

A new bullfrog from southern Africa (Pyxicephalidae, *Pyxicephalus* Tschudi, 1838)

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ABSTRACT

Four species of African bullfrogs are currently recognised. We describe a new species from southern Africa, which can be distinguished on the basis of morphology, advertisement call and DNA sequences. Morphologically it differs from other bullfrogs by a combination of characteristics including a tympanum that is smaller or equal in size to the eye, and smaller in diameter than the space between eye and tympanum, presence of a white dot on the tympanum, longitudinal skin ridges with speckling between dorsal mottles, pale vertebral line usually present, absence of cream coloured lateral stripes, absence of a pale interorbital-bar, upper jaw-barring absent or faint. It has been confirmed from north-eastern Namibia, southern Angola and north-western Botswana. Three further undescribed species are recognised but not formally named, pending further investigation. We confirm the genetic distinctiveness of *P. angusticeps*.

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Introduction

There are four species of African bullfrogs currently recognised (Frost 2023): *Pyxicephalus adspersus* Tschudi, 1838; *P. angusticeps* Parry, 1982; *P. edulis* Peters, 1854; and *P. obbianus* Calabresi, 1927. These are large frogs which are often consumed by humans in certain areas within their distribution (Badenhorst et al. 2002; Lord and Baines 1876; Nelson 2009). Although *P. adspersus* is protected in Gauteng Province of South Africa (Nature Conservation Ordinance 12 of 1983), it is not protected elsewhere, neither is any other species of *Pyxicephalus*. Pickersgill (2007) distinguished three forms of bullfrogs but was uncertain of their taxonomy. To add to the confusion, some juveniles look quite

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different from the adults, and some have been described as separate species (*Phrynopsis boulengeri* Pfeffer, 1893; *Phrynopsis usambarae* Ahl, 1924), both now regarded as synonyms of *P. edulis* (Loveridge 1936).

Bullfrogs display interesting breeding behaviour. Most are explosive breeders, breeding during the daytime (Kok et al. 1989). Males call from shallow water while extending the front limbs to display the striking yellow-orange on the upper arms or the pale white or yellow of the throat (Du Preez and Carruthers 2017). They often do not show any fear during breeding events, with fierce fighting observed in *P. adspersus* and *P. edulis* in South Africa (Engelbrecht et al. 2015) and *P. edulis* in Mozambique (MOR, pers. obs.). Unlike most other species of frogs, male bullfrogs are significantly larger than females.

Some species have been reported to lek. Dominant males chase off other males and attempt to mate with many females (Du Preez and Carruthers 2017). Non-dominant males remain some distance from the aggressive dominant male and form a lek. Males attempt to mate with females attracted to the lek. It is not easy to confirm the identity of the frogs in many of these reports, as we now know that pairs of species can be sympatric, and that there are more species in southern and eastern Africa, rather than just the three reported (*P. adspersus*, *P. edulis* and *P. angusticeps*).

The first report of parental care in bullfrogs showed that the tadpoles were gregarious and guarded by adults (Mitchell 1946). But Loveridge (1950) suggested that the adults were actually eating the tadpoles, as he had recovered tadpoles and small bullfrogs in the stomachs of several adults. Wager (1965) shared the same belief. Balinsky and Balinsky (1954) showed that adult *P. adspersus* in South Africa remain with the eggs and tadpoles. Males may dig channels to permit tadpoles to move from drying peripheral pools to deeper water (Kok et al. 1989; Cook et al. 2001). A detailed multi-year field study did not record even a single male feeding on tadpoles (Cook 1996). Cook's study extended our knowledge of parental care, demonstrating that offspring survival was significantly increased when a male bullfrog was present (Cook 1996). However, apart from observations of the unmistakable *P. adspersus* in South Africa, until bullfrog taxonomy is revised, breeding behaviour may not be correctly attributed to species.

Pyxicephalus adspersus (Figure 1A) was described from "*Promontorium Bonae Spei*" in South Africa (Tschudi 1838), here taken to be inland of the Cape of Good Hope. It appears to be present in central southern Africa, extending to central Kenya. The northern extent of its range is uncertain, as this nomen has been used historically as a catch-all term. Poynton (1964) for example, recognised only this single species of large bullfrog in southern Africa. However, Scott et al. (2013) showed that most East African museum material labelled as *P. adspersus* was *P. edulis*. The natural history of *P. adspersus* was summarised by Du Preez and Cook (2004).

Pyxicephalus edulis (Figure 1B) was described from Tete in Mozambique (Peters 1854). The description mentions types from 'Moçambique', 'Tette' and 'Sena'. Loveridge (1953) restricted the type locality to Tete in Mozambique, and Scott et al. (2013) designated ZMB 50301 as lectotype. It has been recorded from eastern southern Africa, from southern Somalia to northern South Africa (Channing and Rödel 2019). This is a medium-sized species and has been confused with juveniles of *P. adspersus* (see Cook and Minter 2004) and with *P. angusticeps* (Scott et al. 2013). Its life history is summarised in Cook and Minter (2004). Mercurio (2011) recorded *P. edulis* from southern Malawi (as

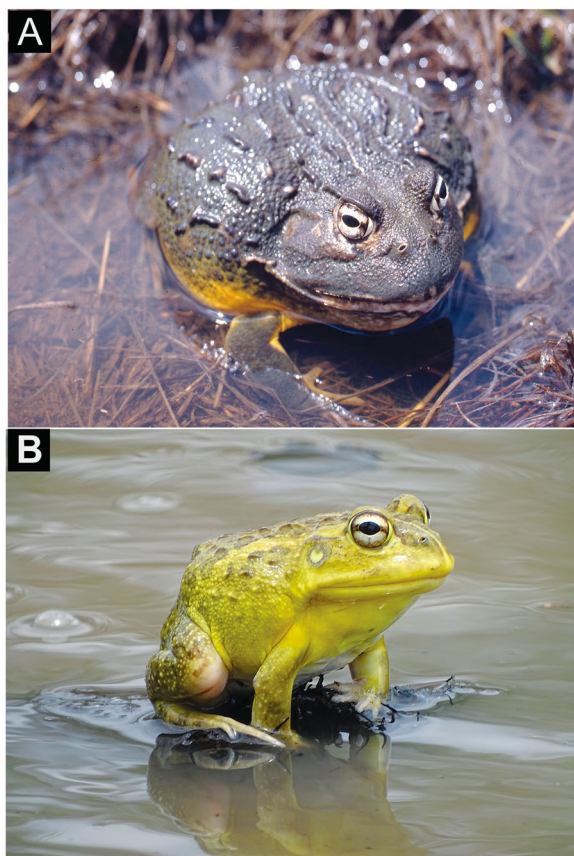


Figure 1. Males of *Pyxicephalus adspersus* (A) from Bloemfontein, South Africa (photo A Channing) and *P. edulis* (B) from Gorongosa National Park, Mozambique (photo M-O Rödel).

P. adspersus), and Marques et al. (2018) identified all southern Angolan material as *P. edulis*, while Baptista et al. (2019) list all Angolan material as *P. adspersus*. Scott et al. (2013) showed that most East African material labelled as *P. edulis* was actually *P. angusticeps*, including one animal of the *P. edulis* type series, ZMB 10056. Lanza (1981) recorded material reaching southern Somalia as *P. edulis*, but it is unknown if it should be assigned to *P. angusticeps*.

Much of the literature concerning bullfrogs in West Africa names them *P. edulis*, although there is evidence that the taxonomy is in need of revision (Rödel 2000; Channing and Rödel 2019). This is beyond the scope of the present paper, but we provisionally refer to this species as *Pyxicephalus* 'edulis west' (Figure 2A).

Pyxicephalus obbianus was described from Dolosbsciò in Somalia (Calabresi 1927). It is restricted to the coastal plain of Somalia, east of 46 degrees E, and north of 4 degrees N (Lanza 1981, 1990). This species remains poorly known.

Pyxicephalus angusticeps (Figure 2B) was described from Beira in Mozambique (Parry 1982). It is known along the coastal plain from southern Kenya to Beira. Its species status was confirmed by Scott et al. (2013), who demonstrated based on morphology, that the species is widespread along the East African coast. Sequences of the species

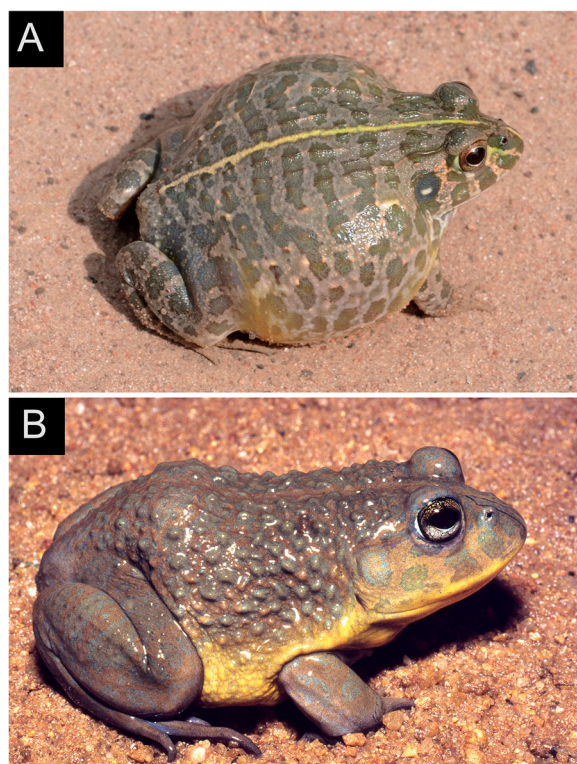


Figure 2. Males of *Pyxicephalus* 'edulis west' (A) from Senegal (photo JF Trape) and *P. angusticeps* (B) from Beira, AMNH A-190102 (photo A Channing).

are known from coastal Tanzania (Roelants et al. 2007, EF107211; Barratt et al. 2018, KY177062), "Africa" (Chen et al. 2005, AF206472), and the pet trade (Frost et al. 2006, DQ283157). Scott identified *P. angusticeps* BM 2002.438, and the 16S rRNA sequence of this specimen (EF107211) forms a clade with the sequences listed above.

Material from north-western Namibia, and the Okavango Delta in Botswana, did not conform to our present knowledge of the described species, and this led to the present study. The aim of this study was to identify the bullfrog species concerned, and if new to science to describe it.

Materials and Methods

Sampling

A list of available specimens that were examined is shown in Table 1. We examined the types of *P. adspersus*, *P. edulis* (including the types of *Phrynopsis Boulengeri* Pfeffer, 1893 and *Phrynopsis usambarae* Ahl, 1924), and *P. angusticeps*, and compared photos and data on *P. obbianus* from Scortecci (1933) and Scott et al. (2013). Material and sequences of the new species was collected in the Khaudum Game Reserve and Divundu area in Namibia and on Chief's Island in the Okavango in Botswana. We did not extend our sampling to the many museum specimens identified as *P. adspersus* or *P. edulis* due to the lack of molecular confirmation of their identities.

Table 1. *Pyxicephalus* specimens examined, giving the taxonomic status and locality. Museum acronyms follow Sabaj (2022)

SPECIES	STATUS	VOUCHER	LOCALITY	LATITUDE	LONGITUDE
<i>P. adspersus</i>	syntype	ZMB 10077	"Cape of Good Hope", South Africa		
<i>P. adspersus</i>	syntype	MNHN 1253-2 1991 1820	"Cap", South Africa		
<i>P. adspersus</i>	syntype	MNHN 1253-2 1991 1821	"Cap", South Africa		
<i>P. adspersus</i>		NMZB 18247	Debshan Ranch, Mahwe, Zimbabwe	19.6499°S	29.2358°E
<i>P. adspersus</i>		NMZB 618	Khami Ruins, Zimbabwe	20.1428°S	28.4232°E
<i>P. adspersus</i>		NMB 618	Bloemfontein, South Africa	29.0948°S	26.1446°E
<i>P. adspersus</i>		NMB 909	Bloemfontein, South Africa	29.0948°S	26.1446°E
<i>P. adspersus</i>		NMB 2078	Bloemfontein, South Africa	29.0948°S	26.1446°E
<i>P. adspersus</i>		NMB 906	Bloemfontein, South Africa	29.0948°S	26.1446°E
<i>P. adspersus</i>		NMB 2192	Bloemfontein, South Africa	29.0948°S	26.1446°E
<i>P. adspersus</i>		NMB 763	Thaba 'Nchu, South Africa	29.0209°S	26.8189°E
<i>P. adspersus</i>		NMB 4692	Bloemfontein, South Africa	29.0948°S	26.1446°E
<i>P. adspersus</i>		NMB 5999	Bloemfontein, South Africa	29.0948°S	26.1446°E
<i>P. angusticeps</i>	Holotype	NMSA 1992	Beira, Mozambique	19.8316°S	34.8371°E
<i>P. angusticeps</i>	Paratype	NMSA 1991	Beira, Mozambique	19.8316°S	34.8371°E
<i>P. angusticeps</i>	Paratype	NMZB 19774-A	Beira, Mozambique	19.8316°S	34.8371°E
<i>P. angusticeps</i>	Paratype	NMZB 19774-B	Beira, Mozambique	19.8316°S	34.8371°E
<i>P. angusticeps</i>		NMZB 31071	Ponte du Pungwe, Mozambique	19.5725°S	34.6460°E
<i>P. beytelli</i>	Holotype	NMNW R11398	Khaudum National Park, Namibia	19.0809°S	20.6966°E
<i>P. beytelli</i>	Paratype	SAIAB 141597	Khaudum National Park, Namibia	19.0809°S	20.6966°E
<i>P. beytelli</i>	Paratype	SAIAB 141598	Khaudum National Park, Namibia	19.0929°S	20.6201°E
<i>P. beytelli</i>	Paratype	NMNW R11399	Khaudum National Park, Namibia	19.0929°S	20.6201°E
<i>P. beytelli</i>	Paratype	PEM A15359	Khaudum National Park, Namibia	19.3728°S	20.4999°E
<i>P. beytelli</i>	Paratype	SAIAB 105031	Chief's Island, Okavango Delta, Botswana	19.21075°S	22.7901°E
<i>P. beytelli</i>	Paratype	SAIAB 141615	Khaudum National Park, Namibia	19.3728°S	20.4999°E
<i>P. edulis</i>		NMZB 15128	Mururuzi Ranch, Gunwe, Zimbabwe	17.9521°S	31.7329°E
<i>P. edulis</i>		NMZB 8036	Doddieburn Ranch, Zimbabwe	21.4667°S	29.4°E
<i>P. edulis</i>		NMZB 12339	Sazale Pan, Gonarezhou, Zimbabwe	21.6833°S	31.7333°E
<i>P. edulis</i>		GNP 432	Gorongosa National Park, Mozambique	-18.6847°S	34.0931°E
<i>P. edulis</i>	Lectotype	ZMB 50301	Tete, Mozambique	16.1328°S	33.6064°E
<i>P. edulis</i>	Paralectotype	ZMB 50260	Mozambique		
<i>P. edulis</i>	Paralectotype	ZMB 3349	Mozambique		
<i>P. edulis</i>	Paralectotype	ZMB 50302	Mozambique		
<i>P. edulis</i>	Paralectotype	ZMB 3350	Mozambique		
<i>P. edulis</i>	Syntype of <i>Phrynopsis boulengeri</i>	ZMB 10984	Quelimane, Mozambique	17.8503°S	36.9219°E
<i>P. edulis</i>	Holotype of <i>Phrynopsis usambarae</i>	ZMB 27637	Usambara, Tanzania		
<i>P. edulis</i>		ZMB 83283	Gorongosa National Park, Mozambique	18.9671°S	34.3634°E

Collected animals were euthanised with a buffered 250 mg/l solution of methanosulphonate (MS222). A small incision was made on the thigh to retrieve a piece of muscle tissue as well as on one side of the animal behind the armpit to retrieve a piece of liver tissue. Alternatively toe tips or pieces of tadpole tail were sampled. Tissue samples were cut in small pieces using sterile dissecting equipment, fixed in absolute EtOH and stored at -80°C . Type and voucher specimens were deposited in the amphibian collection of the South African Institute for Aquatic Biodiversity (SAIAB); herpetological collection of the National Museum of Namibia, Windhoek, Namibia (NMNW); and the herpetological collection, Bayworld, Gqeberha, South Africa (PEM). Tissue samples were entered in the SAIAB collection. Comparative material was investigated at the Museum für Naturkunde, Berlin, Germany (ZMB), the Museum National d'Histoire Naturelle, Paris, France (MNHN), and obtained on loan from the National Museum, Bloemfontein, South Africa (NMB). Measurements obtained from collected material as well as from material from loans are listed in tables and in the text.

Morphology

Measurements and acronyms are according to Watters et al. (2016). Linear morphometric measurements were taken using an electronic calliper, rounded to 0.1 mm. Standard descriptive statistics were calculated using Microsoft Excel. Measurements included the following variables: snout-vent-length (SVL), direct line from tip of snout to posterior margin of vent; snout-urostyle length (SUL), direct line from tip of snout to distal tip of the urostyle; head width (HW), widest point at the angle of the jaws; head length (HL), straight distance from posterior corner of mandible to the anterior tip of the head; mandible to eye distance (MBE), distance from posterior corner of mandible to posterior corner of eye; eye tympanum distance (ETD), shortest distance between anterior margin of the tympanum to the posterior corner of the eye; tympanum diameter (TD), greatest horizontal width of the tympanum; anterior interorbital distance (AIOD), distance between anterior corners of eyes; posterior interorbital distance (PIOD), distance between posterior corners of eyes; eye length (EL), direct distance between anterior and posterior corners of eyes; upper eyelid length (UEL), length of the upper eyelid, measured along the anterior-posterior axis; eye-snout distance (ES), measured as distance from the tip of head to anterior corner of eye; eye-nostril distance (NOD), measured as the shortest distance from the anterior corner of the eye to the margin of the nearest nostril; internarial distance (IND), measured as the shortest distance between the inner margins of the nostrils; nostril-upper lip distance (NL), measured as distance from the closest margin of the nostril to margin of upper lip; odontoid height (OH), measured from the tip of the odontoid to the lower margin of the mandible; inter-odontoid distance (IO), measured as the distance between the tips of the odontoids; hand length (HAL), measured from the tip of the third finger to the proximal edge of the palmar tubercle; femur length (FML), distance from vent to knee; tibia length (TBL), distance from the outer surface of the flexed knee to the heel/tibiotarsal articulation; foot length (FL), distance from proximal edge of inner metatarsal tubercle to tip of longest toe; inner metatarsal tubercle length (IMTL), measured as the greatest length of the inner metatarsal tubercle; webbing formula was determined according to standard usage, e.g. Savage and Heyer (1967).

Notes were made of colour patterns, skin folds and warts, and details of subarticular tubercles. The following body proportions were calculated: HW/SUL, HL/HW, HL/SUL, AIOD/IND, NOD/ES, IO/OH, EL/TD, ETD/TD, NOD/IND, SUL/HAL, TBL/SUL, FL/SUL, FL/TBL, and IMTL/AIOD.

Advertisement calls

Comparative advertisement calls of *P. adpersus*, *P. edulis* and *P. angusticeps* were available. Identification of the calls was confirmed by comparison to sequenced specimens, or in the case of *P. angusticeps*, to topotypical calls, from a vouchered male (AMNH A-190102) identified by Scott et al. (2013). Due to their large size and permit restrictions, few vouchers were collected of calling males. Recordings were made in the field, using directional microphones and various recorders. Some calls were taken from video recordings of breeding aggregations. All recordings by AC were made with a Marantz PMD 660. Older cassette recordings were made with a Sony TCD-5M. A list of advertisement call recordings is shown in Table 2. Calls were digitised where necessary and analysed using Raven Pro 1.6.5 (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology 2023) using an initial window size of 512. We investigated dominant frequency, frequency modulation, note duration, and pulse rate averaged over a note, following Köhler et al. (2017).

Calls of *P. adpersus* were recorded from Bloemfontein (Free State province), farm Bylsteel (Limpopo province), farm Vetkuil (Western Cape province) and Mosdene Private Nature Reserve (Limpopo province), all in South Africa; Harry Alan Golf Course in Bulawayo, and 10 km south-east of Bulawayo, in Zimbabwe. Calls of *P. angusticeps* were recorded just inland of Beira, Mozambique; Silversands Beach, Tanzania; and Tshaneni, eSwatini. *Pyxicephalus* aff. *adpersus* calls were recorded at Chief's Island, Botswana. *Pyxicephalus edulis* calls were recorded at Tsavo East and Voi, Kenya; South Luangwa National Park and Luangwa, Zambia; and Gorongosa National Park, Mozambique.

Table 2. *Pyxicephalus* advertisement calls analysed, showing locality, source and number of vocalizations.

SPECIES	LOCALITY	LATITUDE	LONGITUDE	SOURCE	n
<i>P. adpersus</i>	Beaufort West, South Africa	−32.3029	22.4733	HH Braack	6
<i>P. adpersus</i>	Mosdene Private Nature Reserve, Naboomspruit, South Africa	−24.6749	28.7328	NI Passmore	1
<i>P. adpersus</i>	Farm Bylsteel, Molemole, South Africa	−23.5383	29.5166	D Engelbrecht	5
<i>P. adpersus</i>	Farm Vetkuil, Beaufort West, South Africa	−32.3541	22.293	HH Braack	5
<i>P. adpersus</i>	Harry Alan Golf Course, Bulawayo, Zimbabwe	−20.1595	28.6313	R Hopkins	
<i>P. adpersus</i>	10 km SE Bulawayo, Zimbabwe	−20.2832	28.6311	R Hopkins	
<i>P. adpersus</i>	Bloemfontein, Free State Province, South Africa			A Channing	
<i>P. angusticeps</i>	Beira, Mozambique	−19.7038	34.7436	A Channing	7
<i>P. angusticeps</i>	Silversands, Kunduchi Bahari Beach, Tanzania	−6.6544	39.2144	A Channing	1
<i>P. angusticeps</i>	Tshaneni, eSwatini	−26.0013	31.7594	V Carruthers	1
<i>P. beytelli</i> sp. nov.	Chief's Island, Okavango, Botswana	−19.2118	22.8561	Joubert Films	13
<i>P. edulis</i>	Tsavo East, Kenya	−2.2619	38.3303	A Duff-Mackay	3
<i>P. edulis</i>	Voi, Kenya	−3.973	38.5559	A Duff-Mackay	1
<i>P. edulis</i>	South Luangwa National Park, Zambia	−13.077	31.704	A Channing	4
<i>P. edulis</i>	Feira, Luangwa, Zambia	−15.6211	30.4138	R Sjostedt	4
<i>P. edulis</i>	Gorongosa, Mozambique	−18.6847	34.0931	M-O Rödel	7

Molecular data

A list of molecular material is shown in Table 3. DNA was extracted and the 16S rRNA gene fragment and the nuclear tyrosinase exon 1 (*tyr*) fragment were amplified and sequenced using standard methods (Channing 2022; Kouamé et al. 2022).

Additional sequences were obtained from Genbank and from N. Baptista from southern Angola. The sequences were checked and aligned using Sequencher 5.4 (GeneCodes), and the Maximum Likelihood phylogeny was determined using the web version of IQ-TREE (Nguyen et al. 2015; Trifinopoulos et al. 2016). *Aubria masako* was used as the outgroup. The ultrafast bootstrap uFBoot2 (Hoang et al. 2017) was determined using 1000 replicates. The ModelFinder software (Kalyaanamoorthy et al. 2017) determined the appropriate models for the ML analysis. Support values were calculated as SH-aLRT (%) and the ultrafast bootstrap (%). Acceptable support values are > 95% for ultrafast bootstrap, and > 80% for SH-aLRT. The tree was rooted with *Aubria* and drawn using FigTree 1.4.4 (Rambaut 2018), with *Aubria* not shown. Uncorrected minimum *p*-distances were calculated using PAUP* (Swofford 2002).

The two most likely haplotypes for each individual for the nuclear genes were determined by first submitting the aligned sequences, including IUPAC polymorphism symbols, to SeqPhase step 1 (Flot 2010), which produces an input file for PHASE (Stephens and Donnelly 2003). PHASE calculates the likelihood of possible haplotypes. The output is submitted to SeqPhase step 2, which produces full sequences for each haplotype (accessed at <https://eeg-ebe.github.io/SeqPHASE>).

A haplotype network for *tyr* was determined using TCS 1.21 (Clement et al. 2000) and illustrated using PopArt (Population Analysis with Reticulate Trees; <http://poparty.otago.ac.za>).

Results

We refer some material from northern Namibia, southern Angola and southern Zambia to *Pyxicephalus* aff. *adspersus*. This name served as a placeholder for this study.

Morphology

The species are remarkably similar morphologically. All species have undivided subarticular tubercles, and all have a smoothly rounded canthus rostralis. Tympanum shape varies from round to vertically elliptical, with all *P.* aff. *adspersus* having round tympanums. The upper jaw is barred in *P. angusticeps* and *P. edulis*, but is without visible barring in *P.* aff. *adspersus* and *P. adspersus*. A pale patch on the tympanum is present in *P. angusticeps*, *P. edulis* and *P.* aff. *adspersus*, although sometimes it is not easily visible. A pale interorbital bar is absent in *P. angusticeps*, *P. edulis*, *P. adspersus* and *P.* aff. *adspersus*. The dorsal skin folds are not continuous in *P. angusticeps*, but run the length of the dorsum in the other species, rarely broken in *P. edulis* and *P. adspersus*. The pale vertebral stripe is wide in *P. angusticeps*, but thin in *P. edulis*. The pattern of markings on the thigh and tibia is variable in all species, varying from marbling in *P. angusticeps* to distinct cross bars in *P. edulis*, and *P.* aff. *adspersus*. Cross bars on extremities are usually absent in *P. adspersus*, particularly in larger animals. The SUL of the species may be a useful

Table 3. Available DNA sequences, giving voucher, locality, 16S rRNA and tyrosinase exon 1 Genbank accession numbers, and sources.

SPECIES	VOUCHER	LOCALITY	LATITUDE	LONGITUDE	GENBANK 16S	GENBANK TYR	SOURCE
<i>P. adspersus</i>	ZCAK AF0011	Africa			LC640564		Kambayashi et al. 2022
<i>P. adspersus</i>		Africa			NC044480		Cai et al. 2019
<i>P. adspersus</i>	FB-2006	Africa			DQ347304	DQ347146	Bossuyt et al. 2006
<i>P. adspersus</i>		Africa			LC440402		Yoshida and Kaito 2020
<i>P. adspersus</i>	SAIAB 141790	Khaudum National Park, Namibia	-19.7039	20.4962	OR128366		This study
<i>P. adspersus</i>	SAIAB 141791	Khaudum National Park, Namibia	-19.093	20.6201	OR128367		This study
<i>P. adspersus</i>	No voucher	Komani, South Africa	-31.8975	26.8753	OR128368		This study
<i>P. adspersus</i>	FB 480	Witvlei, Namibia	-22.3947	18.5482	OR128369	OR125114	This study
<i>P. adspersus</i>	FB 482	Witvlei, Namibia	-22.3947	18.5482	OR128370	OR125115	This study
<i>P. adspersus</i>	FB 2033	Waterberg, Namibia	-20.5284	17.2782	OR128371	OR125116	This study
<i>P. adspersus</i>	AC1147	Bloemfontein, South Africa	-29.0498	26.1446	DQ022366		Scott et al. 2005
<i>P. beytelli</i> sp. nov. Paratype	SAIAB 141598	Khaudum National Park, Namibia	-19.093	20.6201	OR128372		This study
<i>P. beytelli</i> sp. nov.	BMNH 2018.5790	Sioma Ngwezi National Park, Zambia	-16.8987	23.5985	MK464306		Bittencourt-Silva 2019
<i>P. beytelli</i> sp. nov.	BMNH 2018.5789	Sioma Ngwezi National Park, Zambia	-16.8987	23.5985	MK464307		Bittencourt-Silva 2019
<i>P. beytelli</i> sp. nov.	BMNH 2018.5788	Sioma Ngwezi National Park, Zambia	-16.8987	23.5985	MK464308		Bittencourt-Silva 2019
<i>P. beytelli</i> sp. nov.	BMNH 2018.5791	Sioma Ngwezi National Park, Zambia	-16.8987	23.5985	MK464305		Bittencourt-Silva 2019
<i>P. beytelli</i> sp. nov.	BMNH 2018.5787	Sioma Ngwezi National Park, Zambia	-16.8987	23.5985	MK464309		Bittencourt-Silva 2019
<i>P. beytelli</i> sp. nov. Paratype	NMNW R11399	Khaudum National Park, Namibia	-19.093	20.6201	OR128373		This study
<i>P. beytelli</i> sp. nov. Paratype	PEM A15359	Khaudum National Park, Namibia	-19.093	20.6201	OR128374		This study
<i>P. beytelli</i> sp. nov. Paratype	SAIAB 141615	Khaudum National Park, Namibia	-19.093	20.6201	OR128375		This study
<i>P. beytelli</i> sp. nov. Holotype	NMNW R11398	Khaudum National Park, Namibia	-19.093	20.6201	OR128376	OR125117	This study
<i>P. beytelli</i> sp. nov. Paratype	SAIAB 141597	Khaudum National Park, Namibia	-19.093	20.6201	OR128377	OR125118	This study
<i>P. beytelli</i> sp. nov.	AE0756	Ondiva, Angola	-17.05	15.7333	(AE0756)	(AE0756)	N Baptista unpublished
<i>P. beytelli</i> sp. nov.	AE0757	Ondiva, Angola	-17.05	15.7333	(AE0757)	(AE0757)	N Baptista unpublished
<i>P. beytelli</i> sp. nov.	AE0758	Ondiva, Angola	-17.05	15.7333	(AE0758)	(AE0758)	N Baptista unpublished
<i>P. beytelli</i> sp. nov. Paratype	SAIAB 105031	Chief's Island, Okavango Delta, Botswana	-19.2118	22.8561	OR128378		This study
<i>P. edulis</i>	CB 13.483	Ndimba Forest Reserve, Tanzania	-9.5833	39.6167	KY177061		Barratt et al. 2018
<i>P. edulis</i>	ZMB 83282	Gorongosa, Mozambique	-18.6847	34.0931	OR128379	OR125119	This study
<i>P. edulis</i>	GNP 177	Gorongosa, Mozambique	-18.6847	34.0931	OR128380		This study
<i>P. edulis</i>	GNP 432	Gorongosa, Mozambique	-18.6847	34.0931	OR128381		This study
<i>P. 'edulis west'</i>		Benin				HQ014445	Van der Meijden et al. 2011
<i>P. 'edulis west'</i>	Be119	Pendjari National Park, Benin	10.8847	1.4925	KF991277		Barej et al. 2014
<i>P. sp. 1</i>	SAIAB 106224	GooMoreme Reserve, Botswana	-22.562	27.4337	OR128382		This study
<i>P. sp. 1</i>	SAIAB 235118	Vivo, Limpopo Province, South Africa	-23.2004	29.4058	OR128383	OR125120	This study
<i>P. sp. 1</i>	SAIAB 235320	Tshipise, Limpopo Province, South Africa	-22.6549	30.0462	OR128384		This study
<i>P. sp. 1</i>	SAIAB 235319	Waterpoort, Limpopo Province, South Africa	-22.8085	29.4664	OR128385		This study

(Continued)

Table 3. Continued.

SPECIES	VOUCHER	LOCALITY	LATITUDE	LONGITUDE	GENBANK 16S	GENBANK TYR	SOURCE
<i>P. sp. 1</i>	SAIAB 235311	Tshipise, Limpopo Province, South Africa	−22.6549	30.0462	OR128386		This study
<i>P. sp. 1</i>	SAIAB 235304	Waterpoort, Limpopo Province, South Africa	−22.8578	29.5599	OR128387		This study
<i>P. sp. 1</i>	SAIAB 106088	Ndumo NR, KwaZulu-Natal Province, South Africa	−26.8934	32.2585	MH115770		Reeder 2019
<i>P. sp. 1</i>	SAIAB 106236	Bonamanzi, Hluhluwe, South Africa	−28.0673	32.3233	MH115771		Reeder 2019
<i>P. sp. 1</i>	SAIAB 106313	Monzi Road, St Lucia, South Africa	−28.3906	32.3149	MH115769		Reeder 2019
<i>P. sp. 1</i>	FMK 66466	KwaMbonambi, South Africa	−28.6038	32.0866	AF215505		Vences et al. 2000
<i>P. sp. 1</i>	SAIAB 106312	Monzi Road, St Lucia, South Africa	−28.4011	32.3195	OR128388		This study
<i>P. sp. 1</i>	SAIAB 105329	Phalaborwa, South Africa	−23.979	31.1816	OR128389	OR125121	This study
<i>P. sp. 1</i>	SAIAB 235120	Karingani GR, Mozambique	−24.3356	32.2617	OR128390		This study
<i>P. angusticeps</i>	AMNH A168412	Tanzania			DQ283157	DQ282941	Frost et al. 2006
<i>P. angusticeps</i>	BMNH 2002.438	Ruvu South, Tanzania	−6.9503	38.8379	EF107211		Roelants et al. 2007
<i>P. angusticeps</i>	CB 13.618	Ruvu North, Tanzania	−6.9239	38.5503	KY177062		Barratt et al. 2018
<i>P. angusticeps</i>		Africa			AF206472		Chen et al. 2005

diagnostic character. The mean SUL for *P. angusticeps* is 60.5 mm, range 37.9–73.9 mm ($n = 5$). *Pyxicephalus edulis* have a mean SUL of 80.4 mm, range 19.1–94.3 mm ($n = 12$). *Pyxicephalus adspersus* is the largest bullfrog, known to reach a SUL of 285 mm (R. Hopkins, pers comm.). Our sample mean is 82.5 mm, range 25.3–126.3 mm ($n = 11$). The mean SUL for *P. aff. adspersus* is 142.8 mm, range 135–200 mm ($n = 7$).

The body proportions are remarkably similar between all these species, with some exceptions. The mean HW/SUL ratio of the larger species is 0.49 in *P. adspersus*, and 0.5 in *P. aff. adspersus*. The smaller species have ratios of 0.43 in *P. edulis* and 0.39 in *P. angusticeps*. The relative eye and tympanum sizes compared by the ratio EL/TD show a mean of 1.01 for *P. aff. adspersus*, while the other species all have larger eyes than tympanums, with mean ratios of 1.47 in *P. angusticeps*, 1.54 in *P. edulis* and 1.6 in *P. adspersus*. The two larger species, *P. adspersus* and *P. aff. adspersus* both have mean values for ETD/TD of 1.3, while the other species vary from 0.74 in *P. angusticeps* and 0.75 in *P. edulis*.

The colour patterns of adults may prove diagnostic, although too few breeding males and females have been observed to use these as diagnostic characters. *Pyxicephalus adspersus* has a uniform dark-green back, with scattered white markings, with bright yellow flanks tinged with orange at the base of the front limbs in breeding males, and no pale mark on the tympanum. *Pyxicephalus angusticeps* has greenish-olive warts on a brown background, with yellow flanks (Channing et al. 1994), but no distinct pale mark on the tympanum. Males of *P. edulis* are brown to olive with darker blotches, and bright yellow lips and throat, with the yellow extending to the arm insertion and the belly, and a pale patch on the tympanum. The upper lip is barred. When breeding, dominant males have a yellow ground colour with darker olive markings. The colour patterns of the new species are described and illustrated below.

Dorsal skin folds are well-developed in *P. adspersus* and *P. edulis* but reduced in the other species, being frequently a series of elongated warts in *P. angusticeps*.

Advertisement call

Bullfrog calls consist of low-pitched ‘whoop’ sounds. They vary by duration, emphasised frequency, degree of frequency modulation, and pulse rate. The pulses of calls with a slow pulse rate can be distinguished by ear. The sources of the recordings are listed in Table 2 and the species calls are compared in Table 4. Sound spectrograms of representative calls are presented in Figure 3.

Pyxicephalus adspersus has the longest call, with a mean duration of 1.26 sec (0.56–1.79 s, $n = 21$). The mean emphasised frequency is 234.2 Hz (86.1–369.5 Hz, $n = 21$). The calls show a small amount of frequency modulation, rising slightly in pitch at the end of the call, but this is not always noticeable. Some males will call at night during rain, with a peak of calling, fighting and mating during the day until mid-morning. The duration and dominant frequency of the calls varies, with overlap of both parameters in South Africa. The calls from the Bulawayo area showed the lowest dominant frequencies (86.1–172.3 Hz), which might be attributable to the larger sizes of the males.

Pyxicephalus angusticeps has a brief high-pitched call with a mean duration of 0.16 sec (0.08–0.21 sec, $n = 9$). The mean emphasised frequency is 573.6 Hz (301.5–775.2 Hz, $n = 9$). The calls are clearly frequency modulated with a rise mid-duration. The calls are not strongly pulsed. The duration of the calls and the dominant frequency overlapped at

Table 4. Comparison of the advertisement calls of *Pyxicephalus* from eastern and southern Africa.

Species	Description	Mean duration	Mean emphasised frequency	Characteristics
<i>Pyxicephalus adspersus</i>	Very long call	1.26 s (0.56–1.79 s, $n = 21$)	234.2 Hz (86.1–369.5 Hz, $n = 21$)	Very slight frequency modulation
<i>Pyxicephalus angusticeps</i>	Brief, high-pitched call	0.16 s (0.08–0.21 s, $n = 9$)	573.6 Hz (301.5–775.2 Hz, $n = 9$)	Rise in frequency at mid duration
<i>Pyxicephalus beytelli</i> sp. nov.	Brief, pulsed call	0.34 s (0.20–0.55 s, $n = 13$)	198.8 Hz (172.3–258.4 Hz, $n = 13$)	Pulsed 74.8 s ⁻¹ (56.1–88.2 s ⁻¹ , $n = 13$)
<i>Pyxicephalus edulis</i>	Brief frequency modulated call	0.17 s (0.05–0.40 s, $n = 20$)	309.3 Hz (237.0–355.3 Hz, $n = 20$)	Distinct rise in frequency at the end of the call
<i>Pyxicephalus obbianus</i>	A very loud bellow (Scortecci 1933)			

all localities, with the lowest and highest dominant frequencies recorded from Beira. The topotypical calls from Beira were produced by a vouchered specimen (AMNH A-190102).

Pyxicephalus aff. *adspersus* calls are each a brief pulsed whoop, with a mean duration of 0.34 sec (0.20–0.55 sec, $n = 13$), and a mean emphasised frequency of 198.8 Hz (172.3–258.4 Hz, $n = 13$). The mean pulse rate is 74.8 s⁻¹ (56.1–88.2 s⁻¹, $n = 13$).

Pyxicephalus edulis has a brief frequency-modulated call. The mean duration is 0.17 sec (0.05–0.40 sec, $n = 20$). The mean emphasised frequency is 309.3 Hz (237.0–355.3 Hz, $n = 20$). Both the duration and dominant frequencies of the calls overlapped at all localities.

Molecular data

The best-fit model chosen for the 16S rRNA analysis was TIM2e + G4 according to BIC, and the model chosen for *tyr* was K2P + I according to BIC. The maximum likelihood tree based

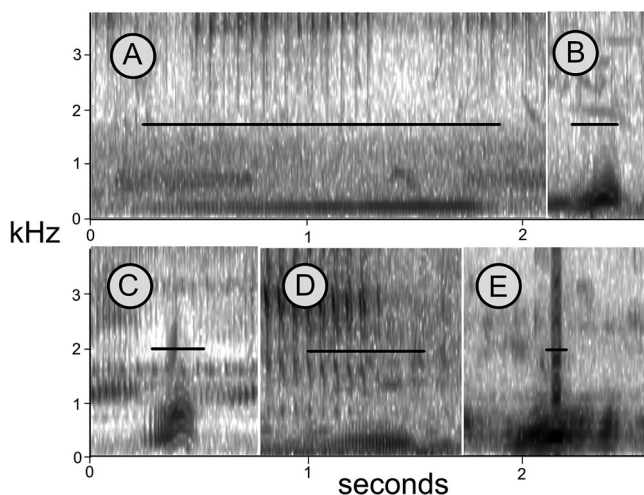


Figure 3. Sound spectrograms of representative bullfrog calls. (A) *Pyxicephalus adspersus* (Vetkuil, Limpopo province, South Africa), (B) *P. angusticeps* (Beira, Mozambique), (C) *P. beytelli* (Chief's Island, Botswana), (D) *P. edulis* (Gorongosa, Mozambique). The black lines indicate the length of each call. Compare frequency of each species' call in Table 4 in order to identify the calls.

on 16S rRNA sequences showed that the sample consisted of six clades. All lineages are monophyletic and regarded as separate evolutionary units. Three of the genotyped taxa, *P. edulis*, *P. angusticeps* and *P. adspersus* are established species. The identification of material of *P. adspersus* is not in doubt, as it occurs alone inland of the Cape of Good Hope, the type locality, from where sequences were obtained. Material is assigned to *P. edulis* based on published identification (Barratt et al. 2017) and the proximity of the samples to Tete, the type locality. Gorongosa National Park is about 200 km from Tete, connected by drainage lines. *Pyxicephalus angusticeps* identification was confirmed by Scott et al. 2013, based on BM 2002.438, which has been sequenced (EF107211). One taxon is described below as new to science, one is insufficiently known (here referred to as *P. sp. 1*), and one (the West African form) is not treated here.

Pyxicephalus adspersus and *P. aff. adspersus* are sister species, with a 16S rRNA *p* distance of 5.5–5.7%. *Pyxicephalus angusticeps* from coastal Africa and *P. 'edulis west'* are sister species with a *p* distance of 7.8–8.2%. *Pyxicephalus sp. 1* and *P. edulis* are sister species with a *p* distance of 2.3–3.6% (Table 5). The maximum likelihood tree is shown in Figure 4.

The nuclear *tyr* gene shows similar relationships, with *P. aff. adspersus* and *P. sp. 1* having a *p* distance from the other bullfrog species of 0.6–1.6% and 0.2–2.0% respectively. The haplotype network (Figure 5) shows that no haplotypes are shared between species. The uncorrected *p* distances between species for 16S rRNA and *tyr* are shown in Table 5.

Taxonomy

We use the concept of species as units of separately evolving metapopulation lineages (De Queiroz 2007). Differences in mitochondrial 16S and nuclear *tyr* sequences, advertisement call, and morphology, indicate that there is one new species from southern Africa, which we describe below. In addition, there is a clade of bullfrogs (*P. sp. 1*) that is presently under investigation. The taxon known as *Pyxicephalus 'edulis west'* (Channing and Rödel 2019) is probably also a good species, but its taxonomy will be dealt with separately.

***Pyxicephalus beytelli* sp. nov.** Du Preez, Netherlands, Rödel and Channing

Beytelli's bullfrog

Figures 6, 7, 8

Referred to above as *Pyxicephalus aff. adspersus*

Zoobank: urn:lsid:zoobank.org:act: 15D48E74-594A-46ED-B31A-74F78C94F5C5

Table 5. Uncorrected *p* distances between species of *Pyxicephalus* for 16S rRNA (below diagonal) and *tyr* (above diagonal).

	<i>P. adspersus</i>	<i>P. beytelli</i> sp. nov.	<i>P. edulis</i>	<i>P. sp. 1</i>	<i>P. angusticeps</i>	<i>P. 'edulis west'</i>
<i>P. adspersus</i>		0.6–0.8%	1.0–1.2%	1.2–2.0%	0.6–1.2%	0.8–1.0%
<i>P. beytelli</i> sp. nov.	5.5–5.7%		1.4%	1.4–1.6%	0.8%	1.0%
<i>P. edulis</i>	4.7–5.9%	6.5–6.8%		0.2%	0.8%	0.8%
<i>P. sp. 1</i>	5.3–5.5%	7.4–7.6%	2.3–3.6%		0.6–1.0%	1.0%
<i>P. angusticeps</i>	7.4–8.1%	10.0%	5.9–7.2%	7.8–8.7%		1.0%
<i>P. 'edulis west'</i>	6.3–6.7%	8.9%	5.5–7.0%	6.5–6.7%	7.8–8.2%	

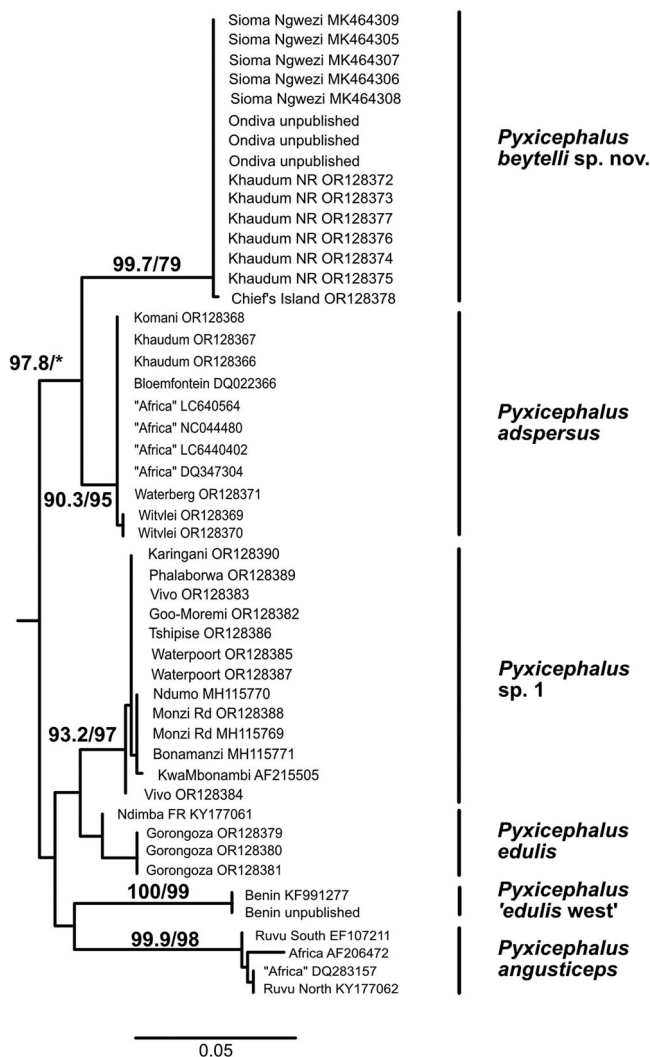


Figure 4. Maximum likelihood consensus tree of available 16S rRNA sequences of *Pyxicephalus*. Scale shows rate of substitutions. Ultrafast bootstrap percentages shown for values above 95% / SH-aLRT percentages shown above 75%.

Holotype

A male, NMNW R11398, (field number AL211204F2), collected by L du Preez and EC Netherlands in Khaudum National Park, Namibia (19.0809°S, 20.6966°E), 4 December 2021 (Figure 6).

Paratypes

Four males collected by LdP and ECN in Khaudum National Park, Namibia: SAIAB 141597, field number AL211204F1, with the same locality details as the holotype; SAIAB 141598, field number AL211204H1 and NMNW R11399, field number AL211204H2 from a muddy pan

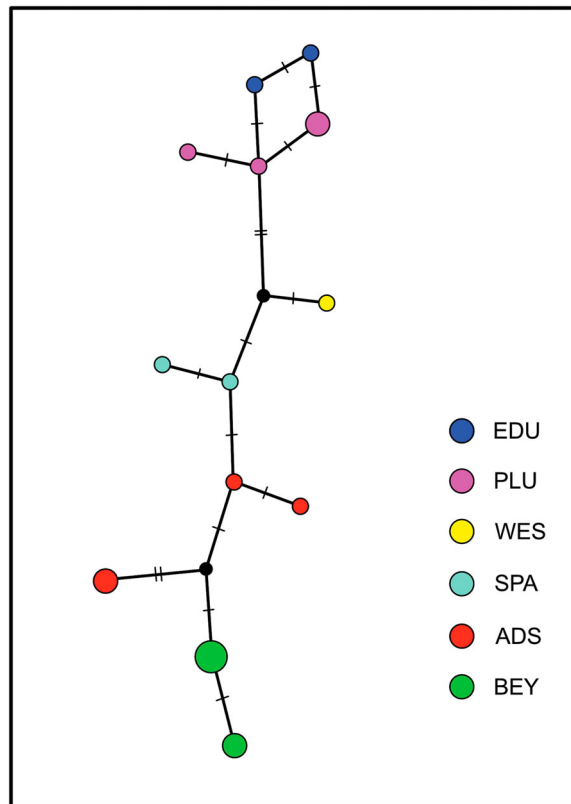


Figure 5. Haplotype network for the tyrosinase exon 1 gene fragment. Tik marks indicate differences, solid black circles indicate hypothetical intermediates. Circle sizes are proportional to number of haplotypes. Species abbreviations: ADS – *P. adspersus*, BEY – *P. beytelli*, EDU – *P. edulis*, WES – *P. 'edulis west'*, SPA – *P. sp. 1*, PLU – *P. sp. 2*.

(19.0929°S, 20.6201°E); PEM A1359, field number AL211204A1, from a dirt road (19.3728°S, 20.4999°E). A male, SAIAB 105031, field number AACRG 1956, collected by Marleen Byron and LdP from Chief's Island, Botswana (19.2108°S, 22.7901°E), 26 November 2009.

Diagnosis

The new species is similar to all other species of bullfrogs in morphology and burrowing behaviour. We assign it to the genus *Pyxicephalus* based on the presence of teeth on the maxilla, two large sharp projections on the lower jaw, the inner metatarsal tubercle strongly flanged, no outer metatarsal tubercle, the outer metatarsals bound into the sole, and the presence of vomerine teeth, all characters that distinguish the genus *Pyxicephalus* from other pyxicephalids (Poynton 1964).

Pyxicephalus beytelli is a large frog, maximum SVL 208 mm. The large size distinguishes it from *P. angusticeps* (maximum SVL 78.7 mm), *P. edulis* (maximum SVL 103 mm) and *P. sp. 2*. (maximum SVL 120 mm). It has strongly developed dorsal skin ridges, differing from the oval dorsal warts of *P. angusticeps* and *P. obbianus*. There is speckling between the dorsal mottles separating it from *P. angusticeps* and *P. obbianus* which

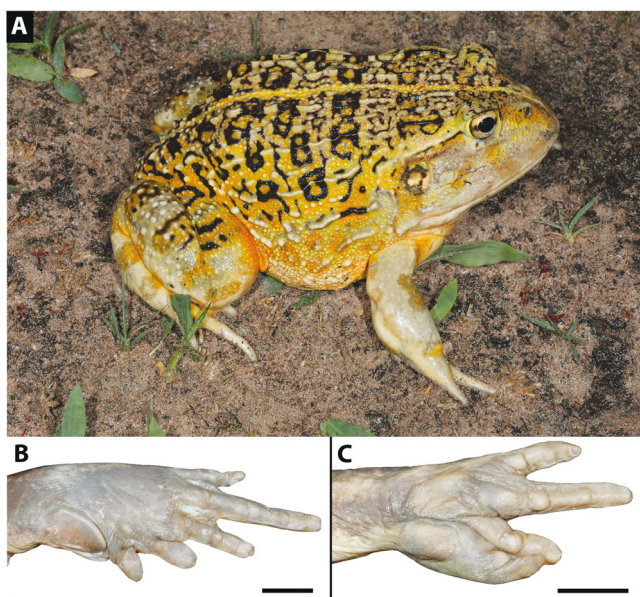


Figure 6. (A) Male Holotype of *Pyxicephalus beytelli*, NMNW R11398. (B) Right foot. (C) Right hand. Scales both 10 mm.

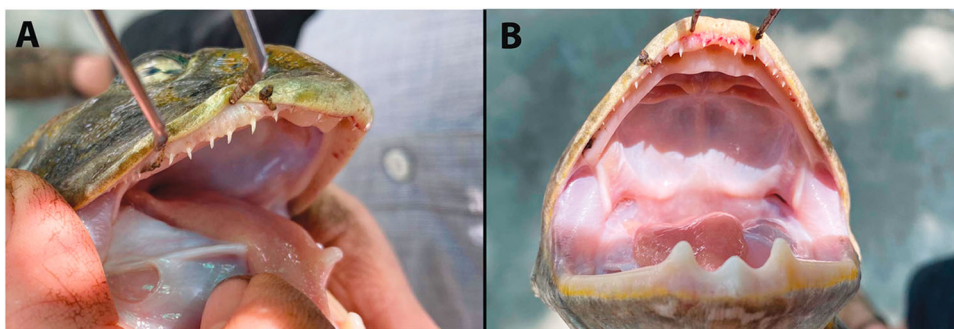


Figure 7. *Pyxicephalus beytelli* male. (A) Widely-spaced recurved teeth. (B) Open mouth showing teeth and odontoids.

have none. The odontoids are longer than wide, distinguishing it from *P. angusticeps* and *P. obbianus*. There is a white spot on the tympanum, distinguishing it from *P. adspersus*. The tympanum is smaller or equal to the eye, distinguishing it from *P. obbianus* with a much larger tympanum. The eye-tympanum distance is more than one tympanum width, distinguishing it from *P. angusticeps* where it is equal to or less than one tympanum, and *P. obbianus* where the tympanum is adjacent to the eye. There are no cream-coloured lateral stripes or mottles, unlike *P. adspersus*, *P. obbianus*, *P. edulis* and *P. sp. 2*. The subarticular tubercles on the feet are very small to barely visible, compared to the relatively distinct tubercles in *P. adspersus* and *P. sp. 2*.

The colour pattern of a golden yellow back with black markings (see below) is different to the other species.

Pyxicephalus beytelli has a mean HW/SUL ratio of 0.5, distinguishing it from *P. edulis* (0.43) and *P. angusticeps* (0.39). The eye and tympanum are nearly equal in diameter, with a mean ratio of EL/TD of 1.01, distinguishing it from the species with relatively larger eyes, with ratios of 1.47 in *P. angusticeps*, 1.54 in *P. edulis* and 1.41 in *P. adspersus*. The mean ratio ETD/TD is 1.3, distinguishing it from *P. angusticeps* (0.74) and *P. edulis* (0.75).

The call is shorter (0.2–0.55 sec) than that of *P. adspersus*, (0.56–1.79 sec), and longer than that of *P. angusticeps* (0.08–0.21 sec), with a lower emphasised frequency (172.3–258.4 Hz) than *P. angusticeps* (301.5–775.2 Hz). The call is strongly pulsed, unlike all the other species (Figure 3).

The uncorrected *p*-distances as percentages of *P. beytelli* compared to all other species of bullfrogs based on 16S rRNA vary from 5.5–10%, and 0.6–1.6% for *tyr* (Table 5).

The new species occurs sympatrically with the other large species, *P. adspersus* in Khaudum National Park in northern Namibia.

Description of holotype (measurements in mm, summarised in Table 6).

A male (Figure 6A) SVL 153, SUL 145; the body is robust; head short (HL/SVL 0.36, HW/SVL 0.47), not wider than trunk, not longer than wide (HL/HW 0.77); snout short (ES/HL 0.46), rounded in dorsal view, bluntly rounded in profile, slightly projecting beyond lower jaw, narrow (ES/IND 2.6); canthus rostralis smoothly rounded; loreal region slightly concave; nostrils situated on slight projections, closer to the eye than snout tip (NOD/NS 0.70); eyes directed anterolaterally, slightly protruding, relatively small (EL/HL 0.19); eye diameter about half of snout length (EL/ES 0.42); anterior interorbital distance is greater than upper eyelid (AIOD/UEL 1.5), and greater than internarial distance (AIOD/IND 2.34); internarial distance subequal to eye diameter (IND/EL 0.9); distance from eye to tympanum nearly twice diameter of tympanum, tympanum slightly larger than eye diameter (TD/ED 1.1); upper jaw with widely spaced recurved teeth (Figure 7); choanae small, round, vocal sac single; dorsal surfaces of head, trunk and limbs with rounded or elongated warts; 10–14 dorsal and lateral longitudinal skin folds that may be continuous or broken, ventral surface of limbs, gular and abdomen smooth.

Front limbs robust (Figure 6C), hand moderately large (HAL/SVL 0.21); tips of fingers not enlarged into discs; relative length of fingers: IV < II < I < III; subarticular tubercles single and distinct, with one on fingers I and II, and two on fingers III and IV; fingers without webbing; thenar tubercle distinct; metacarpals without supernumerary tubercles; pale nuptial pads present on upper surface of fingers I and II.

Hind limbs stout (Figure 6B), tarsal tubercle absent; tibia short (TBL/SVL 0.36); heels not reaching each other when knees are flexed and thighs are held at right angle to body; foot equal to tibia; relative length of toes: I < II < V < III < IV; toes without expanded discs; subarticular tubercles very flat to barely visible: one on toe I and II, two on toe III, three on toe IV and two on toe V; pedal webbing formula I1–2III1.5–2IIII2–3IV3.5–2 V; thin margin of webbing extending to tips; inner metatarsal tubercle prominent and shovel-shaped, continuing as a tarsal ridge, larger than eye diameter (IMTL/EL 1.36); outer metatarsal tubercle absent.

Table 6. Measurements of the holotypes and paratypes of *Pyxicephalus beytelli* plus other type material of *P. adspersus*, *P. angusticeps* and *P. edulis*. See text for abbreviations. Due to preservation condition, not all measures could be taken from all vouchers.

Species	Voucher	Status	SVL	SUL	HW	HL	MBE	ETD	TD	ITOS	AIOD	PIOD	EL	UEL	ES	NOD	IND	NL	OH	IO	FML	TBL	HAL
<i>P. angusticeps</i>	NMSA 1992	Holotype	78.5	71.9	27.1	29		5.1	5.7	5.6	7.8		8.6	9.5	14.5	3.8	5.7					29.2	18.8
<i>P. angusticeps</i>	NMSA 1991	Paratype	78.7	73.9	26.8	28		5.9	6.2	4.3	10		8.7	9.2	13.8	3.1	5.2					29.4	17.1
<i>P. angusticeps</i>	NMZB 19774-A	Paratype		43.1	16.9	14.9		1.7	3	2.5	5.8		5	5.3			3.3					15.8	
<i>P. angusticeps</i>	NMZB 19774-B	Paratype		37.9	15.2	6.8		2	3.2	2	3.8		4.9	5.3			2.4					15.2	
<i>P. adspersus</i>	ZMB 10077	Syntype	28.2	27.5	13.8								4.1						1.5	3.4	10.5	10.3	
<i>P. adspersus</i>	MNHNP 1253-2 1991 1820	Syntype	27.2		12.7	13.3		2.3	1.9	1.5	2.4	6.5	4.4	4.6	5	2.2	2	2.7	1.4	3.1	10.7	10.2	6.5
<i>P. adspersus</i>	MNHNP 1253-2 1991 1820	Syntype	30.7	25.3	13.4	13.3	5.1	1.7	1.7	2.5	2.9	6.3	4.6	4.9	4.8	2.7	1.7	2.5			10.1	8.1	6.8
<i>P. beytelli</i>	NMNW R11398	Holotype	153	145	71.6	55.6	28.9	15	11.8	15.0	21.7	32.4	11	15	25.3	11.5	9.7	15.3	9.9	14.8	66	55.5	33.0
<i>P. beytelli</i>	SAIAB 141598	Paratype	183	150.4	77.2	62.8	33.4	17.9	12.7	12.8	26.6	38	12.5	14.2	31.1	10.8	10	17.1	11.5	16.2	66.7	56	33.6
<i>P. beytelli</i>	NMNW R11399	Paratype	128.9	117.2	58.7	48.2	24.7	10.6	10	15.0	19	29	11.3	13.2	25.4	8.7	8.1	12.5	8.9	10.7	52.5	45	28.2
<i>P. beytelli</i>	SAIAB 141597	Paratype	146.9	135	68.2	57	27.8	13.8	11.7	15.0	24.4	36	11.9	16	26.9	10.8	10.4	14.6	9	12.5	72	54.4	29.0
<i>P. beytelli</i>	SAIAB 141615	Paratype	208	200	92.8	61		26	17	18.2	25.8	41	16.2	16.2	34.4	12	14.6	20.4	6.5	17	79.8	68	30.0
<i>P. beytelli</i>	PEM A15359	Paratype	108.3	100.1	50.5	41.3	20.1	8	9.9	9.0	16.3	25.3	10	12	21.1	7.5	7.3	11.3	6.2	8.2	46	39	24.5
<i>P. beytelli</i>	SAIAB 105031	Paratype	176	152	83.9	65	36.3	22	11.6	18.0	25.6	38	12.6	15	28.5	12	10.8	15.7	11.7	13.7	63	60.2	33.3
<i>P. edulis</i>	ZMB 50301	Lectotype	78	75.1	29.9	29.8	11.6	5.1	6.3	5.5	10.8	17.9	8.8	8.6	13	4.9	4.6	7.1	2.6	4.5	32.2	29.6	16.4
<i>P. edulis</i>	ZMB 50260	Paralectotype	96.3	94.3	40.8	34.4	16.4	6.9	6.7	5	12	23.3	11.6	11.6	15.9	6.9	6.4	9.4	5.2	7.3	32.4	30.2	20.7
<i>P. edulis</i>	ZMB 3349	Paralectotype	93.6	87.6	37.1	31.3	14.2	6	7.5	6.8	10.9	21.9	9.3	11	13.3	6.1	6.1	7.9	4	7	33.9	28.9	23.3
<i>P. edulis</i>	ZMB 50302	Paralectotype	103	95.2	43.3	42.8	16.5	7.8	7.1	6.7	13.7	25.4	11.4	?	18.3	8.4	7.4	10.7	6.2	8.4	39.4	32.9	22.2
<i>P. edulis</i>	ZMB 3350	Paralectotype	76.2	71.3	31.3	27	11.2	4.2	6.7	4.6	10.3	22.1	8.2	10.6	12.4	6.4	6	7.5	2.8	7.5	30.4	24.2	16.6
<i>P. edulis</i>	ZMB 10984	Syntype of <i>Phrynopsis boulengeri</i>	60.3	53.4	25.5	21.2	10.8	3.9	4.3	4.3	8.8	17.5	7.5	9.4	11.7	4.4	4.7	5.8	2.6	6.1	23	20.1	12.9
<i>P. edulis</i>	ZMB 27637	Holotype of <i>Phrynopsis usambarae</i>	23.9	19.1	6.9	8.4	3.6	1	1.7	1.7	2.4	5.2	3.3	3.8	3.7	1.9	2.5	2.2	1.2		7.9	7.1	4.5

Colour in life

Dorsal surface golden-yellow to dark orange in groin (Figure 6A); dark patches with pale centres roughly in transverse bands over the body; interspersed among the patches a reticulate network of thin black lines. Laterally no black blotches and few black lines. Distinct yellow vertebral line present. Interorbital bar edged in black. Tympanum with a prominent white dot in the centre. Thigh and tibia barred and with scattered transverse black lines. Gular region white with scattered grey blotches. Abdomen white, immaculate.

Colour in preservative

Dorsum dark grey with black blotches and lines. Prominent thin white vertebral line from snout to vent. Gular region with light grey blotching. Tympanum distinctly black with white center. Ventrally smooth, abdomen grey, immaculate.

Paratype variation

Dorsal colour patterns of paratypes vary from asparagus green to yellow backgrounds with silver-blue blotches (Figure 8). All the paratypes have distinct upper jaw barring and a prominent vertebral line with a tympanum as large as the eye. All specimens have a prominent white tympanic patch. A specimen collected in the Okavango was

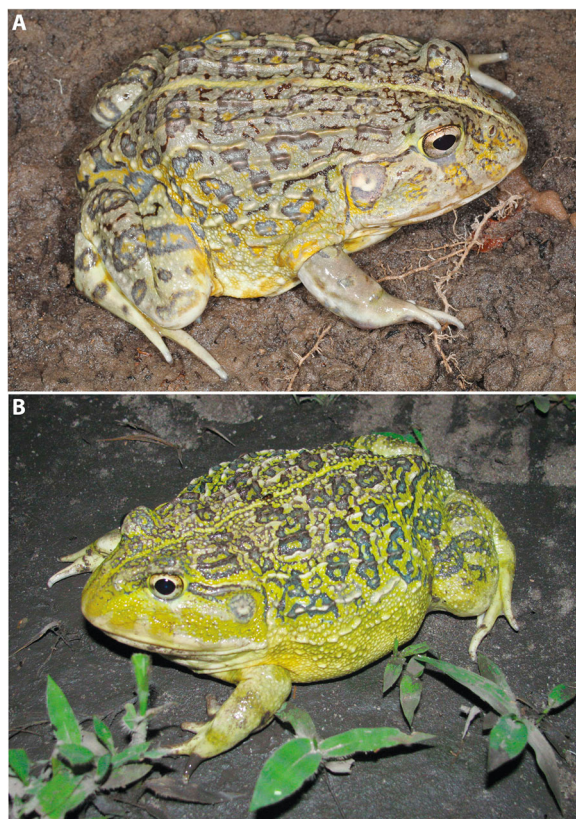


Figure 8. Colour pattern variation in *Pyxicephalus beytelli*: (A) male specimen from the Okavango, Botswana; (B) from Khaudum National Park, Namibia (Photos L du Preez).

lime green with dark grey-blue blotches. The SUL range of the paratypes is 108.3–146.9 mm. No females were collected.

Advertisement call

The advertisement call is brief and distinctly pulsed (Figure 3, Tables 2, 4). The call duration varies 0.2–0.55 sec, with a dominant frequency 172–258 Hz ($n = 13$). The pulse rate of each note varies 56–88 sec⁻¹. Breeding takes place during the day.

Tadpoles

The tadpoles were described by Channing et al. (2012) as *P. edulis* from South Luangwa, Zambia.

Habitat and biology

Following a rainfall event, specimens were found at night in shallow water in temporary pans or foraging in savanna woodland and grassland near pans. The type locality is illustrated in Figure 9. No breeding activity was observed, and no tadpoles were seen. They shared the habitats with *Cacosternum boettgeri* (Boulenger, 1882), *Kassina senegalensis* (Duméril and Bibron, 1841), *Ptychadena mossambica* (Peters, 1854), *P. anchietae*

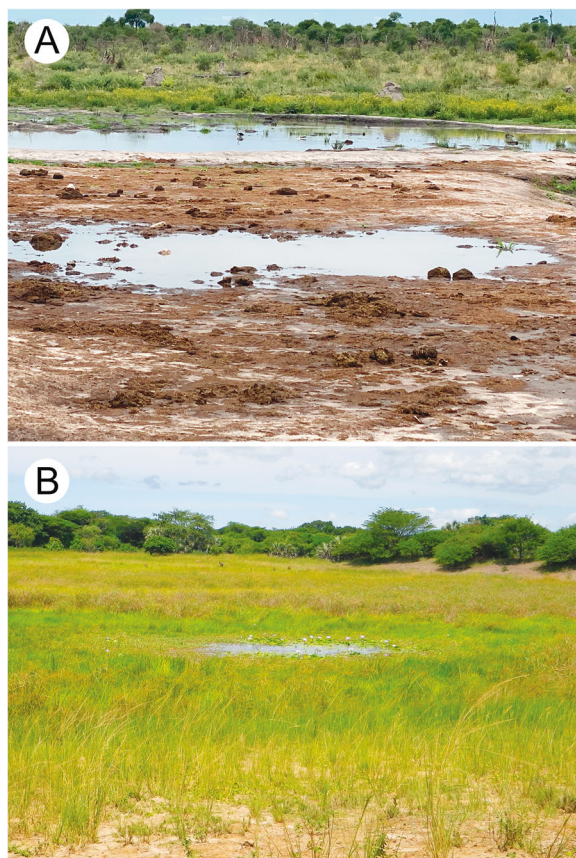


Figure 9. A-type locality of *Pyxicephalus beytelli*, Khaudum National Park, Namibia.

(Barboza du Bocage, 1868), *Poyntonophrynus kavangensis* (Poynton and Broadley, 1988) and *Tomopterna tandyi* (Channing and Bogart, 1996). This species is collected during breeding events and sold along the side of the road, in Namibia and in Angola (Figure 10) (WR Branch pers. comm.).

Distribution

Based on sequence data this species is known from western Botswana, north-eastern Namibia, south-western Zambia and southern Angola. We expect that its range will be found to be much larger, as more data become available. Based on its habitat preference we believe that this species may occur throughout northern and north-western Botswana, northern and north-eastern Namibia, south-western Zambia and we expect the species to be found in south-eastern Angola and north-eastern Zimbabwe. The identity of bullfrogs from this area that have previously been identified as *P. adspersus* will have to be confirmed using molecular data and we expect many to be *P. beytelli*.

Etymology

We have the pleasure in naming this species for the late Mr Ben Beytell, Director of Parks and Wildlife Management in Namibia. He was instrumental in the proclamation of the Khaudum National Park, Namibia.



Figure 10. *Pyxicephalus beytelli* being sold along the road in southern Angola (Photo WR Branch).

Discussion

In the last 60 years the number of recognised southern and eastern African *Pyxicephalus* species of bullfrogs has increased from only one, *P. adspersus* Tschudi, 1838 (Poynton 1964) with the second *P. edulis* Peters, 1854 added by Channing et al. (1994), and then *P. angusticeps* Parry, 1982 (Scott et al. 2013). The present paper adds one more species.

Identification of bullfrogs has been based on the presence of a large tympanum (*P. obbianus*), a narrow head (*P. angusticeps*), large size without a white spot on the tympanum (*P. adspersus*) or a smaller frog with a white spot on the tympanum (*P. edulis*). The present study shows that there are two large-sized species (*P. adspersus* and *P. beytelli*) and two smaller species (*P. edulis* and *P. angusticeps*). Three species have longitudinal ridges on the back (*P. adspersus*, *P. beytelli* and *P. edulis*), whereas one has only rows of elongated warts (*P. angusticeps*). Only *P. adspersus* lacks a white mark on the tympanum. It was suggested that introgression between *P. adspersus* and *P. edulis* was responsible for the blurring of the characters used for identification (Lambiris 1989). However, our study and the genetic evidence provided, shows a clear distinction between all the species and included overlooked taxa.

The integrated approach, using morphology, acoustics and genetics permitted the differentiation of the species. The advertisement call of *P. angusticeps*, recorded at the type locality from a vouchered specimen, has a much higher dominant frequency than *P. edulis*, and can be further distinguished by the short dorsal skin folds. *Pyxicephalus angusticeps* is known to breed at night without lekking (Channing et al. 1994, as *P. edulis*), while *P. edulis* is known to lek, and breeds during the day (Braack and Maquire 2005).

Pyxicephalus edulis has a breeding system similar to that of *P. adspersus*, including males fighting during daytime gatherings, and dominant males chasing other males out of their territories (Engelbrecht et al. 2015; Braack and Maquire 2005). *Pyxicephalus beytelli* is active during the day, and is expected to have a similar breeding system, but this has not yet been documented. Breeding behaviour of the South African *P. adspersus* has been assumed for bullfrogs in other parts of Africa (e.g. Spawls et al. 2006), but original observations are required to show possible differences and similarities.

All four presently recognised bullfrogs have been classified as Least Concern by the IUCN, but *P. adspersus* will need to be revised, following the description of *P. beytelli* with which it has been confused. Bullfrog numbers and habitat size are declining (Jacobsen 1989; Harrison et al. 2001; IUCN 2013). It appears that only *P. adspersus* has been investigated for conservation (Yetman and Ferguson 2011; Yetman et al. 2012; Thomas et al. 2014).

Southern African bullfrogs are a traditional food source, eaten in Mozambique (Channing et al. 1994), Botswana (Turner 1987), northern Namibia (Okeyo et al. 2015; Hatutale 2022), Angola (this paper) and South Africa (Badenhorst et al. 2002; Lord and Baines 1876; Nelson 2009). However, we propose that the new species be regarded as Least Concern, as it is widely distributed and found in protected areas. The new species also occurs in the widespread savanna habitat. Despite their use as food, bullfrogs are found in the vicinity of villages and farms, even in degraded habitats.

The distribution of 796 records of *Pyxicephalus* based on museum catalogues (American Museum of Natural History AMNH, California Academy of Science CAS,

Museum of Comparative Zoology, Harvard MCZ, Port Elizabeth Museum, Bayworld PEM), literature (Bocage 1895; Parker 1936; Mitchell 1946; Poynton 1964; Parry 1982; Morgan-Davies et al. 1984; Poynton and Broadley 1985; Poynton and Haacke 1993; Channing et al. 1994; Pickersgill 2007; Mercurio 2011; Scott et al. 2013; Marques et al. 2018; Baptista et al. 2019; Spawls et al. 2023) and citizen scientists (iNaturalist and the ADU FrogMap) shows that the genus is widespread in the drier interior of southern Africa and the Sahel, excluding the Guinea-Congolian tropical forests (Figure 11). Mapping the sequences and calls shows the probable distribution and sympatry of the southern African species (Figure 12). *Pyxicephalus adspersus* appears to be found in South Africa, Namibia, Botswana, Zimbabwe, extending into Zambia. *Pyxicephalus angusticeps* is restricted to the broad coastal plain from eSwatini (based on a call record) to Kenya. We expect *P. beytelli* to be widespread in northern Namibia, southern Angola, northern Botswana, Zimbabwe and Zambia, although it may extend further north. *Pyxicephalus edulis* is widespread in Mozambique, eastern Zambia, Tanzania, Kenya, probably extending into Uganda and Somalia. Although not dealt with here, *P. obbianus* is known from Somalia, and expected to extend into eastern Ethiopia.

The following cases of sympatry within the genus are known: *P. adspersus* and *P. beytelli* in northern Namibia (this study), *P. edulis* and *P. angusticeps* along the eastern coastal

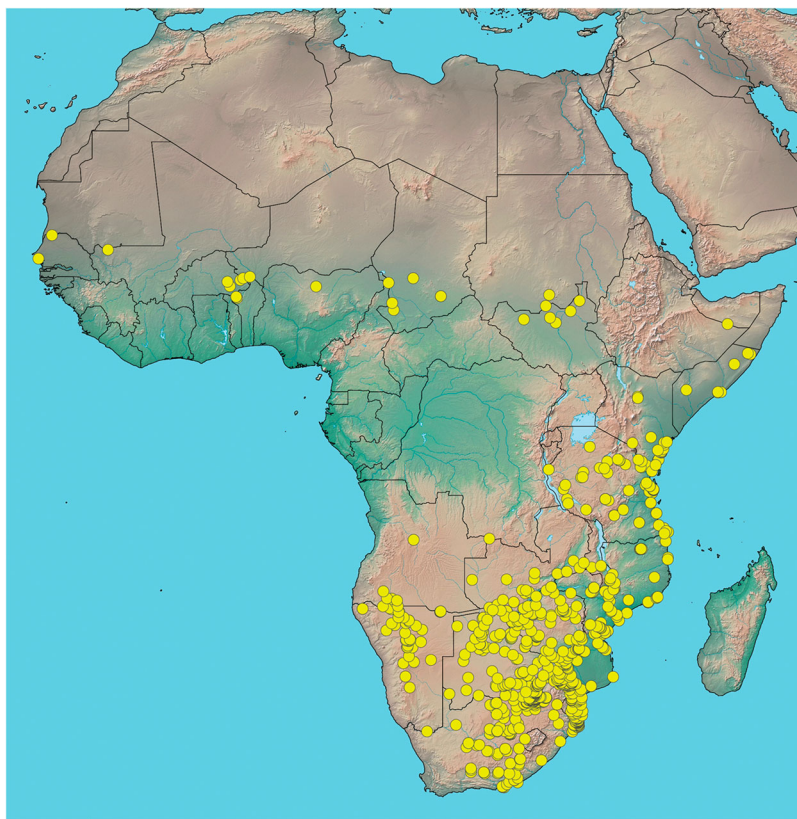


Figure 11. Records of *Pyxicephalus*. See text for sources.

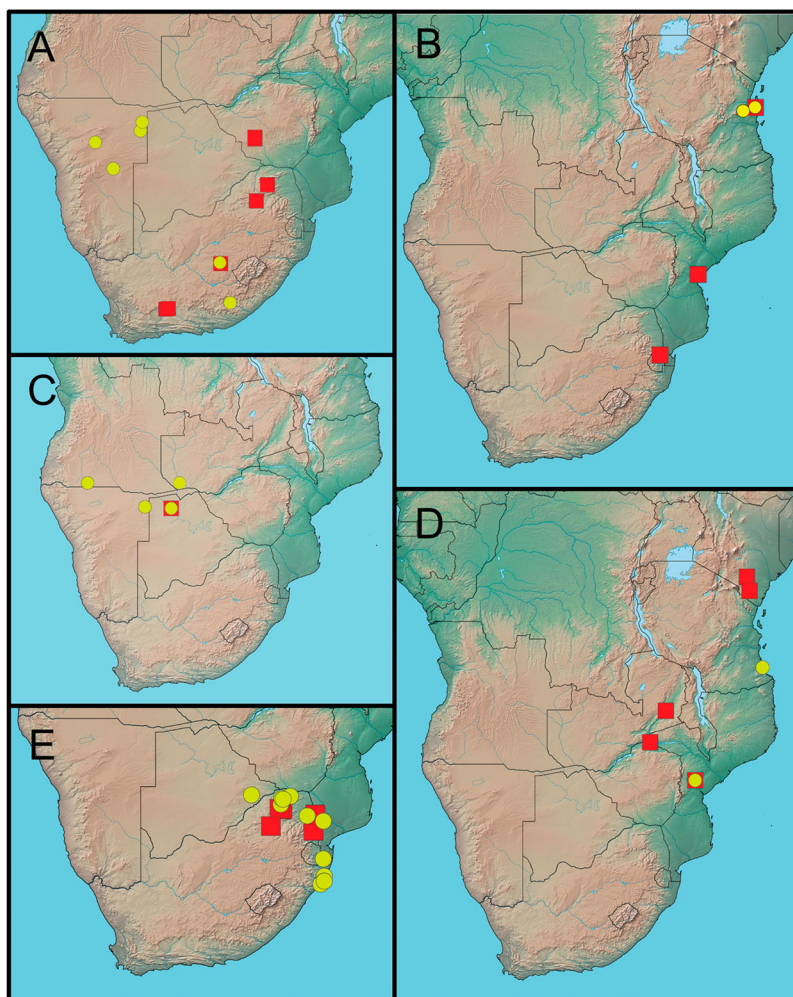


Figure 12. Localities of calls (red squares) and sequences (yellow circles) of southern and eastern African *Pyxicephalus* species. (A) *P. adspersus*, (B) *P. angusticeps*, (C) *P. beytelli*, (D) *P. edulis*, (E) *P. sp. 1*.

plain from Mozambique to Kenya (this study), *P. adspersus* and *P. sp. 1* from South Africa (Channing et al. 1994).

Scott et al. (2013) doubted that *P. adspersus* and *P. edulis* were sympatric, stating that Channing et al. (1994) had based their observation on “an unspecified recording from Naboomspruit (Limpopo province, South Africa), wherein both species were allegedly present”. However, Scott had the recordings in her possession, obtained from AC, made by NI Passmore at Mosdene Private Nature Reserve near Naboomspruit, where both the long calls of *P. adspersus* and the brief calls of *P. edulis* can be distinguished based on the high-quality recording.

We expect that many more observations of sympatry will be made as the DNA evidence for identification becomes available. The use of molecular evidence to confirm the identification of *P. beytelli*, *P. angusticeps* and *P. edulis* is recommended as morphological identification might not always be easy or possible, especially

where juveniles are concerned. Nevertheless, we provide an identification field key below, but recommend that tissues are collected and calls recorded in cases of uncertainty.

Key to the described species of southern and eastern African bullfrogs

- 1a. Tympanum larger than eye, known from Somalia *Pyxicephalus obbianus*
- 1b. Tympanum equal or smaller than eye 2
- 2a. White mark on tympanum (sometimes faint), dorsal vertebral stripe usually present 3
- 2b. No white mark on tympanum, no dorsal vertebral stripe *Pyxicephalus adspersus*
- 3a. Longitudinal dorsal ridges present, with speckling between dorsal mottles 4
- 3b. Oval warts on dorsum, no speckles between dorsal mottles *Pyxicephalus angusticeps*
- 4a. No pale crossbar between eyes, upper jaw barring absent (or faint), no cream-coloured lateral stripes or mottles, tympanum smaller than eye-tympanum distance *Pyxicephalus beytelli*
- 4b. Pale crossbar between eyes, sometimes incomplete; upper jaw barring present, cream-coloured lateral stripes or mottles present, tympanum larger than eye-tympanum distance *Pyxicephalus edulis*

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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