony phylogram for the ND2 data set. B) Representative phylogram from the nDNA data set. Values on selected branches refer to parsimony bootstrap values above the branch and Bayesian posterior probabilities below.

***Ctenotus ora* sp. nov.**
Coastal Plains Skink
(Figures 5j, 6, 7)

Holotype. WAM R131983. Type locality: Cape Naturaliste at 33°32`21"S, 115°01`13"E. Collected by M. D. Shapiro on 4th November 1997.

Paratypes. R73591 – Yalgorup National Park, 32°50`00"S, 115°39`00"E; R81601 – Eaton, 33°21`00"S, 115°42`00"E; R119059 – Lake Mealup (15km WSW Pinjarra), 32°40`00"S, 115°43`00"E; R141244 – Yallingup Brook, 33°38`39"S, 115°02`15"E.

Diagnosis. *Ctenotus ora* is distinguished from sister taxon *C. lancelini* by its smaller size, generally darker colouration and lack of vertebral stripes (see Ford 1969). It is distinguishable from *C. gemmula*, *C. delli* and *C. catenifer* by a continuous white dorsolateral line, and from *C. youngsoni* by its smaller size and sharper dorsal patterning (Figure 5). *C. ora* can be distinguished from *C. labillardieri* by its smooth copper-brown dorsum and absence of white specks above the dorsolateral line.

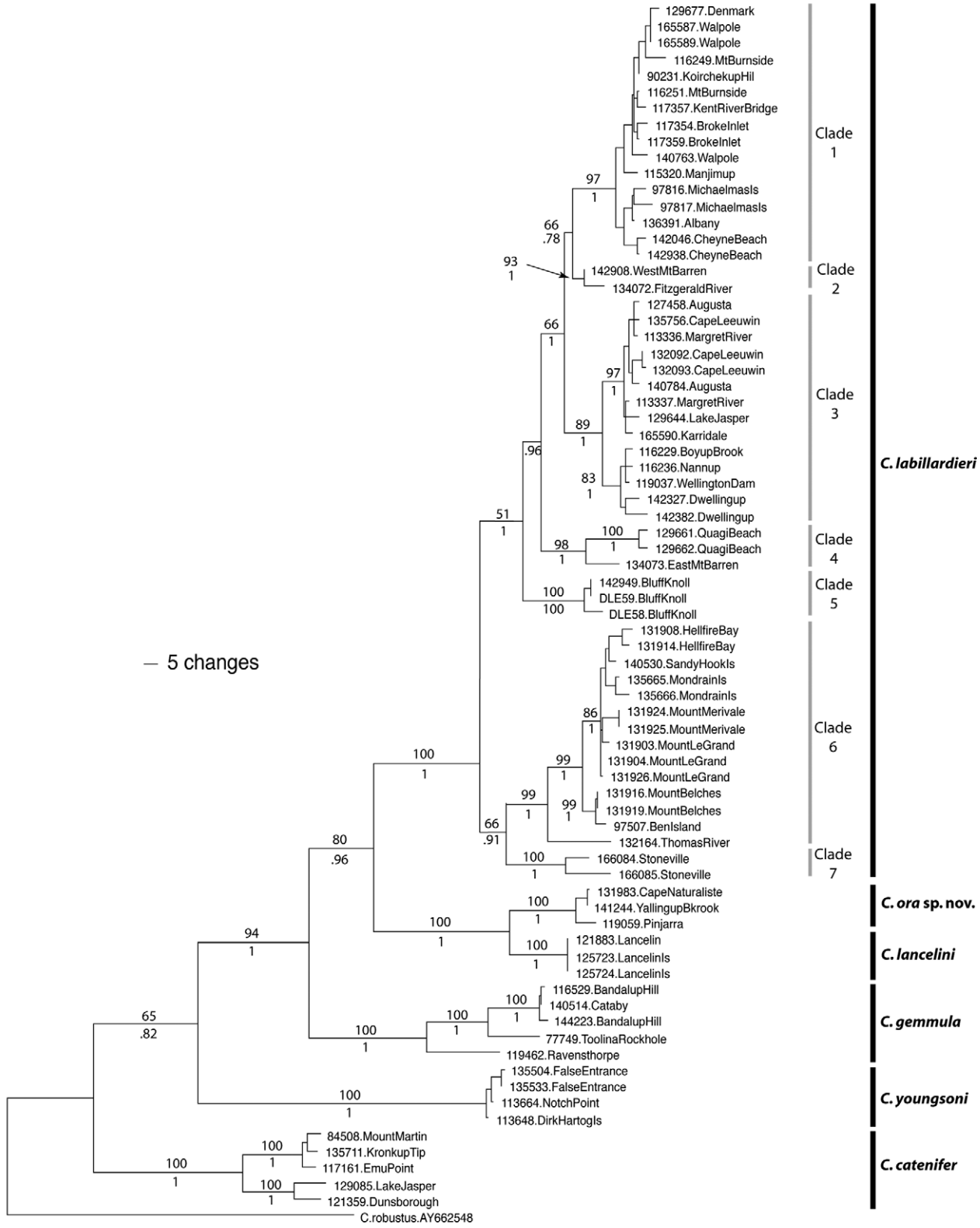

FIGURE 3. Phylogram based on analysis of the combined data set. Values on selected branches refer to parsimony bootstrap values above the branch and Bayesian posterior probabilities below.

Description. A small to medium-sized (maximum SVL 60mm) member of the *Ctenotus labillardieri* species group. Measurements for 19 morphological characters are summarized in Table 6. In addition to these: external ear opening prominent, small and ovate, about half the diameter of eye; snout triangular in profile with nose rounded; body slender, pentadactyl limbs; forelegs extend beyond the eye when adpressed; hindlimbs long, reaching beyond

two-thirds of the axilla-groin length when adpressed; digits moderately long and slender; finger length: 4>3>2>5>1; toe length: 4>3>5>2>1; tail round in cross-section with very gradual taper to its pointed tip; head scales smooth; nasals separated; prefrontals separated; supraoculars four, with first two in contact with frontal; ear lobules three, occasionally four, with either the 1st or 2nd the largest.

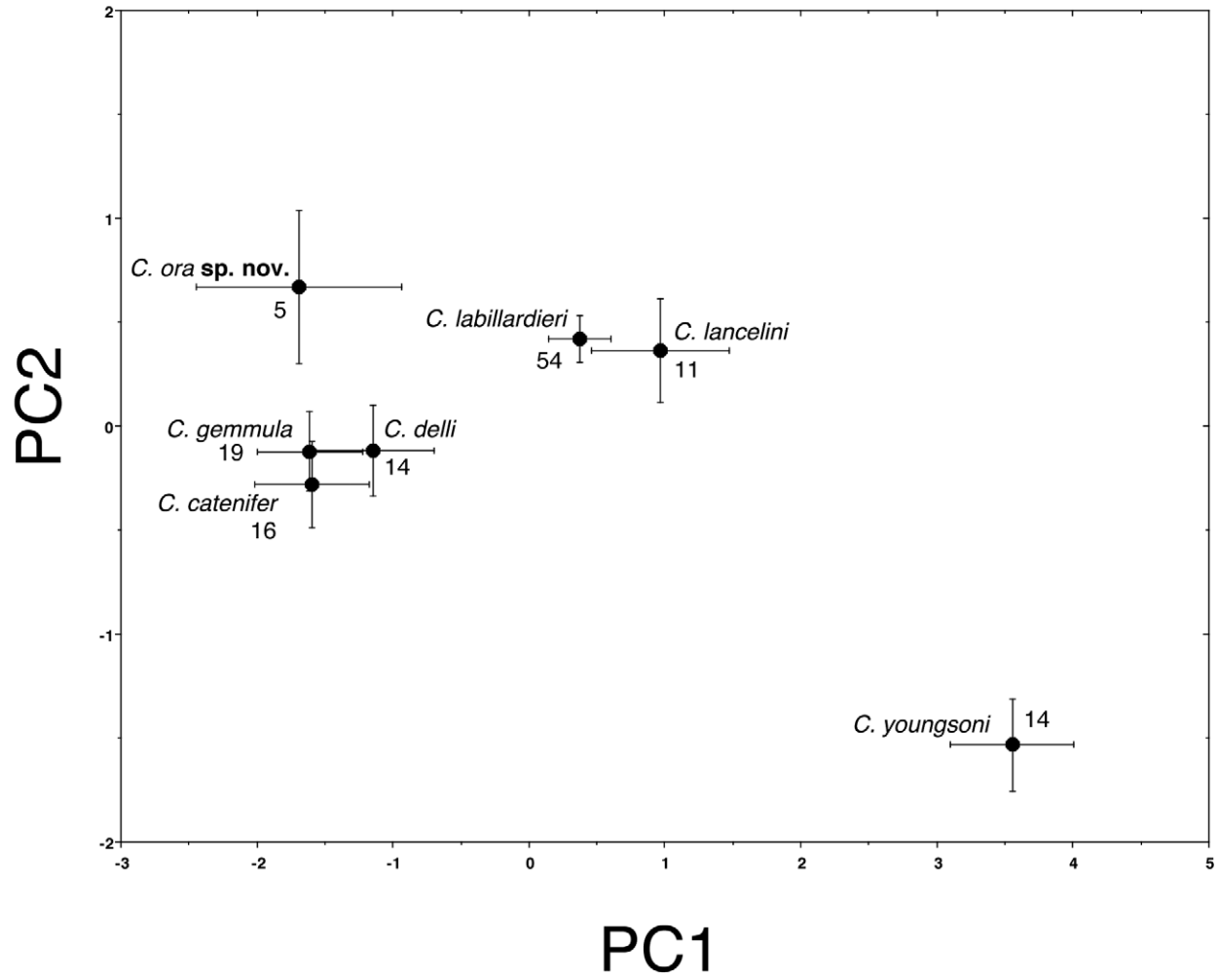

FIGURE 4. Summary of results for the principal component analyses (PCAs) of the morphological data. Mean principal component scores and standard deviations are shown with sample sizes noted.

Colouration. Dorsal surface bronze-brown, without any black pigmentation within the bronze-brown ground colour, creating a smooth appearance; white dorsolateral, midlateral and ventrolateral stripes, the latter much less sharp and defined than the former two; below each white stripe is a dark brown-black band, the most ventral of which is narrowest and least defined (Figure 5); some fine white flecks between the dorsolateral and upper lateral stripes; chin and throat uniform whitish-grey in preserved specimens; digital lamellae with slightly darker pigmentation; legs reddish-orange with black patches covering nearly half of each leg (Figure 7).

Description of holotype.

SVL – 58mm; HeadL – 14.4mm; HLL – 23mm; FLL – 15mm; ILL – 35mm; TailL – 88mm; LorH:W – .91; MidB – 22; Lam4Toe – 24; VentS – 48; NasSS – Yes; PrefSS – Yes; SOS – 4; SOSContact – 2; SupCil – 8; Palp – 11; UppLab – 8; EarL – 3; EarLPos – 2.

Variation. Table 6 presents the morphological variation for the 19 characters measured. Juveniles show the same overall colour patterns, but with somewhat finer black blotching on legs. There appears to be little geographic variation.

Habitat. Specimen WAM R119059 was found under a banksia log in open eucalypt woodland over *Banksia attenuata* and *Banksia grandis* on white sand. Specimen WAM R73591 was found in *Corymbia calophylla* over heath in sandy soil. The species seems to have a preference for sandy substrates with low vegetation with open *Eucalyptus* woodland over *Banksia* (B. Maryan, pers. comm.)

Distribution. This species appears to be restricted to the SWA coastal plain west of the Darling Range, south of Perth, Western Australia. In addition to the specimens examined and listed in Table 1, we examined photographs of specimens from Lake Clifton on the Swan Coastal Plain and these appear to also represent *C. ora* (WAM R17966-68). It is known to occur as far north as Pinjarra and south as far as Yallingup Brook, where it occupies coastal dunes. Across its range it occurs in very low densities, in contrast to neighbouring *C. labillardieri* populations, which are found in great abundance throughout the Darling Range (B. Maryan, pers. comm.).

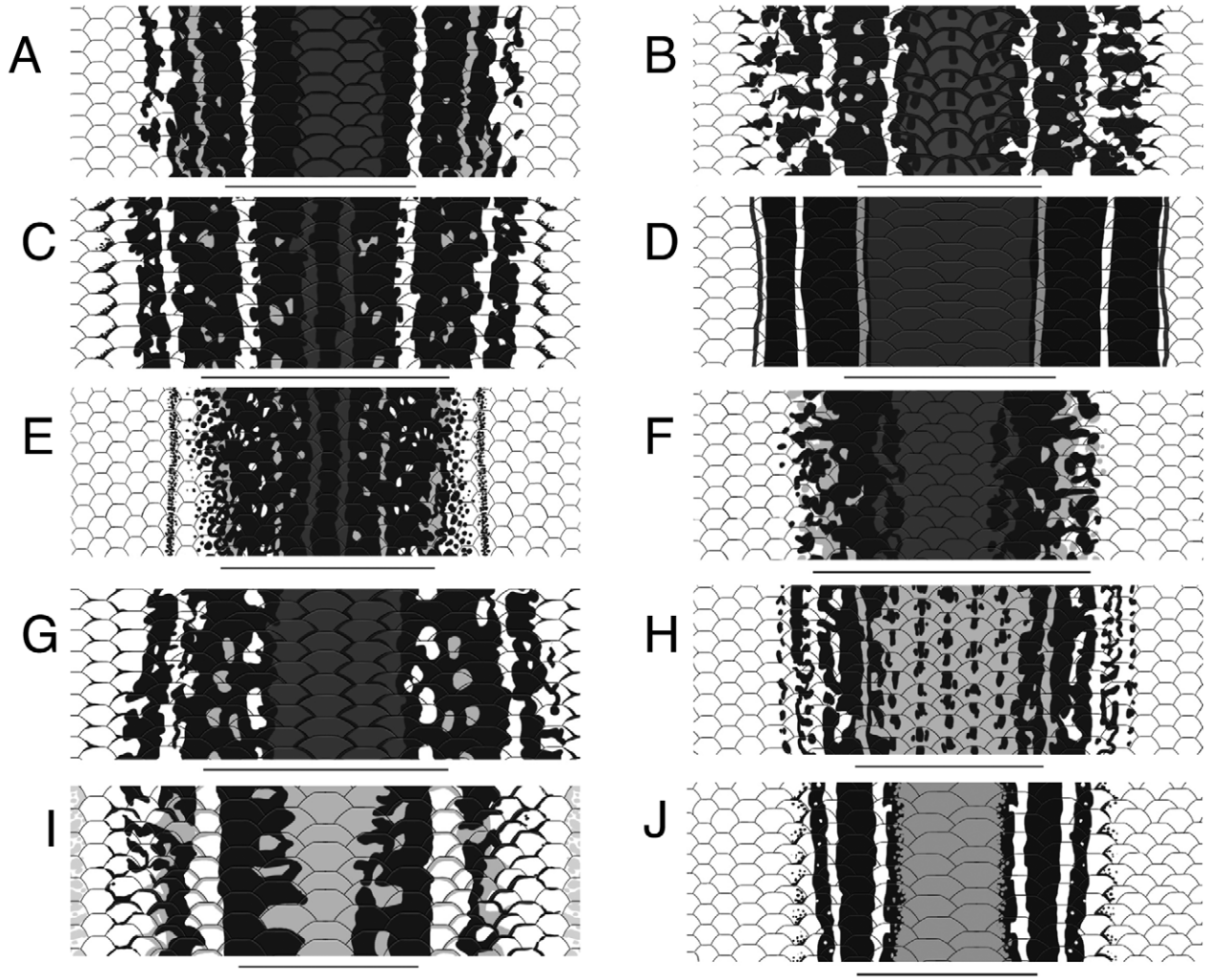

FIGURE 5. Back patterns in the *Ctenotus labillardieri* species group. A–D illustrate geographic variation in *C. labillardieri* and E–J show representative back patterns in the other species in the group. The variation shown within *C. labillardieri* is not confined to the clades illustrated. A) *C. labillardieri* (R135665; lineage 6), B) *C. labillardieri* (R117354, lineage 1), C) *C. labillardieri* (R142908, lineage 2), D) *C. labillardieri* (166085, lineage 7), E) *C. catenifer* (R163143), F) *C. delli* (R13444), G) *C. gemmula* (R150245), H) *C. lancelini* (R18874), I) *C. youngsoni* (R66208), J) *C. ora* **sp. nov.** (R131983). Scale bars are all 1cm.

Etymology. *ora* is Latin for "coast", "seaside" or "shore" and is in reference to the coastal distribution of the species.

Similar species. Despite large morphological variation within *C. labillardieri* in color patterns, *C. ora* can be readily distinguished by its smooth copper-brown dorsum and absence of white specks above the dorsolateral line. All *C. labillardieri* (with the exception of Clade 2) shared varying degrees of melanism on the dorsal surface scales, creating either an unconnected pattern of dark flecks, or a connected set of one or two vertebral stripes (Figure 5). Furthermore, *C. ora* lacks the heavily speckled flanks present in all *C. labillardieri* clades, with the exception of *C. labillardieri* Clade 2. Instead, both *C. ora* and *C. labillardieri* Clade 2 possess two dark brown and one white ventrolateral stripes with Clade 2 being distinguished from *C. ora* by the most ventral brown zone much more solid and defined. In addition, *C. labillardieri* Clade 2 lacks any white specks between the dorso-lateral and mid-lateral stripes, giving these individuals a uniquely "immaculate" appearance overall, in contrast to the subtle

white speckling in *C. ora*. Finally, the ventral surface of *C. ora* is whitish and clean where *C. labillardieri* Clade 2 individuals have dark flecks under the chin and throat.

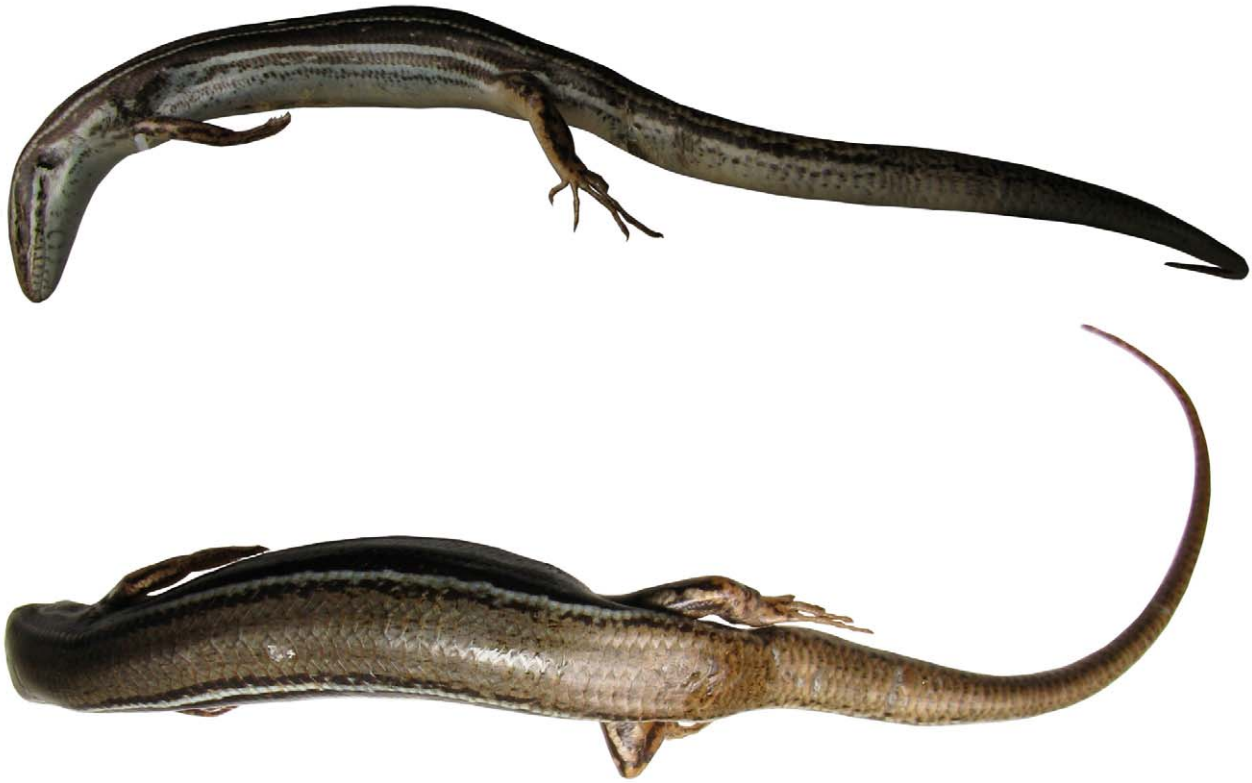

FIGURE 6. Photographs of the holotype of *Ctenotus ora* **sp. nov.** (WAM R131983).

FIGURE 7. Photograph in life of *Ctenotus ora* **sp. nov.** from Pinjarra, Western Australia (WAM R119059).

Discussion

Molecular phylogeny of the *C. labillardieri* species group

A morphological revision of *Ctenotus* by Storr (1974), which included *C. labillardieri*, resulted in the recognition of several new species, including *C. catenifer*, *C. delli*, and *C. gemmula*, which together with *C. youngsoni* and the threatened *C. lancelini* form the larger '*labillardieri* group' (Storr 1974; Storr *et al.* 1999). Our molecular data provides the first detailed phylogeny for these taxa and strong support for the species status of all members for which we could obtain tissues (no tissues available for *C. delli*). Our molecular and morphological data further provide important information on phylogeographic structuring and morphological variation in *C. labillardieri*, the first such published data set for a reptile in SWA, and evidence for a new species of *Ctenotus*. Each of the species in the *C. labillardieri* species group is 10-18% divergent in mtDNA from the other species, with the exception of *C. lancelini* and *C. ora* **sp. nov.**, which are approximately 4% divergent. We first outline the phylogeographic structure and morphological variation in *C. labillardieri*, followed by some brief comments on *C. catenifer* and *C. gemmula*. Lastly, we consider the newly described species *C. ora* **sp. nov**.

Phylogeography of *C. labillardieri* clades

Our molecular phylogeny shows that *C. labillardieri* displays considerable intraspecific genetic diversity with strong support for seven genetic clades (Figure 1, 3). The genetic distances between many of these clades (Table 4 and 5) are equivalent to species-level differences in other reptiles using mitochondrial genes that evolve at a similar rate (e.g. ND4, Scott & Keogh 2000), but importantly, our morphological data show high levels of geographic variation that do not correspond well to the genetic patterns. Therefore, we maintain that *C. labillardieri* should be considered a single but morphologically variable species.

Clades 1, 3 and 7 are restricted to the High Rainfall Zone (HRZ) of southwestern Australia. The biogeographic break between Clades 1 and 3 coincides with boundaries recognised in multiple taxonomic groups, including plants (*Lambertia orbifolia* [Byrne *et al.* 1999]), frogs (*Geocrinia rosea* species complex [Wardell-Johnson & Roberts 1993]; *Geocrinia leai* [Edwards 2007b]; *Metacrinia nichollsi* [Edwards *et al.* 2008]) and freshwater invertebrates (*Engaewa subcoerulea* and *E. similis* [Horwitz & Adams 2000]; *Cherax preissii* [Gouws *et al.* 2006]). Contraction to moist refugia during arid pulses of the Pliocene/Pleistocene (Dodson & Macphail 2004) is thought to have promoted much of this divergence. This seems like a plausible explanation for the patterns seen in our study given the obvious ecological preference of *C. labillardieri*, where dependence on wet restricted sites such as granite outcrops is likely to have resulted in limited gene flow between populations during arid periods. Indeed, a previous study of *C. labillardieri* suggested that the isolation of populations to granite outcrops accounted for observed morphological differences throughout SWA (Ford 1969). However, habitat differentiation may also be important for diversification in this taxon. For example, the distribution of Clade 1 appears to largely coincide with the Warren biogeographic subregion, defined under the Interim Biogeographical Regionalisation of Australia (IBRA), further supporting its recognition as a distinct bioregion (Thackway & Cresswell 1995). In addition, the eastern extent of the distribution of Clade 1 coincides with the Transitional Rainfall Zone (TRZ) / HRZ climatic boundary, recognized as a significant climatic barrier for plants (Hopper 1979; Hopper & Gioia 2004) and frogs (Edwards *et al.* 2007, 2008). However, the close relationship between Clade 2 in the TRZ and Clades 1 and 3 in the HRZ suggests a more recent genetic connection with subsequent geographic isolation across the TRZ / HRZ boundary. Both Clades 4 and 5 appear to be geographic isolates within the TRZ. This is consistent with climatic data suggesting long-term geographic isolation as the primary driver for the huge diversity of endemic species and populations found in the Stirling Ranges (Clade 5) (Erika *et al.* 1993; Main 1996; Hopper & Gioia 2004; Edwards *et al.* 2008; Rix & Harvey 2012). Similarly, Clade 4 may have experienced a prolonged period of geographic isolation based on strong support by the nuclear data. This is consistent with patterns in plants (Byrne & Hines 2004) and frogs (Edwards *et al.* 2007a) which implicate a xeric barrier east of the Bremer Bay region as the primary cause for this isolation. The distributions of clades 2 and 4 also fall within different IBRA subregions of the Esperance Plains; the Fitzgerald (Clade 2) and Recherche (Clade 4). Both regions comprise subdued coastal sandplains on the coast, but differ in environmental aspects such as rainfall and geological substrate (Environment Australia 2004).

Biogeographic comments on *C. catenifer* and *C. gemmula*

While limited sampling precludes any detailed phylogeographic comments, we found substantial genetic structure in *C. catenifer* and *C. gemmula* (Table 4). Interestingly, the two clades recovered in *C. catenifer* have geo-

graphic distributions much like *C. labillardieri* clades 1 and 3, adding further support that this region has historically been an important biogeographic barrier to moist-adapted taxa. Our sampling of *C. gemmula* recovered two divergent individuals, each represented by a single individual. A third clade contains closely related individuals from Cataby near Perth, and Bandalup, west of Esperance. This pattern is remarkably similar to *C. labillardieri* clades 6 and 7 (albeit with low support in *C. labillardieri*) suggesting recent connectivity despite large geographic distances across the TRZ.

***Ctenotus ora,* a new species of immediate conservation concern**

Ctenotus ora **sp. nov.** comprises specimens that previously were allocated to the widely distributed and morphologically variable *C. labillardieri*. Our genetic data demonstrate that it instead is the sister taxon to the threatened *C. lancelini*. *Ctenotus lancelini* is distributed only on Lancelin Island and the immediate adjacent mainland. A recovery plan for this species outlined unpublished allozyme data (Mark Adams, South Australian Museum) and morphological evaluation (Ken Aplin, Western Australian Museum) which suggested 'the existence of a taxon closely related or the same as *C. lancelini*' and made a call for detailed genetic and morphological data to sort out this problem (Pearson & Jones 2000). Our molecular and morphological data resolve this issue and demonstrate that *C. lancelini* and *C. ora* **sp. nov.** are closely related but genetically and morphological quite distinct species, each of which are habitat specialists. *Ctenotus lancelini* is threatened and a recovery plan is in place for its long-term survival (Pearson & Jones 2000). *Ctenotus ora* **sp. nov.** is restricted in distribution to the sandy Swan Coastal Plain subregion south of Perth, a region now under heavy development from population growth from Perth, and an area of biogeographic significance (Horwitz & Adams 2000; Wheeler & Byrne 2006). *Ctenotus ora* **sp. nov.** is known to be very sparsely distributed in the region and may already be under threat. The conservation status of this new species should be assessed immediately.

Acknowledgements

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