

The King of the Dwarves: a new cryptic species of Dainty Frog (Anura: Pyxicephalidae: *Cacosternum*) from the eastern Great Escarpment of South Africa

WERNER CONRADIE^{1, 2, 3}

¹Port Elizabeth Museum, P.O. Box 13147, Humewood, 6013, South Africa

²South African Institute for Aquatic Biodiversity, P/Bag 1015, Grahamstown, 6140, South Africa

³Corresponding author. E-mail: werner@bayworld.co.za

Abstract

Phylogenetic reconstruction using the mitochondrial 16S marker shows the presence of a cryptic species of *Cacosternum* (Anura: Pyxicephalidae) from the eastern Great Escarpment of South Africa, supporting the Greater Maputaland-Pondoland-Albany region of vertebrate endemism. Bioacoustic and morphological characteristics, in conjunction with colouration differences, allow the description of this cryptic species. Tadpoles and details of life history are described.

Key words: Amphibia, endemism, hotspot, grassland, cryptic species

Introduction

The genus *Cacosternum* has recently been revised by Channing *et al.* (2013) and now consists of 15 recognised species with more cryptic species expected to occur in the *Cacosternum boettgeri* complex (Poynton *et al.* 2004; Channing *et al.* 2005; Channing & Schmitz 2008; Du Preez & Carruthers 2009). During a recent search to find the elusive Amatola Toad (*Vandijkophrynus amatolicus*) (see Conradie & Tarrant 2011; Tarrant & Cunningham 2011) in the Hogsback area, South Africa, a frog species referable to the genus *Cacosternum* was collected. Various features of the ventral colouration, morphology, vocalisation, and level of genetic divergence all indicated that these individuals are not referable to any known *Cacosternum* species, and I therefore take this opportunity to describe them as a new species.

Material and methods

Sampling. Specimens were collected on the escarpment around Hogsback, Eastern Cape Province, South Africa in early October 2011 (Fig. 1). Specimens were humanely euthanized by submerging them in tricaine methanesulfonate (MS222) solution, after which they were formalin-fixed for 48 hours and transferred to 70% ethanol for long-term storage in the herpetological collection of the Port Elizabeth Museum (PEM), South African Institute for Aquatic Biodiversity (SAIAB) and National Museum Bloemfontein (NMB). Prior to formalin fixation, tissue samples from thigh muscle or tail clippings (tadpoles) were taken, and stored in 96% ethanol (Table 1). Two breeding pairs collected spawned overnight. Eggs were kept and raised to metamorphosis at the PEM and preserved in two day intervals in 10% buffered formalin.

Morphological analysis. Specimens were measured to the nearest 0.1 mm using digital callipers under a dissecting microscope. The same set of measurements as in Channing *et al.* (2013) were taken: snout-urostyle length (SUL, distance from the tip of the snout to the end of the urostyle); the maximum tibia length (TIB, measured on the bent hind limb); the length of the foot (FOT, measured from the proximal end of the inner metatarsal tubercle to the tip of the fourth toe on the fully extended foot); the eye-nostril distance (EN, measured

Highlands may produce more cryptic species. In isolated montane regions (forest and grasslands) species over time speciate resulting in the formation of narrow endemics and candidate new species, especially evident in the Afromontane forested inselbergs of northern Mozambique (reptiles—Branch and Bayliss 2009; Branch & Tolley 2010; Portik *et al.* 2013, bats—Taylor *et al.* 2010, crustaceans—Daniels & Bayliss 2012). Similar trends are shown for the Great Escarpment of southern African, eg. Berg adders (*Bitis atropos*—Kelly *et al.* in process) and Short-headed Legless Lizards (*Acontias breviceps*—Conradie *et al.* in process).

Clark *et al.* (2009) described the Sneeuberg region as a floristic centre of endemism. Furthermore Clark *et al.* (2011) define the Sneeuberg, Great Winterberg-Amatolas and Stormberg as the Cape Midlands Escarpment, which have a high level of both floristic and vertebrate endemism. Perera *et al.* (2011) suggested the inclusion of sections of the Great Escarpment, from the Amatola-Winterberg-Sneeuberg Mountains (AWS), into the larger Maputaland-Pondoland-Albany (MPA) biodiversity hotspot on grounds of high level of vertebrate endemism. This new species adds another endemic species to the AWS list of endemic to near endemic species; eg. *Afroedura amatolica* (Amatola Flat Gecko)—Least Concern, *Afroedura karroica* (Karoo Flat Gecko)—Least Concern, *Afroedura tembulica* (Tembu Flat Gecko)—Least Concern, *Bitis inornata* (Plain Adder)—Endangered, *Anhydrophryne rattrayi* (Hogsback Chirping Frog)—Vulnerable, *Vandijkophrynus amatolicus* (Amatola Toad)—Critically Endangered, *Barbus amatolicus* (Amatola Barb)—Vulnerable, and *Barbus trevelyanii* (Border Barb)—Endangered. The AWS also have an endangered endemic damselfly, Amatola Malachite (*Chlorolestes apricans*), associated with it (Tarboton & Tarboton 2005), thus further supporting the suggestion of Perera *et al.* (2011) to include the AWS into the newly defined Greater Maputoland-Pondoland-Albany (GMPA) region.

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References

- Altig, R. (2007) A primer for the morphology of anuran tadpoles. *Herpetological Conservation and Biology*, 2, 71–74.
- Boycott, R.C., De Villiers, A.L. & Scott, E. (2002) A new species of *Cacosternum* Boulenger (Anura: Rainidae: Petropedtina) from the Karoo region of South Africa, with notes on the ecology and distribution of *Cacosternum namaquense* Werner. *Journal of Herpetology*, 35, 333–341.
- Branch, W.R. & Bayliss, J. (2009) A new species of *Atheris* (Serpentes: Viperidae) from northern Mozambique. *Zootaxa*, 2113, 41–54.
- Branch, W.R. & Tolley, K.A. (2010) A new species of chameleon (Sauria: Chamaeleonidae: *Nadzikambia*) from Mount Mabu, central Mozambique. *African Journal of Herpetology*, 59, 157–172.
<http://dx.doi.org/10.1080/21564574.2010.516275>
- Channing, A. & Schmitz, A. (2008) Hiding in plain sight: another cryptic dainty frog from the highlands of Kenya (Anura: Pyxicephalidae: *Cacosternum*). *African Journal of Herpetology*, 57, 75–84.
<http://dx.doi.org/10.1080/21564574.2008.9635570>
- Channing, A. (2001) *Amphibians of Central and Southern Africa*. Cornell University, New York, 447 pp.
- Channing, A., Brun, C., Burger, M., Febvre, S. & Moyer, D. (2005) A new cryptic dainty frog from East Africa (Anura: Ranidae: *Cacosternum*). *African Journal of Herpetology*, 54, 139–148.
<http://dx.doi.org/10.1080/21564574.2005.9635527>
- Channing, A., Rödel, M.-O. & Channing, J. (2012) *Tadpoles of Africa*. Frankfurt-am-Main: Edition Chimaira, 402 pp.
- Channing, A., Schmitz, A., Burger, M. & Kielgast, J. (2013) A molecular phylogeny of African Dainty Frogs, with the

- description of four new species (Anura: Pyxicephalidae: *Cacosternum*). *Zootaxa*, 3701 (5), 518–550.
<http://dx.doi.org/10.11646/zootaxa.3701.5.2>
- Clark, V.R., Barker, N.P. & Mucina, L. (2011) The Great Escarpment of southern Africa: a new frontier for biodiversity exploration. *Biodiversity Conservation*, 20, 2543–2561.
<http://dx.doi.org/10.1007/s10531-011-0103-3>
- Clark, V.R., Barker, N.P. & Mucina, L. (2009) The Sneueberg: a new centre of floristic endemism on the Great Escarpment, South Africa. *South African Journal of Botany*, 75, 196–238.
- Conradie, W. & Tarrant, J. (2011) Amatola Toad AWOL: Thirteen years of futile searches. *FrogLog*, 97, 24.
- Daniels, S.R. & Balliss, J. (2012) Neglected refugia of biodiversity: mountainous regions in Mozambique and Malawi yield two novel freshwater crab species (Potamonautidae: *Potamonautes*). *Zoological Journal of the Linnean Society*, 164, 498–509.
<http://dx.doi.org/10.1111/j.1096-3642.2011.00773.x>
- Du Preez, L.H. & Carruthers, V.C. (2009) *A Complete Guide to the Frogs of Southern Africa*. Struik Nature, Cape Town, 488 pp.
- Ferguson, J.W.H. (2002) On the use of genetic divergence for identifying species. *Biological Journal of the Linnean Society*, 75, 509–516.
<http://dx.doi.org/10.1046/j.1095-8312.2002.00042.x>
- Fouquet, A., Gilles, A., Vences, M., Marty, C., Blanc, M. & Gemmell, N.J. (2007) Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLoS ONE*, 2 (10), e1109.
<http://dx.doi.org/10.1371/journal.pone.0001109>
- Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 183–190.
- Gridi-Papp, M. (2003–2007) SoundRuler: Acoustic analysis for research and teaching. Available from: <http://soundruler.sourceforge.net> (accessed 5 March 2014)
- IUCN (2001) *IUCN Red List Categories and Criteria: Version 3.1*. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK, ii + 30 pp.
- McDiarmid, R.W. & Altig, R. (1999) *Tadpoles: The Biology of Anuran Larvae*. University of Chicago Press, Chicago, London, 444 pp.
- Measey, G.J. (2011) *Ensuring a future for South Africa's frogs: a strategy for conservation research*. SANBI Biodiversity Series 19. South African National Biodiversity Institute, Pretoria, 92 pp. Available from: <http://www.sanbi.org/documents/biodiversity-series-19-ensuring-future-sas-frogs> (accessed 5 March 2014)
- Palumbi, S.R., Martin, A., Romano, W., McMillan, O., Stice, L. & Grabowski, G. (1991) *The simple fools guide to PCR*. Department of Zoology, Special Publication, University of Hawaii, Honolulu, 44 pp.
- Perera, S.J., Ratnayake-Perera, D. & Procheş, Ş. (2011) Vertebrate distributions indicate a greater Maputaland-Pondoland-Albany region of endemism. *South African Journal of Science*, 107, 1–15.
<http://dx.doi.org/10.4102/sajs.v107i7/8.462>
- Portik, D.M., Travers, S.L., Bauer, A.M. & Branch, W.R. (2013) A new species of *Lygodactylus* (Squamata: Gekkonidae) endemic to Mount Namuli, an isolated ‘sky island’ of northern Mozambique. *Zootaxa*, 3710 (5), 415–435.
<http://dx.doi.org/10.11646/zootaxa.3710.5.2>
- Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.
<http://dx.doi.org/10.1093/bioinformatics/14.9.817>
- Poynton, J., Largen, M. & Scott, E. (2004) *Cacosternum boettgeri*. IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. Available from: <http://www.iucnredlist.org> (accessed 30 October 2013)
- Rambaut, A. & Drummond, A.J. (2007) Tracer v1.4. Available from: <http://beast.bio.ed.ac.uk/Tracer> (accessed 5 March 2014)
- Rödel, M.-O. (2000) *Herpetofauna of West Africa. Vol. I. Amphibians of the West African savanna*. Edition Chimaira, Frankfurt am Main, 332 pp.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Scott, E. (2004) *Cacosternum boettgeri* (Boulenger, 1882). In: Minter, L.R., Burger, M., Harrison, J.A., Braack, H.H., Bishop, P.J. & Kloepfer, D. (Eds.), *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland. SI/MAB Series #9*. Smithsonian Institution, Washington, DC, pp. 224–227.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599.
<http://dx.doi.org/10.1093/molbev/msm092>
- Tarboton, W. & Tarboton, M. (2005) *A Fieldguide to the Damselflies of South Africa*. PJ's Printing Services, Johannesburg, 95 pp.
- Tarrant, J. & Cunningham, M.J. (2011) Rediscovery of the elusive Amatola Toad *Vandijkophrynus amatolicus*: twice seen in twenty-six years. *African Herp News*, 55, 1–6.
- Taylor, P.J., Stoffberg, S., Mondjem, A., Schoeman, M.C., Bayliss, J. & Cotterill, F.P.D. (2012) Four New Bat Species (*Rhinolophus hildebrandtii* Complex) Reflect Plio-Pleistocene Divergence of Dwarfs and Giants across an Afromontane

- Archipelago. *PLoS ONE*, 7 (9), e41744.
<http://dx.doi.org/10.1371/journal.pone.0041744>
- Vences, M., Thomas, M., van der Meijden, A., Chiari, Y. & Vieites, D.R. (2005) Comparative performance of the rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, 2, 5.
- Vieites, D.R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F. & Vences, M. (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrated amphibian inventory. *Proceedings of the National Academy of Science of the United States of America*, 106, 8267–8272.
- Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. dissertation, The University of Texas at Austin, 115 pp.