Evolution of foraging behaviour: deep intra-generic genetic divergence between territorial and non-territorial southern African patellid limpets

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ABSTRACT

Southern Africa is a biodiversity hotspot of patellid limpets, with three genera (*Helcion*, *Cymbula* and *Scutellastra*) identified and described in the region. *Scutellastra* is the most diverse and most frequently studied of these and, along with *Cymbula*, includes species with territorial and non-territorial foraging behaviours. We used three mitochondrial markers (12S rRNA, 16S rRNA and COI) and one nuclear marker (ATPSβ intron) to assess evolutionary relationships

amongst species of *Cymbula* and *Scutellastra* with these two foraging behaviours and to identify which foraging mode is the more ancient. Maximum Likelihood and Bayesian Inference phylogenetic analyses revealed that the species sharing a foraging type are monophyletic in both genera. Territoriality is a derived character, as the clades with this foraging type are nested within a tree that otherwise comprises non-territorial taxa. These include *Helcion*, which was recovered as sister to the *Cymbula/Scutellastra* clade, and the next basal genus, *Patella*, which is ancestral to all southern African patellogastropos. Deep genetic divergence between the two foraging traits reflects strong adaptive effects of resource partitioning in the evolution of southern African patellid limpets.

Keywords:

Ecological adaptations, Evolutionary divergence, Molecular phylogeny, Patellid limpets, Southern Africa, Territorial/non-territorial forager

1. Introduction

The evolution of ecological specialization and generalization remains one of the most enigmatic phenomena in evolutionary biology (Berenbaum, 1996; Poisot et al., 2011) and previous studies have indicated that evolutionary transitions could possibly occur in either direction (Poisot et al., 2011). The patellogastropods (patellid limpets) have evolved two major types of foraging mechanisms, namely ecological specialization, often associated with territorial foraging, and ecological generalization, usually associated with non-territorial foraging (Branch, 1985). While the molecular phylogenetics of patellogastropods are very well understood (Nakano and Sasaki,

2011), it is not clear whether their foraging traits followed the transitional model of generalists evolving into specialists or vice versa. For example, Lindberg (2007) superimposed the foraging traits of patellid limpets on their 16S rRNA phylogenetic tree and suggested that territoriality is an ancestral trait, because territorial species were grouped in the basal clades. However, this has not been tested using multiple markers and a diversity of sympatric species with different foraging traits. The southern African shoreline is an epicentre of diversity of patellid limpets, and this high biodiversity makes it particularly suitable to investigate the evolutionary relationships between territorial and non-territorial foragers.

Patellid limpets have been re-reclassified into seven families: Eoacmaeidae, Lepetidae, Lottidae, Nacellidae, Neolepetopsidae, Pectinidontidae and Patellidae (Nakano and Sasaki, 2011). The family Patellidae Rafinesque, 1815 is the best studied group, and so far, 39 patellid species have been described and identified worldwide (Nakano and Sasaki, 2011). Amongst these, 20 species are endemic to the southern African shoreline, from Angola on the west coast to the extreme north-east of South Africa on the east coast (Branch et al., 2010). Molecular and morphological phylogenetic analyses of the family Patellidae have identified four main monophyletic genera: *Patella, Helcion, Cymbula* and *Scutellastra,* the evolutionary relationships of which have been comprehensively documented (Harasewych and McArthur, 2000). *Scutellastra* Quoy and Gaimard, 1834 is the most broadly distributed and taxonomically problematic genus within the patellogastropods, as the evolutionary relationships of its species are poorly understood (Ridgway et al., 2000; Nakano and Sasaki, 2011). Molecular phylogenetic analysis subdivided the genus into three monophyletic subclades corresponding to their spatial range in southern Africa, temperate southern Australia and the tropical Indo-Pacific (Lindberg, 2007). Although

some of the species of *Scutellastra* (*S. argenvillei*, *S. granularis* and *S. miliaris*) were classified as monophyletic, the genus is globally paraphyletic (Lindberg, 2007; Nakano and Sasaki, 2011). To date, 17 species are recognised within this genus, and these range from southern Africa to the Pacific coast of Mexico (Nakano and Sasaki, 2011). The southern African shoreline is inhabited by 11 described territorial and non-territorial species of *Scutellastra*, of which five are endemic to South Africa (Branch et al., 2010). The evolutionary relationships amongst the southern African scutellastrid species remain unresolved due to the lack of synapomorphic characters that could be used to infer common ancestry. The aim of the present study was to use multiple markers to assess the evolution of foraging traits amongst species of *Cymbula* and *Scutellastra* along the southern African shoreline, giving a test, replicated across two related taxa, of whether ecological specialization in this group is a derived or an ancestral trait.

2. Materials and methods

2.1. Study species and sampling

Territorial and non-territorial species of both *Cymbula* and *Scutellastra* were selected based on their abundance, broad range and well-documented foraging behaviours (Branch, 1985). Specimens were identified from their shell morphology (Branch et al., 2010) and collected from 21 sites throughout their distribution ranges along the Angolan and South African coastlines. For each ingroup species, four to nine individual specimens were collected along the species' range plus two additional samples of *Helcion concolor* from Port St. Johns (Table 1). Samples were immediately preserved in 100% ethanol before commencement of the molecular protocol.

Table 1: The details of the samples analysed and corresponding GenBank Accession Numbers.

Species	Sample	Location	GenBank Accession Numbers			
			COI	12S rRNA	16S rRNA	ATPSβ
Scutellastra barbara[T]	CCB Sb2	Cape Columbine	LC075629	LC074885	LC081245	LC092118
	GRM Sb1	Groenriviermond	LC075630	LC074886	LC081246	LC092119
	LMB Sb3	Lambert Bay	LC075631	LC074887	LC081247	LC092120
	MSB Sb4	Mossel Bay	LC075632	LC074888	LC081248	LC092121
	TSK Sb4	Tsitsikamma	LC075633	LC074889	LC081249	LC092122
	PSJ Sb4	Port St. Johns	LC075634	LC074890	LC081250	LC092123
S. cochlear[T]	CPA Sc1	Cape Agulhas	LC075635	LC074892	LC081251	LC092124
	CMB Sc9	Camps Bay	LC075636	LC074893	LC081252	LC092125
	GRM Sc5	Groenriviermond	LC075637	LC074894	LC081253	LC092126
	KOS Sc12	Kenton-on-Sea	LC075638	LC074895	LC081254	LC092127
	HAG Sc4	Haga Haga	LC075639	LC074896	LC081255	LC092128
	TSK Sc1	Tsitsikamma	LC075640	LC074897	LC081256	LC092129
	MSB Sc2	Mossel Bay	LC075641	LC074898	LC081257	LC092130
S. longicosta[T]	CPA Sl1	Cape Agulhas	LC075642	LC074899	LC081258	LC092131
	HAG Sl9	Haga Haga	LC075643	LC074900	LC081259	LC092132
	KOS Sl5	Kenton-on-Sea	LC075644	LC074901	LC081260	LC092133
	TSK Sl2	Tsitsikamma	LC075645	LC074902	LC081261	LC092134
	MSB Sl3	Mossel Bay	LC075646	LC074903	LC081262	LC092135
S. argenvillei[NT]	CCB Sa7	Cape Columbine	LC075647	LC074906	LC081263	LC092136
	HAG Sa1	Haga Haga	LC075648	LC074908	LC081264	LC092137
	GRM Sa2	Groenriviermond	LC075649	LC074904	LC081265	LC092138
	KOS Sa2	Kenton-on-Sea	LC075650	LC074909	LC081266	LC092139
	LMB Sa6	Lamberts Bay	LC075651	LC074905	LC081267	LC092140
	MSB Sa2	Mossel Bay	LC075652	LC074907	LC081268	LC092141
S. granularis[NT]	HAG Sg14	Haga Haga	LC075653	LC074918	LC081269	LC092142
	KOS Sg9	Kenton-on-Sea	LC075654	LC074917	LC081270	LC092143
	SDB Sg7	Sardinia Bay	LC075655	LC074916	LC081271	LC092144
	TSK Sg9	Tsitsikamma	LC075656	LC074915	LC081272	LC092145
	MSB Sg6	Mossel Bay	LC075657	LC074914	LC081273	LC092146
	CPA Sg10	Cape Agulhas	LC075658	LC074913	LC081274	LC092147
	PTN Sg11	Paternoster	LC075659	LC074912	LC081275	LC092148
	DRB Sg13	Doring Bay	LC075660	LC074911	LC081276	LC092149
	GRM Sg10	Groenriviermond	LC075661	LC074910	LC081277	LC092150
S. natalensis[NT]	PED Sn3	Port Edward	LC075662	LC074919	LC081278	LC092151
	PKR Sn7	Park Rynie	LC075663	LC074921	LC081279	LC092152
	BLT Sn25	Ballito	LC075664	LC074920	LC081280	LC092153
	PSJ Sn13	Port St. Johns	LC075665	LC074922	LC081281	LC092154
S. miliaris[NT]	BAB Sm8	Baia de Bengo	LC075666	LC074924	LC081282	LC092155
	FMG Sm8	Flamingo	LC075667	LC074925	LC081283	LC092156
	LBT Sm2	Lobito	LC075668	LC074923	LC081284	LC092157
	LBT Sm7	Lobito	LC075669	LC074926	LC081285	LC092158
Cymbula granatina[NT]	DRB Cg9	Doring Bay	LC075670	LC074927	LC081286	LC092159
	GRM Cg7	Groenriviermond	LC075671	LC074928	LC081287	LC092160

	CMB Cg7	Camps Bay	LC075672	LC074930	LC081288	LC092161
	CCB Cg6	Cape Columbine	LC075673	LC074931	LC081289	LC092162
	PTN Cg11	Paternoster	LC075674	LC074932	LC081290	LC092163
C. oculus [NT]	PED Co1	Port Edward	LC075675	LC074933	LC081291	LC092164
	KOS Co7	Kenton-on-Sea	LC075676	LC074934	LC081292	LC092165
	SDB Co14	Sardinia Bay	LC075677	LC074935	LC081293	LC092166
	TSK Co1	Tsitsikamma	LC075678	LC074936	LC081294	LC092167
	MSB Co18	Mossel Bay	LC075679	LC074937	LC081295	LC092168
	CPA Co10	Cape Agulhas	LC075680	LC074938	LC081296	LC092169
	MZB Co12	Muizenberg	LC075681	LC074939	LC081297	LC092170
	CMB Co8	Camps Bay	LC075682	LC074940	LC081298	LC092171
C. miniata [T]	MSB Cm1	Mossel Bay	LC075683	LC074943	LC081299	LC092172
	GRM Cm5	Groenriviermond	LC075684	LC074947	LC081300	LC092173
	CCB Cm5	Cape Columbine	LC075685	LC074945	LC081301	LC092174
	CPA Cm1	Cape Agulhas	LC075686	LC074946	LC081302	LC092175
	MZB Cm5	Muizenberg	LC075687	LC074944	LC081303	LC092176
	PSJ Cm7	Port St. Johns	LC075688	LC074942	LC081304	LC092177
	HAGCm6	Haga Haga	LC075689	LC074941	LC081305	LC092178
C. compressa [T]	STD Cc1	Strandfontein	LC075690	LC074948	LC081306	LC092179
	STD Cc2	Strandfontein	LC075691	LC074949	LC081307	LC092180
	DRB Cc5	Doring Bay	LC075692	LC074950	LC081308	LC092181
	PTN Cc9	Paternoster	LC075693	LC074951	LC081309	LC092182
	CCB Cc3	Cape Columbine	LC075694	LC074952	LC081310	LC092183
	CMB Cc6	Camps Bay	LC075695	LC074953	LC081311	LC092184
	GRM Cc14	Groenriviermond	LC075696	LC074954	LC081312	LC092185
Helcion concolor [NT]	PSJ Hc1	Port St. Johns	LC088216	LC074955	LC081313	LC092186
H. concolor	PSJ Hc2	Port St. Johns	LC088217	LC074956	LC081314	LC092187
Patella aspera [NT]	Outgroup	Portugal	AJ291545	AF058203	AF058249	-
P. depressa [NT]	Outgroup	Spain	EF462972	AF058208	JF682569	-
P. vulgata [NT]	Outgroup	United Kingdom	AB238580	AF058213	AB238445	-

[T] = Territorial forager, [NT] = Territorial forager.

2.2 .DNA extraction, PCR and sequencing

Muscle tissue was excised from the foot of each specimen using alcohol-sterilized razor blades. The tissue was rinsed with double-distilled water and left in Tris-EDTA buffer overnight. Total genomic DNA was then extracted using the cetyltrimethyl ammonium bromide (CTAB) protocol by Doyle and Doyle (1987). Partial fragments of three mitochondrial markers (12S rRNA, 16S rRNA and COI) and one nuclear marker (ATPSβ intron) were amplified by Polymerase Chain Reaction (PCR) for each sample. The primer sequences for each region are presented in Table 2. The universal COI primers by Folmer et al. (1994) failed to amplify COI in the territorial *Scutellastra* species, so internal primers (Scut F1 and Scut R1) that specifically amplify a portion of the gene in these species were designed using the primer designer software *CLC Main Workbench 6.7* (CLC Bio). PCR amplifications, purifications and sequencing were conducted following protocols explained in Mmonwa et al. (2015) except higher annealing temperatures of 54 °C for 12S rRNA and 16S rRNA and 58 °C for ATPSβ. The GenBank accession numbers of all three mitochondrial markers (12S rRNA, 16S rRNA and COI) and nuclear ATPSβ for each sample are presented in Table 1.

2.3. Phylogenetic analyses

A partition-homogeneity test (Farris et al., 1995) in the program PartitionFinder (Lanfear et al., 2012) confirmed compatibility of the three mitochondrial markers (COI, 12S rRNA and 16S rRNA) and, consequently, the markers were concatenated and treated as a single partition. The

Table 2: Primers used in this study to amplify limpet DNA.

Marker	Primers	Primer Sequences	Reference
COI	LCO1490	5 '- TCAACAAATCAYAAAGAYATTGG - 3'	Folmer et al., 1994
	HCO2198	5' - AATTAAAATRTAWACTTCTGG - 3′	
COI*	Scut F1		
	Scut R1	5`-TCWGGYYTAGTHGGRAC-3`	This study
		5`- CAYARWASCATAGTRATDGC-3`	
12S rRNA	12Sar	5`- CTGGGATTAGATACCCCACTA-3`	Kocher et al., 1989
	12Sbr	5`-TGAGGAGGGTGACGGGCGGT-3`	
16S rRNA	16Sar	5’- CGCCTGTTTATCAAAAACAT-3’	Palumbi, 1996
	16Sbr	5’- GCCGGTCTGAACTCAGATCACGT-3’	
ATPSβ	ATPSβf1	5'-TGRATTCCCTGATGTTTTTGTGAG-3'	Jarman et al., 2002
	ATPSβf2	5'- CGGGCACGGGCRCCDGGNGGTTCGT-3'	

*Territorial *Scutellastra* species.

ATPSβ sequences of both *Cymbula* and *Scutellastra* were so different that they could not be aligned with each other. A ClustalW (Thompson et al., 1994) alignment performed in MEGA 6 (Tamura et al., 2013) resulted in an alignment of 342 bp, followed by Gblocks treatment to remove poorly aligned sections (Talavera and Castresana, 2007), resulting in a final alignment of 232 bp, and phylogenies constructed using this alignment were poorly resolved (not shown). This suggests that the intron originated independently in each genus, so we analysed the nuclear marker separately from the mtDNA sequences, and separately for each genus. Phylogenies for both the concatenated mtDNA data set and the ATPSβ data were reconstructed using a maximum likelihood (ML) approach with RAxML HPC 7.2.6 (Stamatakis, 2006), and Bayesian Inference (BI) in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). For both analyses, the GTR + I + G substitution model (Rodríguez et al., 1990) was specified as determined by ModelTest 3.7 (Posada and Crandall, 1998). Gaps were treated as missing data and uncertainties were coded as ambiguity characters. Support for nodes in the ML phylogeny was based on 1000 bootstrap replications (Felsenstein, 1985). For BI, two independent analyses, each with four chains (one cold chain and three hot chains) were run for 20,000,000 generations, with trees sampled every 2000 generations. A final majority rule consensus phylogram was generated after removing the first 10% of the trees as burn-in, and node support was based on posterior probabilities of the remaining trees having a particular node. Genetic divergence of COI sequences amongst subclades was estimated using Kimura-2-Parameter (K2P) model of distance correction (Kimura 1980) in MEGA 6 (Tamura et al., 2013). The mtDNA trees were rooted using *Patella* spp. (*P. aspera*, *P. depressa*, and *P. vulgata*) from different regions, depending on the available sequences in GenBank (Table 1), while midpoint rooting was applied to the ATPSβ data.

3. Results

The concatenated mtDNA data set comprised 70 ingroup taxa with a final alignment length of 1518 base pairs, of which 639 were variable and 599 parsimony informative. The ATPSβ data set comprised the same 70 ingroup taxa with a final alignment length of 232 base pairs, 162 variable characters and 161 parsimony informative characters. The ML and BI phylogenies were largely congruent, and consequently only the ML trees are presented with bootstrap (ML) and posterior probability (BI) values of major subclades indicated above and below branch nodes respectively (Figs. 1 and 2). The mtDNA data (Fig. 1) and the ATPSβ data (Fig. 2) recovered the following four main subclades or evolutionary lineages: *Cymbula* A, *Cymbula* B, *Scutellastra* A and *Scutellastra* B. *Cymbula* and *Scutellastra* were recovered as monophyletic sister taxa (Figs. 1 and 2). *Helcion concolor* was recovered as the sister taxon of a subclade comprising all species of *Cymbula* and *Scutellastra* (Fig. 1) whereas the ATPSβ tree recovered it as the sister taxon of *Cymbula* subclade B (it could not be aligned with *Scutellastra*) (Fig. 2a). With the exceptional cases of *C. miniata*, *S. argenvillei* and *S. barbara*, the strongly supported monophyletic subclades A and B conformed to the species' foraging traits (Figs. 1 and 2). *Cymbula* A comprised territorial foragers (*C. compressa* and *C. miniata*) while *Cymbula* B comprised non-territorial foragers (*C. oculus* and *C. granatina*). *Scutellastra* A comprised territorial foragers (*S. barbara*, *S. cochlear* and *S. longicosta*) while *Scutellastra* B comprised non-territorial foragers (*S. granularis*, *S. miliaris* and *S. natalensis*). The non-territorial *S. argenvillei* (Bustamante and Branch 1996) clustered among non-territorial foragers in the mtDNA trees (Fig. 1) but among the territorial foragers in the ATPSβ tree (Fig. 2b).

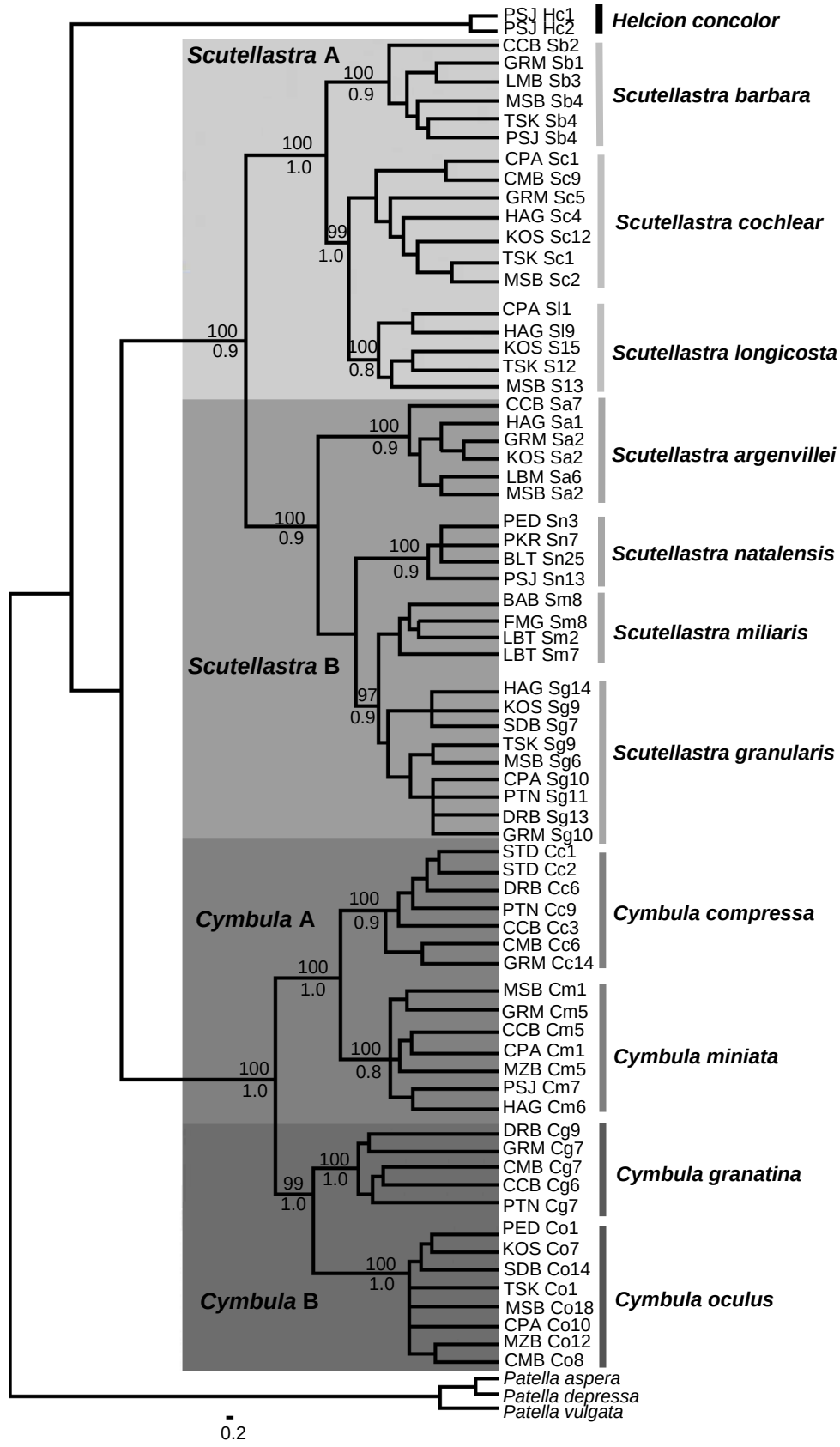
PSJ Hc1
PSJ Hc2
Helcion concolor
Scutellastra A
CCB Sb2
GRM Sb1
LMB Sb3
MSB Sb4
TSK Sb4
PSJ Sb4
Scutellastra barbara
CPA Sc1
CMB Sc9
GRM Sc5
HAG Sc4
KOS Sc12
TSK Sc1
MSB Sc2
Scutellastra cochlear
CPA Sl1
HAG Sl9
KOS S15
TSK S12
MSB S13
Scutellastra longicosta
CCB Sa7
HAG Sa1
GRM Sa2
KOS Sa2
LBM Sa6
MSB Sa2
Scutellastra argenvillei
PED Sn3
PKR Sn7
BLT Sn25
PSJ Sn13
Scutellastra natalensis
BAB Sm8
FMG Sm8
LBT Sm2
LBT Sm7
Scutellastra miliaris
Scutellastra B
HAG Sg14
KOS Sg9
SDB Sg7
TSK Sg9
MSB Sg6
CPA Sg10
PTN Sg11
DRB Sg13
GRM Sg10
Scutellastra granularis
STD Cc1
STD Cc2
DRB Cc6
PTN Cc9
CCB Cc3
CMB Cc6
GRM Cc14
Cymbula compressa
Cymbula A
MSB Cm1
GRM Cm5
CCB Cm5
CPA Cm1
MZB Cm5
PSJ Cm7
HAG Cm6
Cymbula miniata
DRB Cg9
GRM Cg7
CMB Cg7
CCB Cg6
PTN Cg7
Cymbula granatina
PED Co1
KOS Co7
SDB Co14
TSK Co1
MSB Co18
CPA Co10
MZB Co12
CMB Co8
Cymbula oculus
Cymbula B
Patella aspera
Patella depressa
Patella vulgata
100
0.9
100
1.0
99
1.0
100
0.8
100
0.9
100
0.9
100
0.9
100
0.9
97
0.9
100
0.9
100
1.0
100
0.8
100
1.0
100
1.0
99
1.0
100
1.0
100
1.0
0.2

Fig. 1. The Maximum Likelihood phylogenetic reconstruction based on the concatenated mtDNA data set (12S rRNA, 16S rRNA and COI) recovered four major subclades indicated by different colours. The values above and below the branch nodes are respectively the likelihood support and posterior probability values of the diverging taxa.

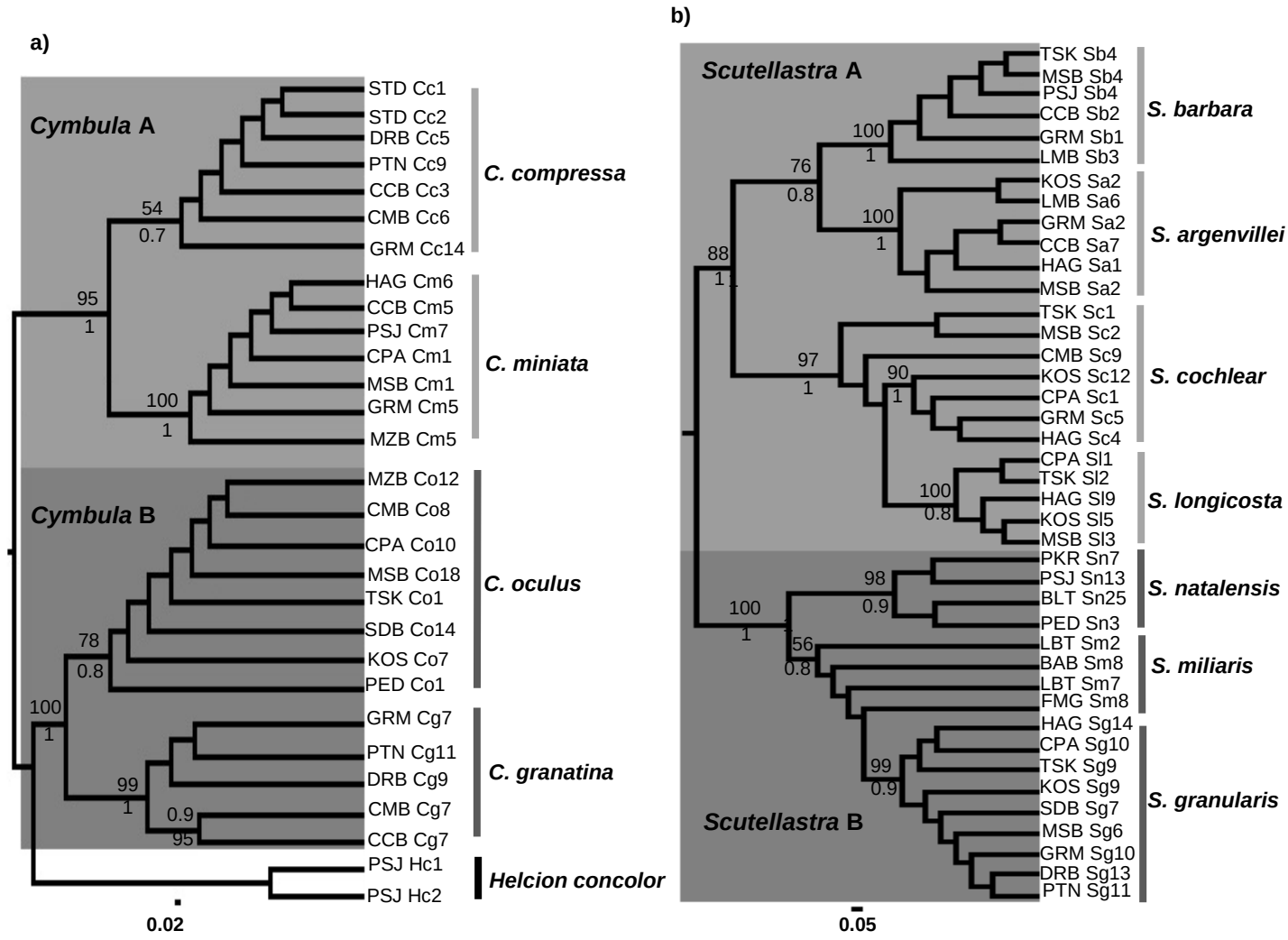

Fig. 2. The Maximum Likelihood phylogenetic reconstruction using ATPSβ data recovered two major subclades (indicated by different colours) corresponding to territorial and non-territorial foragers in both a) *Cymbula* and b) *Scutellastra* species complexes. The values above and below the branch nodes are respectively the likelihood support and posterior probability values of the diverging taxa.

4. Discussion

4.1. Phylogenetic reconstruction

Phylogenetic reconstruction revealed strong divergence and a sister taxon relationship between *Cymbula* A + B and *Scutellastra* A + B. There is a very deep and stable evolutionary divergence between territorial and non-territorial foragers within both genera. Although the divergence between the two foraging types is phylogenetically clear-cut, ecologically it is contentious for three species. Firstly, this study classified *Cymbula miniata* as a territorial forager, which exclusively forages on the encrusting alga *Spongites yendoi* but does not defend foraging territories (Ridgway et al., 2000) whereas the territorial *C. sanguinans,* a sibling taxon of *C. miniata,* creates and defends territorial gardens of the encrusting alga *Hildenbrandia rubra* (Ridgway et al., 2000). Secondly, the clustering of *Scutellastra argenvillei* within the non-territorial subclade in the mtDNA tree but within the territorial subclade in the ATPSβ tree could imply that it evolved as a territorial species from a non-territorial ancestor within *Scutellastra* subclade B, or vice versa. Thirdly, while *S. barbara* was classified as a territorial forager, west coast populations of this species do not exhibit territorial foraging (Branch, 1985). Since not all South African patellid species were examined, this raises uncertainty regarding the phylogenetic resolution of foraging traits for the remaining species. *Helcion* was classified as a paraphyletic sister taxon to *Cymbula* and *Scutellastra* (ML and BI analyses of the mtDNA data set, Fig. 1). This genus comprises only four southern African endemics of which *Helcion concolor*, *H. pruinosus* and *H. pectunculus* are all known to be non-territorial (Henninger and Hodgson, 2001). The phylogenies were not dated since the focus of this study was on the evolutionary

relationships between foraging traits, not how and when these traits evolved. Nonetheless, the molecular dating and fossil records suggest these subclades radiated approximately 90–50 Mya in the Tethys Sea during the late Cretaceous, followed by transoceanic dispersal to other continents (Nakano and Sasaki, 2011).

A sequence divergence of 10% was identified between the COI sequences of the territorial *Scutellastra* A and the non-territorial *Scutellastra* B under the K2P model, which corresponds to the level of divergence recognised amongst genera of marine bivalves (Layton et al., 2014), marine gastropods (Jennings et al., 2010) and geographic clades of limpets (González-Wevar et al., 2010). The corresponding genetic divergence between territorial and non-territorial *Cymbula* spp. was 7%, not as high as the divergence recorded between other limpet genera (González-Wevar et al., 2010). The robust congruence amongst four different markers and such deep genetic divergence between territorial and non-territorial foragers reflects strong underlying effects of resource partitioning and adaptation of foraging behaviour in the evolution of patellogastropods. For example, Maneveldt et al. (2006) discovered remarkable anatomical differences in radular teeth between the territorial *S. cochlear* and non-territorial *S. granularis,* and this was strongly associated with adaptation to their respective foraging behaviours.

4.2. Evolution of territoriality in patellid limpets

Territorial foraging is a well-studied and understood ecological trait within the patellogastropods, but it is not yet clear which foraging trait is ancestral (Lindberg, 2007). Our study indicates that territoriality or resource partitioning amongst southern African patellid limpets arose

independently in two different genera. The deep divergence at the very base of the phylogenies in both *Cymbula* and *Scutellastra* makes it difficult to ascertain which foraging trait is ancestral within each genus, because no species displaying a particular foraging trait were nested within a subclade comprising species with the other foraging trait, which would indicate that the former trait is derived. The sister relationship and synchronized evolution of territorial and non-territorial foragers suggest their concurrent radiation from the ancestral taxa. However, the foraging traits of the next basal lineages support the idea that non-territorial foraging is the ancestral trait within the patellogastopods. *Helcion* (the sister genus of the ingroup) is non-territorial, and the fossil record indicates that the next basal genus, *Patella*, is ancestral to all southern African patellid limpets (Nakano and Sasaki, 2011). Its species are also presumed to be non-territorial foragers based on their high shore migration, generalized algal diet and lack of foraging territories (Lewis and Bowman, 1975). This reflects an evolutionary scenario whereby non-territorial taxa radiated twice and independently into multiple territorial and non-territorial taxa.

5. Conclusion

The results of this study indicate that foraging traits evolved from generalist to specialist, not once, but twice within two closely related groups, and highlight how the evolution of ecological adaptations can result in deep genetic divergence amongst closely related, co-distributed marine invertebrates. The evolution of resource partitioning, or territoriality and non-territoriality, within this group is so ecologically distinct and so evolutionarily divergent that it is even recognisable at the phylogenetic level. We believe that the integration of phylogenetic tools with species'

ecological traits is essential not only to comprehend evolutionary relationships within closely related taxa, but also to unravel cryptic biodiversity.

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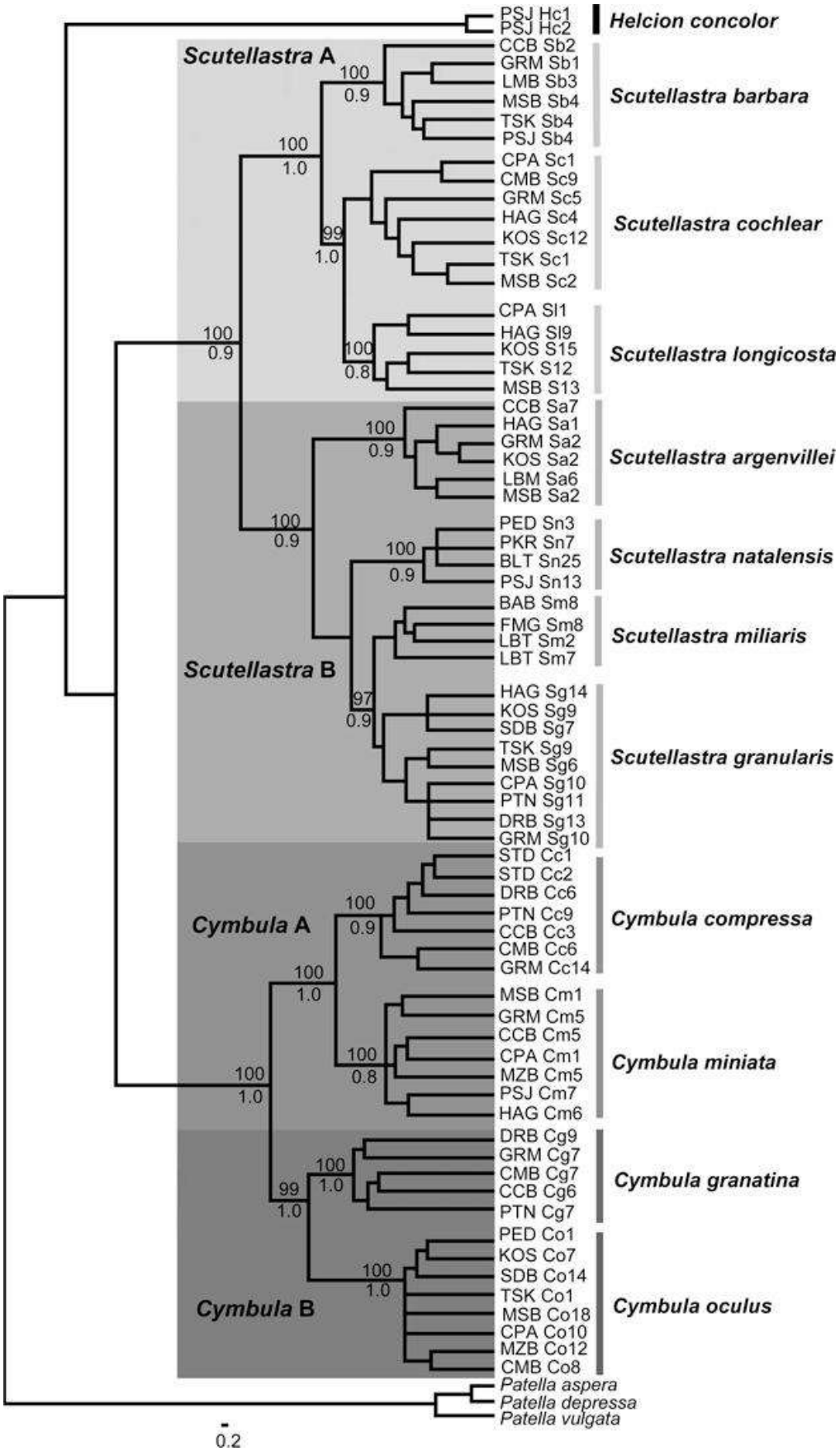
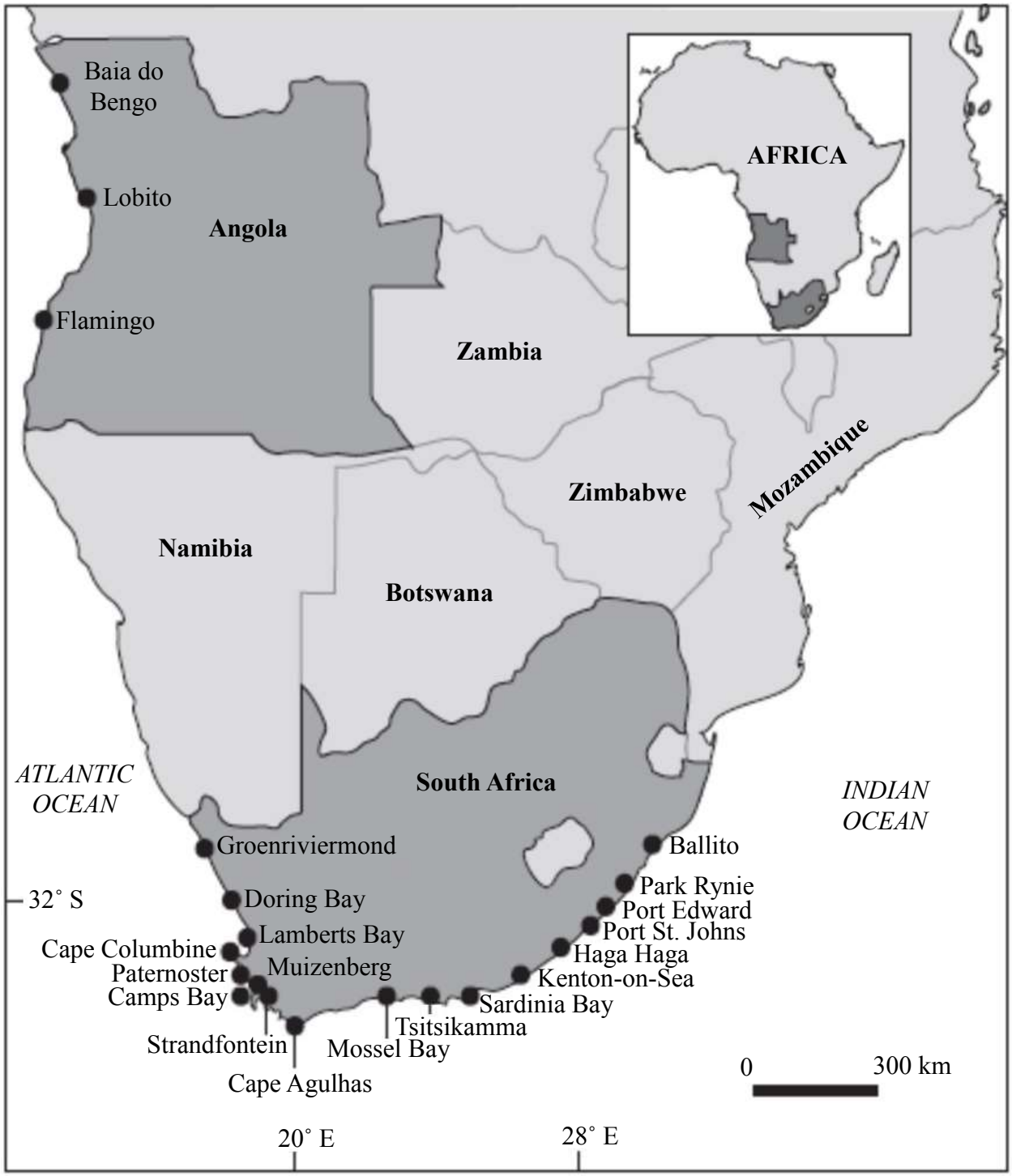
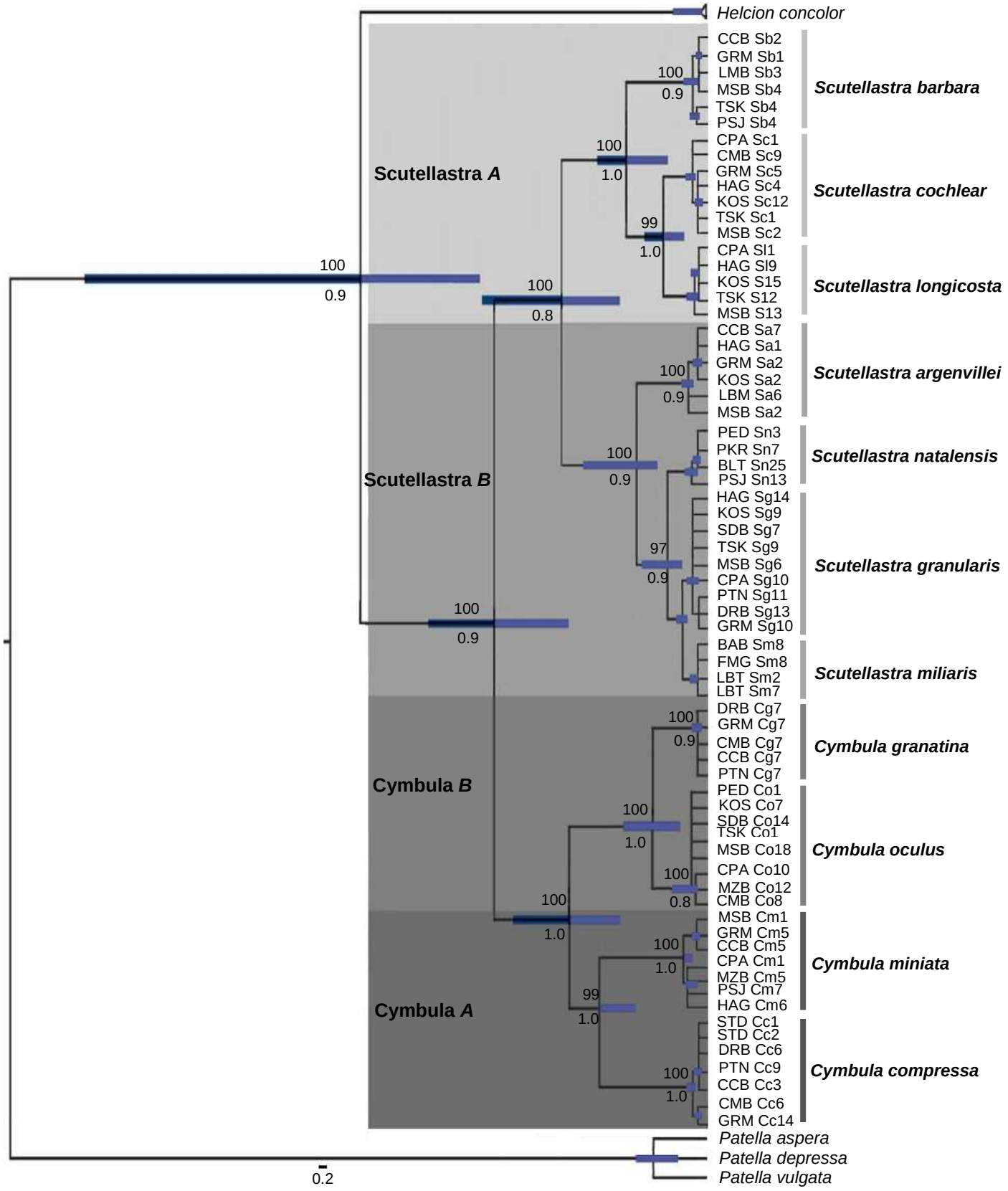
Graphical abstract



Supplementary Material



Appendix A: Map of southern Africa showing sampling sites. The insert shows the location of South Africa and Angola on the African continent.


Helcion concolor
Scutellastra A
Scutellastra B
Cymbula B
Cymbula A
CCB Sb2
GRM Sb1
LMB Sb3
MSB Sb4
TSK Sb4
PSJ Sb4
Scutellastra barbara
CPA Sc1
CMB Sc9
GRM Sc5
HAG Sc4
KOS Sc12
TSK Sc1
MSB Sc2
Scutellastra cochlear
CPA Sl1
HAG Sl9
KOS S15
TSK S12
MSB S13
Scutellastra longicosta
CCB Sa7
HAG Sa1
GRM Sa2
KOS Sa2
LBM Sa6
MSB Sa2
Scutellastra argenvillei
PED Sn3
PKR Sn7
BLT Sn25
PSJ Sn13
Scutellastra natalensis
HAG Sg14
KOS Sg9
SDB Sg7
TSK Sg9
MSB Sg6
CPA Sg10
PTN Sg11
DRB Sg13
GRM Sg10
Scutellastra granularis
BAB Sm8
FMG Sm8
LBT Sm2
LBT Sm7
Scutellastra miliaris
DRB Cg7
GRM Cg7
CMB Cg7
CCB Cg7
PTN Cg7
Cymbula granatina
PED Co1
KOS Co7
SDB Co14
TSK Co1
MSB Co18
CPA Co10
MZB Co12
CMB Co8
Cymbula oculus
MSB Cm1
GRM Cm5
CCB Cm5
CPA Cm1
MZB Cm5
PSJ Cm7
HAG Cm6
Cymbula miniata
STD Cc1
STD Cc2
DRB Cc6
PTN Cc9
CCB Cc3
CMB Cc6
GRM Cc14
Cymbula compressa
Patella aspera
Patella depressa
Patella vulgata
0.2

Appendix B: A majority rule Bayesian chronogram generated in BEAST based on the concatenated mtDNA data (12S rRNA, 16S rRNA and COI) recovered four major clades indicated by distinct colours. The shaded bold line indicates 95% credibility intervals for each estimate of divergence time measured in millions years ago (Mya). The values above and below the branch nodes are respectively the likelihood support and posterior probability values of the diverging taxa. Broadly overlapping confidence intervals shows contemporaneous radiation of territorial and non-territorial foragers in both *Cymbula* and *Scutellastra*.