SALAMANDRA **53**(3) | 461–465 | 15 August 2017 | ISSN 0036–3375

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Tomopterna damarensis (Anura: Pyxicephalidae) is broadly distributed in Namibia and Angola

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Manuscript received: 13 May 2016
Accepted 26 September 2016 by Stefan Lötters

The pyxicephalid genus *Tomopterna*, or Sand Frogs, includes 15 recognized species of small to medium-sized frogs (Frost 2016). Most *Tomopterna* species are morphologically similar, making it difficult to identify species solely on the basis of external morphological characters (Dawood & Channing 2002, Dawood & Uqubay 2004, Zimkus & Larson 2011). Most recently described species have been identified largely on the basis of acoustic and genetic data (e.g., Wasonga & Channing 2013). In Southern Africa, the species *Tomopterna cryptotis* (Boulenger, 1907), *T. damarensis* Dawood & Channing, 2002, *T. delalandii* (Tschudii, 1838), *T. krugerensis* Passmore & Carruthers, 1975, and *T. tandyi* Channing & Bogart, 1996, which we refer to here as the *T. cryptotis* complex, are especially similar in morphology. These five species vary in colour pattern, skin texture, and subarticular tubercle arrangement, but variation in these traits broadly overlaps between species (Channing 2001, du Preez & Carruthers 2009).

We have sought to clarify the status of some *Tomopterna* populations in Namibia and Angola in conjunction with our herpetological field and museum research programs. On a November 2011 trip to the Kunene Region, northwestern Namiba, we collected two specimens of *Tomopterna* from along the banks of the Kunene River in the vicinity of the Epupa Falls. These were tentatively identified as *T. krugerensis* because the specimens lack prominent skin tubercles and have a colour pattern without dorsolateral stripes. A third specimen collected at the same locality was similar, but was not identified to species level. Subsequently, during a December 2013 expedition to Namibe Province, southwestern Angola, we collected an unidentified specimen of *Tomopterna* from a sand bank of a pool of the Pediva Hot Springs (recently identified as the Damaraland Sand Frog, *T. damarensis* in Ceríaco et al. 2016), and a June 2014 trip to Erongo Province, Namibia yielded an additional juvenile *Tomopterna*. These specimens are now deposited in the Museum of Comparative Zoology, Harvard University (MCZ), Cambridge/MA, or the California Academy of Sciences (CAS), San Francisco/CA. We have also examined additional specimens in the collection of the Zoologische Staatssammlung München (ZSM). The purpose of our study is to demonstrate that all of these specimens from widely spread localities in southwestern Angola and northwestern Namibia belong to *T. damarensis*, a species previously known only from the type locality at Khorixas, Namibia

Preserved specimens were examined to determine diagnostic features of *Tomopterna* species occurring in southwestern Africa, including snout–vent length (SVL), state of the subarticular tubercles (single or divided), presence/absence of a glandular ridge below the tympanum, distinctiveness of the tympanic membrane, skin texture, dorsal colour pattern, and presence of dark gular pigmentation in mature males. For one specimen each collected from the Kunene Region, Namibia, Erongo Region, Namibia, and Namibe Province, Angola, a fragment of the mitochondrial 16S gene was sequenced, using primers 16SA and 16SB (Palumbi 1996). Sequences for other *Tomopterna* species, plus *Amietia angolensis*, were obtained from GenBank (Table 1), aligned with Clustal W (Larkin et al. 2007), and the best-

Table 1. GenBank accession numbers of sequences used in the phylogenetic analyses.

Species	Locality	Accession Number
Amietia angolensis	Tanzania: Mazumbai, Tanga Region	DQ022350
Tomopterna cryptotis	South Africa: Bloemfontein, Free State	AY255099
Tomopterna damarensis	Namibia: Khorixas, Kunene	AF215419
Tomopterna damarensis	Angola: Pediva Hot Springs, Namibe	KU662310
Tomopterna damarensis	Namibia: Farm Omandumba, Erongo	KX869909
Tomopterna damarensis	Namibia: Epupa Falls, Kunene	KX869908
Tomopterna delalandii	South Africa: Stellenbosch, Western Cape	AY454372
Tomopterna elegans	Somalia: Buq Village, Puntland	HQ700692
Tomopterna gallmanni	Kenya: Lewa Wildlife Conservancy, Central Province	JX088642
Tomopterna kachowskii	Somalia: 4 km N Borama, Woqooyi Galbeed	HQ700690
Tomopterna krugerensis	South Africa: Tembe Elephant Park, KwaZulu-Natal	AY255098
Tomopterna luganga	Tanzania: Dodoma Region	AY547276
Tomopterna marmorata	South Africa: Lekgalameetse Nature Reserve, Limpopo	AF371233
Tomopterna natalensis	South Africa: Mafefe Road, Limpopo	AY205286
Tomopterna sp. "Beira"	Mozambique: Beira, Sofala Province	AY255093
Tomopterna sp. "Mauritania"	Mauritania	AY014383
Tomopterna sp. "Shankara"	Namibia: Shankara, Kavango East	AY255095
Tomopterna tandyi	South Africa: Blouberg Nature Reserve, Limpopo	AF436072
Tomopterna tuberculosa	Namibia: Ongongo, Kunene	AY255100
Tomopterna wambensis	Kenya: Wamba, Rift Valley Province	JX088651

fitting model of evolution found with jModelTest 2 (Darriba et al. 2012). A Bayesian phylogeny was estimated in a 1-million generation run in MrBayes 3.2 (Ronquist et al. 2012), with convergence confirmation based on effective sample sizes (ESS) of all parameters being >1,700 as calculated in Tracer 1.5 (Rambaut et al. 2014). A maximum likelihood phylogeny was produced in MEGA 7 (Kumar et al. 2015) with 1000 bootstrap replicates. MEGA 7 was also used to calculate uncorrected pairwise sequence distances between species.

Specimen information: ZSM 80/1960/1–7, seven adult specimens, SVL not taken; Namibia: Osire, Otjozondjupa Region (21°03'56.8" S, 17°21'53.6" E), 24 March 1960, by W. Hoesch. MCZ A-148577, adult male, SVL 36 mm; MCZ A-148582, adult female, SVL 35 mm; MCZ A-148585, subadult male, SVL 29 mm; Epupa Falls, Kunene Region (17°00'07.4" S, 13°14'44.1" E), 27 November 2011, by M. P. Heinicke, A. M. Bauer, J. Marais, S. V. Nielsen & S. L. Travers. MCZ A-149503, juvenile; Farm Omandumba, Erongo Mountains, Erongo Region (21°29'51.1" S,

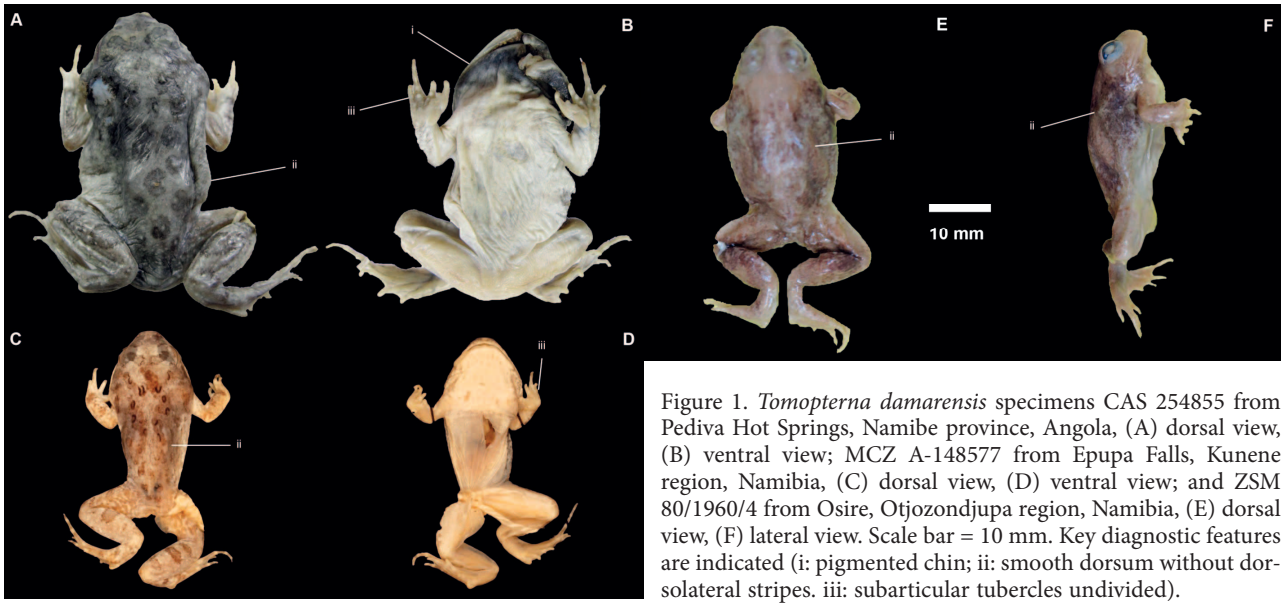

Figure 1. *Tomopterna damarensis* specimens CAS 254855 from Pediva Hot Springs, Namibe province, Angola, (A) dorsal view, (B) ventral view; MCZ A-148577 from Epupa Falls, Kunene region, Namibia, (C) dorsal view, (D) ventral view; and ZSM 80/1960/4 from Osire, Otjozondjupa region, Namibia, (E) dorsal view, (F) lateral view. Scale bar = 10 mm. Key diagnostic features are indicated (i: pigmented chin; ii: smooth dorsum without dorsolateral stripes. iii: subarticular tubercles undivided).

15°37'47.9" E), 12 June 2014, by M. P. Heinicke, A. M. Bauer, W. R. Branch, J. Childers & J. Marais. CAS 254855, adult male, SVL 41 mm; Angola: Pediva Hot Springs, Iona National Park, Namibe Province (16°17'4.62" S, 12°33'47.86" E), 3 December 2013, by L. M. P. Ceríaco, E. L. Stanley, A. L. Kuhn, J. V. Vindum, S. de Sá, S. Bandeira & H. Valério.

In preservative, the dorsal colour pattern consists of dark grey/brown spots or blotches on a somewhat paler background (Fig. 1). There are no dorsolateral or vertebral stripes. The upper eyelid has three darker blotches, with the central blotch extending across the forehead. Ventrally, all specimens are pale, except the largest male (CAS 254855) that has a dark throat. In body shape, all specimens represent typical *Tomopterna*. Of the diagnostic characters that distinguish species in the genus, the dorsal and ventral skin textures are relatively smooth, with the exception of MCZ A-149503, which has some small, widely spaced, randomly arranged tubercles (Fig. 2). There is a glandular ridge below the well-defined tympanum, but a supratympanic ridge is absent, and the subarticular tubercle on the first finger is single, not bifurcated. SVL in adult specimens ranges from 35–41 mm.

The above characters suggest that none of the specimens listed above is referable to *T. cryptotis, T. krugerensis*, *T. tandyi*, or *T. tuberculosa*. *Tomopterna tuberculosa* differs in colour pattern, typically displaying dark markings outlined with white, and has a dorsum that is heavily tuberculated or warty rather than smooth or sparsely tuberculated. *Tomopterna krugerensis* is similar in colour pattern and has a smooth skin texture, too, but has a bifurcated subarticular tubercule on the first finger. Both *T. cryptotis* and *T. tandyi* typically have a rough or warty skin texture and often a colour pattern including dorsolateral lines. Based on morphological characters, our specimens best match the diagnostic features of *T. damarensis*. One adult male, MCZ A-148577, does not have the dark gular region characteristic of *T. damarensis*, though (Dawood & Channing 2002). However, this specimen is 5 mm smaller than the larger adult male, and was collected at an earlier date, suggesting that it may not have been reproductively active. The juvenile specimen, MCZ A-149503, has small, widely spaced warts, but its skin texture is otherwise smooth, and it is unambiguously a member of the species *T. damarensis* based on our phylogenetic analyses (Fig. 3). All newly sequenced specimens are recovered in a clade with topotypic *Tomopterna damarensis* (identified as *T. damarensis* in Dawood & Uqubay 2004) with strong node support (Bayesian posterior probability/ML bootstrap = 0.91/93). The *T. damarensis* sequences are nearly identical, sharing 99.4–100% sequence identity (Table 2). In contrast, sequence identity between *T. damarensis* and its closest relatives, *T. cryptotis* and *T. tandyi*, is ~97.5%.

In their original description, Dawood & Channing (2002) suggested that *T. damarensis* likely has a larger range in Damaraland than just the vicinity of Khorixas. The specimens described in the present paper demonstrate that the range of *T. damarensis* extends far beyond Damaraland, to Namibe Province, Angola, 538 km northwest of Khorixas (Fig. 4), which represents a new country (and provincial) record. Namibian specimens from the Erongo Mountains (143 km S of the type locality) and Osire (258 km SE of the type locality) likewise represent new records for the Erongo and Otjozondjupa Regions, respectively. These additional localities also show that *T. damarensis* is tolerant of a variety of habitats as these are situated in three different terrestrial ecoregions. The primary requirement appears to be access to permanent water bodies – specimens collected by the authors were found at warm springs (Pediva), on the

Figure 2. *Tomopterna damarensis*, juvenile specimen MCZ A-149503, lateral view in life.

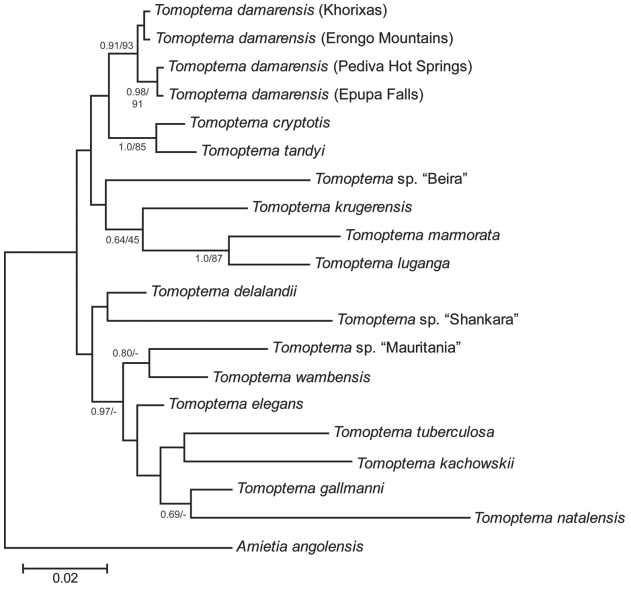

Figure 3. Bayesian phylogeny of *Tomopterna*, rooted with *Amietia angolensis*, based on data from the mitochondrial 16S gene. Bayesian posterior probabilities and ML bootstrap values are indicated at nodes. See Table 1 for GenBank accession numbers.

Table 2. Percent sequence identity between samples used in phylogenetic analyses.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. *T. cryptotis*																		
2. *T. damarensis* (Epupa)	97.5																	
3. *T. damarensis* (Erongo)	98	**99.6**																
4. *T. damarensis* (Khorixas)	97.7	**99.4**	**99.8**															
5. *T. damarensis* (Pediva)	97.5	**100**	**99.6**	**99.4**														
6. *T. delalandii*	96.8	98	98	97.8	98													
7. *T. elegans*	96.6	97.8	98.1	97.9	97.7	97.6												
8. *T. gallmanni*	96.6	97.2	97.2	97	97.1	97.6	97.9											
9. *T. kachowskii*	94.6	96	95.6	95.4	96	95.2	96.1	95.3										
10. *T. krugerensis*	96.2	96.4	96.8	96.6	96.4	95.7	96.2	95.4	93.2									
11. *T. luganga*	94.6	95.5	95.5	95.3	95.5	95	94.8	94.3	92.6	94.6								
12. *T. marmorata*	93.7	94.6	94.6	94.3	94.6	93.9	94.1	93.2	92	95	95.7							
13. *T. natalensis*	93	94	94.4	94.2	93.9	93.6	94.2	94.2	93.1	92.3	90.5	91.1						
14. *T.* sp. "Beira"	94.4	94.4	94.4	94.1	94.4	94.8	94.6	93.4	91.6	93	93.5	92.3	90.3					
15. *T.* sp. "Mauritania"	94.5	95.9	95.9	96.1	95.8	96.2	96.4	96.2	94.8	93.4	92.9	92.5	93	92.9				
16. *T.* sp. "Shankara"	93.2	93.9	93.9	93.7	93.9	94.6	94.1	93.8	92.1	93.2	91.6	92.3	90.1	91.4	92			
17. *T. tandyi*	98.4	97.6	97.9	97.8	97.5	97.2	97.2	96.6	94.8	95.3	95	93.5	93.8	94.1	95.3	93.2		
18. *T. tuberculosa*	94.1	94.6	94.6	94.6	94.6	95.9	95.5	95.9	93.7	93.7	92.1	91.4	92.3	92.8	94.3	93	94.1	
19. *T. wambensis*	96.1	97.4	97.4	97.2	97.3	97	97.9	97	95.5	95.2	93.8	93.4	93.2	93.8	96.4	92.9	97	94.5

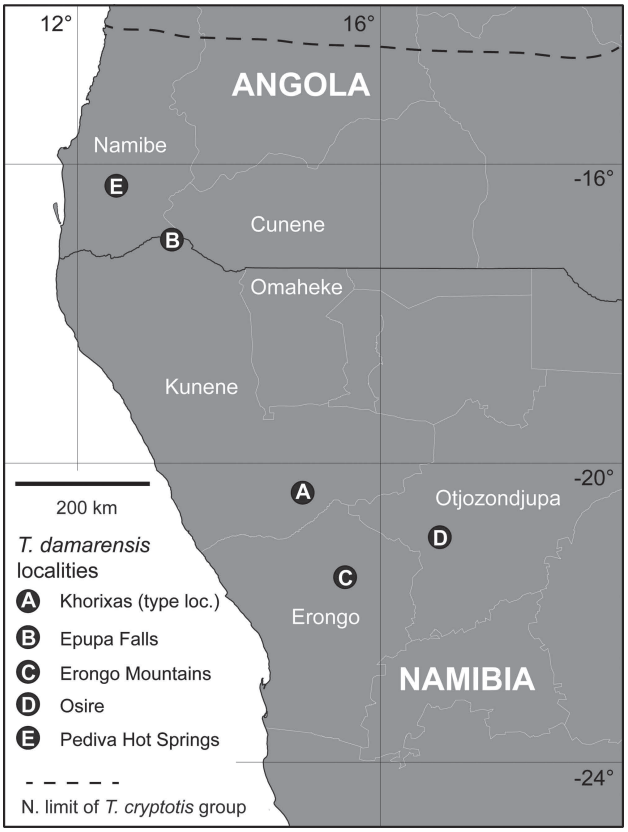

Figure 4. Map of northwestern Namibia and southwestern Angola, showing the type locality and newly confirmed localities for *Tomopterna damarensis*. Regions mentioned in the text and the northern range limit of the *Tomopterna cryptotis* complex in Angola and Namibia are shown.

edge of a farm dam (Erongo), and along a large perennial river (Epupa Falls), respectively.

Additional localities of *T. damarensis* will likely be found in other similar areas of dry, sandy habitat near permanent water. In Namibia, focal regions for future surveys should include Erongo, Kunene, Omaheke, and Otjozondjupa. In Angola, surveys should focus on both Cunene and Namibe Provinces. Museum collections already hold a minimum of 478 *Tomopterna* specimens from 86 localities in these areas; it is likely that at least some of these specimens correspond to *T. damarensis*.

The new confirmed records for *T. damarensis* help to clarify the conservation requirements of this species. Currently, the IUCN Red List status of *T. damarensis* is Data Deficient, primarily because of poorly documented range limits (IUCN 2016). Our new records demonstrate that the expanse of occurrence of *T. damarensis* is at least 70,000 km², although the continuity of the range of *T. damarensis* within this area remains unknown. Much of this area enjoys nominal protection in the form of a series of community conservancies in Namibia and as Iona National Park in Angola. Because species of *Tomopterna* are not of significant economic importance, *T. damarensis* does not face obvious threats other than those affecting the availability of surface water for reproduction. We recommend that the conservation status of *T. damarensis* be updated to Least Concern.

Acknowledgements

Permits for field collection of specimens were provided by the Namibia Ministry of Environment and Tourism (permit numbers 1626/2011 and 1894/2014) and the Angola National Institute of Biodiversity and Conservation Areas (INBAC; permit not num-

bered). We thank Joseph Martinez and José Rosado (Harvard University) for providing photographs. This research was funded by the JRS Biodiversity Foundation (to DCB, AMB, and MPH), the University of Michigan (MPH), and NSF grants DEB-1019443 and DEB-1556255 (to AMB), DEB-1202609 and DEB-1556559 (to DCB), and DEB-1556585 (to MPH).

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