

SUPPLEMENTAL INFORMATION

**EXTENSIONS TO THE KNOWN GEOGRAPHIC DISTRIBUTIONS OF
REPTILES IN THE GREAT KAROO, SOUTH AFRICA**

*NICOLAS S. TELFORD, GRAHAM J. ALEXANDER, FRANCOIS S. BECKER, WERNER CONRADIE,
ADRIAAN JORDAAN, LUKE KEMP, ANJA LE GRANGE, ALEXANDER D. REBELO, PAULA STRAUSS,
JODY M. TAFT, JOSHUA WEEBER, AND KRYSTAL A. TOLLEY*

The following material is provided by the authors and was not subjected to editing by
Herpetological Conservation and Biology.

TABLE S1. Reptile species list for the Karoo fracking area based on interpreted distributions from the IUCN. Species recorded from the area prior to, and during this study are indicated with an x. Those species without an x are inferred to be in the study area based on their range maps but have not been recorded.

Order	Species	Pre-2016	This study
Squamata - lizards			
	Agamidae		
	<i>Agama aculeata</i>	x	x
	<i>Agama atra</i>	x	x
	<i>Agama hispida</i>	x	x
	Chamaeleonidae		
	<i>Bradypodion gutturale</i>	x	
	<i>Bradypodion ventrale</i>	x	x
	<i>Chamaeleo namaquensis</i>	x	x
	Cordylidae		
	<i>Chamaesaura aenea</i>	x	
	<i>Chamaesaura anguina</i>	x	x
	<i>Cordylus aridus</i>		
	<i>Cordylus cloetei</i>	x	x
	<i>Cordylus cordylus</i>	x	x
	<i>Cordylus mclachlani</i>		
	<i>Cordylus minor</i>	x	x
	<i>Hemicordylus capensis</i>	x	
	<i>Karusasaurus polyzonus</i>	x	x
	<i>Ouroborus cataphractus</i>	x	x
	<i>Pseudocordylus melanotus</i>	x	
	<i>Pseudocordylus microlepidotus</i>	x	x
	Gekkonidae		
	<i>Afroedura amatolica</i>	x	x
	<i>Afroedura halli</i>	x	x
	<i>Afroedura karroica</i>	x	x
	<i>Chondrodactylus angulifer</i>	x	x
	<i>Chondrodactylus bibronii</i>	x	x
	<i>Goggia braacki</i>	x	x

Order	Species	Pre-2016	This study
	<i>Goggia essexi</i>	x	
	<i>Goggia hewitti</i>		
	<i>Goggia hexapora</i>	x	
	<i>Goggia incognita</i>		
	<i>Goggia microlepidota</i>		
	<i>Hemidactylus mabouia</i>	x	x
	<i>Lygodactylus capensis</i>	x	x
	<i>Pachydactylus capensis</i>	x	x
	<i>Pachydactylus formosus</i>	x	x
	<i>Pachydactylus geitje</i>	x	x
	<i>Pachydactylus kladaroderma</i>	x	x
	<i>Pachydactylus latirostris</i>	x	
	<i>Pachydactylus maculatus</i>	x	x
	<i>Pachydactylus mariquensis</i>	x	x
	<i>Pachydactylus oculatus</i>	x	x
	<i>Pachydactylus purcelli</i>	x	x
	<i>Pachydactylus rugosus</i>	x	x
	<i>Pachydactylus weberi</i>	x	
	<i>Ptenopus garrulus</i>	x	x
	Gerrhosauridae		
	<i>Cordylosaurus subtessellatus</i>	x	x
	<i>Gerrhosaurus flavigularis</i>	x	x
	<i>Gerrhosaurus typicus</i>	x	x
	<i>Tetradactylus seps</i>	x	
	<i>Tetradactylus tetradactylus</i>	x	x
	Lacertidae		
	<i>Meroles knoxii</i>	x	
	<i>Meroles suborbitalis</i>	x	x
	<i>Nucras holubi</i>		x
	<i>Nucras intertexta</i>		
	<i>Nucras lalandii</i>	x	x
	<i>Nucras livida</i>	x	x
	<i>Nucras taeniolata</i>	x	
	<i>Nucras tessellata</i>	x	x
	<i>Pedioplanis burchelli</i>	x	x
	<i>Pedioplanis laticeps</i>	x	x

Order	Species	Pre-2016	This study
	<i>Pedioplanis lineocellata</i>	x	x
	<i>Pedioplanis namaquensis</i>	x	x
	<i>Tropidosaura montana</i>	x	x
	Scincidae		
	<i>Acontias breviceps</i>	x	x
	<i>Acontias gracilicauda</i>	x	x
	<i>Acontias lineatus</i>	x	x
	<i>Acontias occidentalis</i>	x	x
	<i>Acontias orientalis</i>	x	x
	<i>Scelotes caffer</i>	x	x
	<i>Trachylepis capensis</i>	x	x
	<i>Trachylepis homalocephala</i>	x	x
	<i>Trachylepis occidentalis</i>	x	x
	<i>Trachylepis punctatissima</i>	x	x
	<i>Trachylepis sulcata</i>	x	x
	<i>Trachylepis varia</i>	x	x
	<i>Trachylepis variegata</i>	x	x
	Varanidae		
	<i>Varanus albigularis</i>	x	x
	<i>Varanus niloticus</i>	x	x
<hr/>			
Squamata - snakes			
	Atractaspididae		
	<i>Aparallactus capensis</i>	x	
	<i>Homoroselaps lacteus</i>	x	
	Colubridae		
	<i>Crotaphopeltis hotamboeia</i>	x	x
	<i>Dasypeltis scabra</i>	x	x
	<i>Dispholidus typus</i>	x	x
	<i>Philothamnus occidentalis</i>		
	<i>Philothamnus semivariatus</i>	x	x
	<i>Telescopus beetzii</i>	x	
	Elapidae		
	<i>Aspidelaps lubricus</i>	x	x
	<i>Hemachatus haemachatus</i>	x	x
	<i>Naja nivea</i>	x	x
	Lamprophiidae		

Order	Species	Pre-2016	This study
	<i>Boaedon capensis</i>	x	x
	<i>Boaedon mentalis</i>	x	x
	<i>Lamprophis aurora</i>	x	x
	<i>Lamprophis fiskii</i>	x	
	<i>Lamprophis fuscus</i>		
	<i>Lamprophis guttatus</i>	x	x
	<i>Lycodonomorphus inornatus</i>	x	
	<i>Lycodonomorphus laevisissimus</i>	x	
	<i>Lycodonomorphus rufulus</i>	x	
	<i>Lycophidion capense</i>	x	x
	Leptotyphlopiidae		
	<i>Leptotyphlops nigricans</i>	x	x
	<i>Leptotyphlops scutifrons</i>	x	x
	<i>Namibiana gracilior</i>	x	
	<i>Prosymna sundevallii</i>	x	x
	Psammophiidae		
	<i>Dipsina multimaculata</i>	x	x
	<i>Psammophis crucifer</i>	x	x
	<i>Psammophis notostictus</i>	x	x
	<i>Psammophis trinisalis</i>	x	x
	<i>Psammophylax rhombeatus</i>	x	x
	Pseudaspidae		
	<i>Pseudaspis cana</i>	x	x
	Pseudoxyrhopiidae		
	<i>Amplorhinus multimaculatus</i>	x	x
	<i>Duberria lutrix</i>	x	x
	Pythonidae		
	<i>Python natalensis</i>	Population extirpated from the region	
	Typhlopidae		
	<i>Afrotyphlops bibronii</i>		
	<i>Rhinotyphlops lalandei</i>	x	x
	<i>Rhinotyphlops schinzi</i>		
	Viperidae		
	<i>Bitis arietans</i>	x	x
	<i>Bitis atropos</i>	x	
	<i>Bitis caudalis</i>	x	x

Order	Species	Pre-2016	This study
	<i>Bitis cornuta</i>	x	
	<i>Bitis inornata</i>	x	x
	<i>Bitis rubida</i>	x	x
	<i>Causus rhombeatus</i>	x	x
<hr/>			
Testudines			
	Pelomedusidae		
	<i>Pelomedusa galeata</i>	x	x
	Testudinae		
	<i>Chersina angulata</i>	x	x
	<i>Chersobius boulengeri</i>	x	x
	<i>Homopus areolatus</i>	x	x
	<i>Homopus femoralis</i>	x	x
	<i>Psammobates tentorius</i>	x	x
	<i>Stigmochelys pardalis</i>	x	x

TABLE S2. New records collected during the Great Karoo fracking area surveys that contributed to the extensions and refinement of interpreted distribution maps and collection data (PEM – Port Elizabeth Museum, iNaturalist – <https://www.inaturalist.org/>).

Common Name	Species	Date	Field/Catalogue No.	Coordinates	Type of record	Source
Western Legless Skink	<i>Acontias occidentalis</i>	02/28/2018	PEM R23757	-31.099°S, 24.041°E	Voucher	Current Study
Western Legless Skink	<i>Acontias occidentalis</i>	03/01/2018	PEM R23770	-31.110°S, 24.025°E	Voucher	Current Study
Western Legless Skink	<i>Acontias occidentalis</i>	02/28/2018	PEM R23776	-31.111°S, 24.022°E	Voucher	Current Study
Western Legless Skink	<i>Acontias occidentalis</i>	03/17/2016	iNaturalist: 5374244	-30.843°S, 23.995°E	Photograph	iNaturalist
Dwarf Plated Lizard	<i>Cordylosaurus subtessellatus</i>	10/27/2017	P024	-31.894°S, 20.780°E	DNA sample	Current Study
Dwarf Plated Lizard	<i>Cordylosaurus subtessellatus</i>	11/03/2017	K047, K067, K068	-32.249°S, 21.349°E	DNA sample	Current Study
Namaqua Plated Lizard	<i>Gerrhosaurus typicus</i>	02/22/2018	PEM R23666	-31.976°S, 23.848°E	Voucher	Current Study
Namaqua Plated Lizard	<i>Gerrhosaurus typicus</i>	02/27/2018	PEM R23748	-31.099°S, 24.069°E	Voucher	Current Study
Braack's Dwarf Leaf-toed Gecko	<i>Goggia braacki</i>	04/28/2016	FP063	-31.929°S, 22.881°E	DNA sample	Current Study
Braack's Dwarf Leaf-toed Gecko	<i>Goggia braacki</i>	02/02/2018	PEM R23605	-32.129°S, 22.346°E	Voucher	Museum
Holub's Sandveld Lizard	<i>Nucras holubi</i>	03/03/2018	PEM R23788, PEM R23802	-30.911°S, 25.013°E	Voucher	Current Study
Thin-skinned Gecko	<i>Pachydactylus kladaroderma</i>	11/01/2017	P199	-32.111°S, 21.436°E	DNA sample	Current Study
Thin-skinned Gecko	<i>Pachydactylus kladaroderma</i>	11/01/2017	P202	-32.098°S, 21.432°E	DNA sample	Current Study
Thin-skinned Gecko	<i>Pachydactylus kladaroderma</i>	11/08/2017	P297	-32.305°S, 21.674°E	Observation	Current Study
Thin-skinned Gecko	<i>Pachydactylus kladaroderma</i>	04/05/2018	P455	-31.704°S, 22.230°E	Observation	Current Study
Thin-skinned Gecko	<i>Pachydactylus kladaroderma</i>	03/01/2017	S474	-31.961°S, 21.418°E	DNA sample	Current Study
Thin-skinned Gecko	<i>Pachydactylus kladaroderma</i>	03/02/2017	S510	-31.967°S, 21.415°E	DNA sample	Current Study
Common Rough Gecko	<i>Pachydactylus rugosus</i>	04/21/2016	FP010C	-31.202°S, 20.501°E	DNA sample	Current Study

Common Name	Species	Date	Field/Catalogue No.	Coordinates	Type of record	Source
Common Rough Gecko	<i>Pachydactylus rugosus</i>	07/23/2020	iNaturalist: 54045446	-31.346°S, 20.914°E	Photograph	iNaturalist
Common Barking Gecko	<i>Ptenopus garrulus</i>	10/31/2017	PEM R23118–21	-33.048°S, 24.961°E	Voucher	Current Study
Cape Dwarf Burrowing Skink	<i>Scelotes caffer</i>	10/27/2017	PEM R23129	-32.536°S, 25.291°E	Voucher	Current Study
Cape Dwarf Burrowing Skink	<i>Scelotes caffer</i>	10/23/2017	PEM R23132	-32.539°S, 25.221°E	Voucher	Current Study
Cape Dwarf Burrowing Skink	<i>Scelotes caffer</i>	05/07/2016	PEM R22299	-32.148°S, 24.776°E	Voucher	Current Study
Cape Dwarf Burrowing Skink	<i>Scelotes caffer</i>	05/08/2016	PEM R22302	-32.185°S, 24.788°E	Voucher	Current Study

PHYLOGENETIC METHODS AND RESULTS

DNA Extraction and Fragment Amplification

We digested approximately 10 mg of tissue using standard proteinase K/SDS procedures (Palumbi 1996) and extracted genomic DNA using a standard salt extraction (Aljanabi and Martinez 1997). We used Polymerase Chain Reaction (PCR) to amplify a segment of mitochondrial (mtDNA) NADH dehydrogenase subunit 2 (ND2), nuclear recombination activating gene 1 (RAG-1) and Phosducin (PDC). A 25 μ l PCR mix contained 2 μ l DNA template with a 20 ng/ μ l concentration, a thermophilic buffer (20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT), 1.5 mM MgCl₂, 0.08 μ M of each primer, 0.2 μ M dNTPs, and 0.3 U/ μ l SuperTherm *Taq* DNA polymerase. The thermocycling profile started with 5 minutes of denaturation at 95 °C followed by 35 cycles each of: 1 minute at 95 °C for denaturing, 45 s for primer annealing (Groth and Barrowclough 1999; Cunningham and Cherry 2003; Bauer et al. 2007), and a 1-minute extension at 72 °C, with a final extension of 10 minutes at 72 °C. We sequenced successful PCR product at Macrogen Inc. (Amsterdam) and edited and aligned the trace files in Geneious Pro v11.1.5 (Kearse et al. 2012).

Phylogenetic analysis

To provide a preliminary identification, we carried out DNA barcoding for the ND2 gene on tissue from the shed tail and submitted the resulting sequence to the BLAST function (Basic Local Alignment Search Tool; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>). BLAST indicated 95.7 % similarity to the gekkonid genus *Goggia*. To make a positive identification, we included the sample in a phylogenetic analysis. Three genes (ND2, RAG-1 and PDC) were sequenced (Genbank accession numbers: MZ997471 – MZ997473), which we aligned and analyzed

together with a published dataset for the genus downloaded from GenBank (see Heinicke et al. 2017). To infer phylogenetic relationships, we carried out a maximum likelihood (ML) phylogenetic analysis in RAxML v.8.2.8 (Stamatakis 2014) for the dataset partitioned by gene, with 1000 bootstrap replicates, and nodes with at least 70% bootstrap were considered supported.

Results: identification of an unknown gecko

Phylogenetic analysis of the unidentified gecko confirmed the species to be *Goggia braacki* (Fig. S1), which allowed for a significant revision of the species known geographic area of distribution.

LITERATURE CITED

- Aljanabi, S.M., and I. Martinez. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25:4692–4693.
- Bauer, A.M., A. de Silva, E. Greenbaum, and T.R. Jackman. 2007. A new species of day gecko from high elevation in Sri Lanka, with a preliminary phylogeny of Sri Lankan *Cnemaspis* (Reptilia: Squamata: Gekkonidae). *Mitteilungen aus dem Museum für Naturkunde in Berlin. Zoologische Reihe*, 83:22–32.
- Cunningham, M., and M.I. Cherry. 2004. Molecular systematic of African 20-chromosome toads (Anura: Bufonidae). *Molecular Phylogenetics and Evolution*, 32:671–685.
- Growth, J.G., and G.F. Barrowclough. 1999. Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. *Molecular Phylogenetics and Evolution*, 12:115–123.

- Heinicke, M.P., D. Turk, and A.M. Bauer. 2017. Molecular phylogeny reveals strong biogeographic signal and two new species in a Cape Biodiversity Hotspot endemic mini-radiation, the pygmy geckos (Gekkonidae: Goggia). *Zootaxa*, 4312:449–470.
- Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, A. Drummond, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28:1647–1649.
- Palumbi SR. 1996. Nucleic acids II: The polymerase chain reaction. Pp. 205–247. *In* Molecular Systematics. Hillis, D.M., C. Moritz, and B.K. Mable (Eds.). Sinauer Associates Inc., Sunderland, UK.

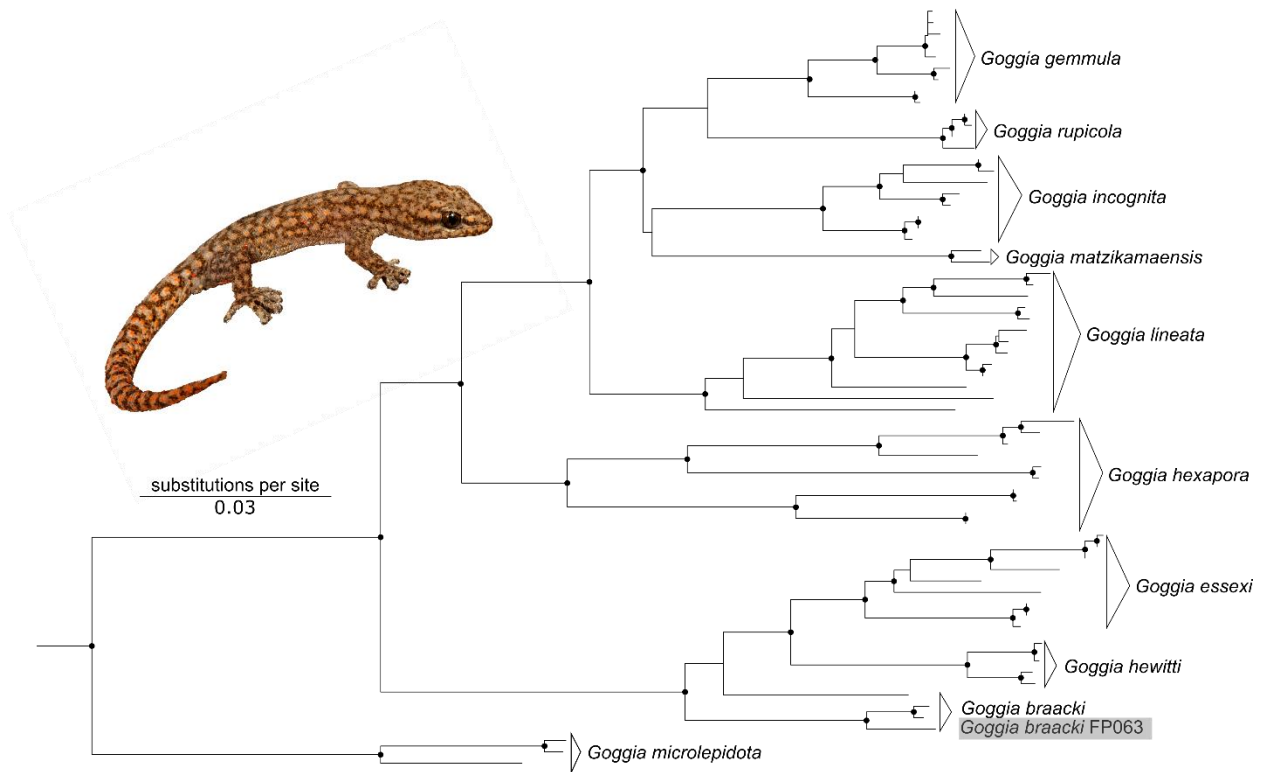


FIGURE S1. Maximum likelihood phylogeny of the genus *Goggia* confirming the identity of the sample as *Goggia braacki* (denoted by the grey box). Black circles at the nodes represent maximum likelihood posterior probability support of $\geq 75\%$. Photo inset: *Goggia braacki*. Photo by Werner Conradie.