Description of a tetraploid *Tomopterna* (Anura: Ranidae) from South Africa

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A new tetraploid species of sand frog, genus *Tomopterna*, is described from the eastern Cape. On the basis of mtDNA sequences, allozymes and advertisement calls, it appears to have arisen as a hybrid between the adjacent diploid populations of *T. delalandii* and *T. cryptotis*. The hybridization event is estimated to have occurred 1,5 MYA. Excluding *Xenopus*, this is the only allotetraploid member of an advanced frog family that is known.

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The genus *Tomopterna* consists of six African species, six Asian species and one species known from Madagascar (Frost 1985). The African species are all burrowing frogs that occur along sandy riverbeds and in temporary pools. During the dry season they hibernate half a meter or more below the surface of the sand.

They are adapted for burrowing by possessing a large flattened flange on the heel that enables them to dig rapidly backwards into the ground. Identification of these frogs is not easy, and calls are probably the most reliable means of identifying some species in the field.

Bogart & Tandy (1976) reported the presence of a tetraploid population of *Tomopterna delalandii* from the eastern Cape, South Africa. This was based on tetraploid specimens collected from Jamestown, Queenstown, Cathcart and Grahamstown. No diploid specimens were found at these localities.

Analysis of calls recorded in the present study showed that only one call is present, indicating the presence of only one species. Chromosome spreads of testes from the males analysed in this study confirmed that the Eastern Cape *Tomopterna* are tetraploid.

We have re-investigated this tetraploid, and compared the advertisement call, distribution, allozymes and mtDNA sequences with the adjacent diploid populations of *T. delalandii* in the south-west and *T. cryptotis* in the north. On the basis of these studies we describe this tetraploid as a new species and discuss its relationships to the adjacent diploid populations.

Tomopterna tandyi n. sp.

Holotype: A male, collected at Bedford in the Eastern Cape, South Africa (32°42'S, 26°04'E) by M. Snyman, 27 January 1993. The specimen is housed in the Port Elizabeth Museum, PEM A 2283.

Paratypes: Collected from Bedford at the same time as the holotype: PEM A 2279-2285, 2287-2288, 2537.

Diagnosis: This species is recognizable in the field on the basis of the advertisement call, which differs in emphasized frequency from the adjacent species of *Tomopterna*.

Description (Figure 1). The major measurements are SVL 38, tibia 16.5, length of foot including inner metatarsal tubercle 19.0, length of hand including outer metacarpal tubercle and third finger 10.0, horizontal diameter of tympanum 2.7, eye 4.8, posterior corner of eye to tympanum 1.0, interocular distance 6.9, internarial distance 3.0, distance from anterior corner of eye to naris 2.3.

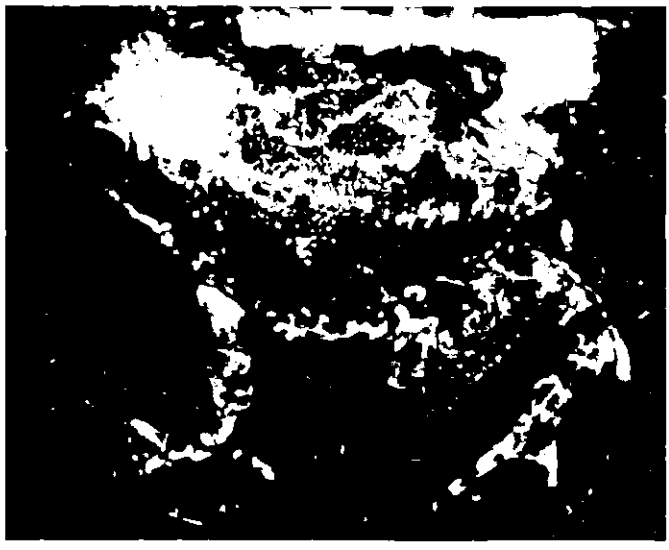

Figure 1 A male *Tomopterna tandyi* from Bedford.

The body is smooth dorsally, with a slightly granular throat. The undersurface of the thighs is corrugated, with the posterior face below the vent very granular.

The nostrils are situated on slight projections of the canthus. The eyelids are smooth with a slight wartiness on the posterior margin. The upper lip becomes a pale glandular uninterrupted ridge running below the tympanum and then turning to terminate in the direction of the arm insertion. The hand (Figure 2) has well-developed single subarticular tubercles, with flattened palmer tubercles.

The foot (Figure 3) is half body length. The inner metatarsal tubercle is large (4.0) and flattened. The subarticular tubercles are single, small, and rounded. Three phalanges of the fourth toe are free of web on both sides.

In preservative the following colours remain: the dorsal pattern consists of a series of darker blotches, edged with dark brown, on a paler grey background. The grey background consists of very fine black stippling. A small pale cervical spot is present. Stripes are absent. The legs are transversely

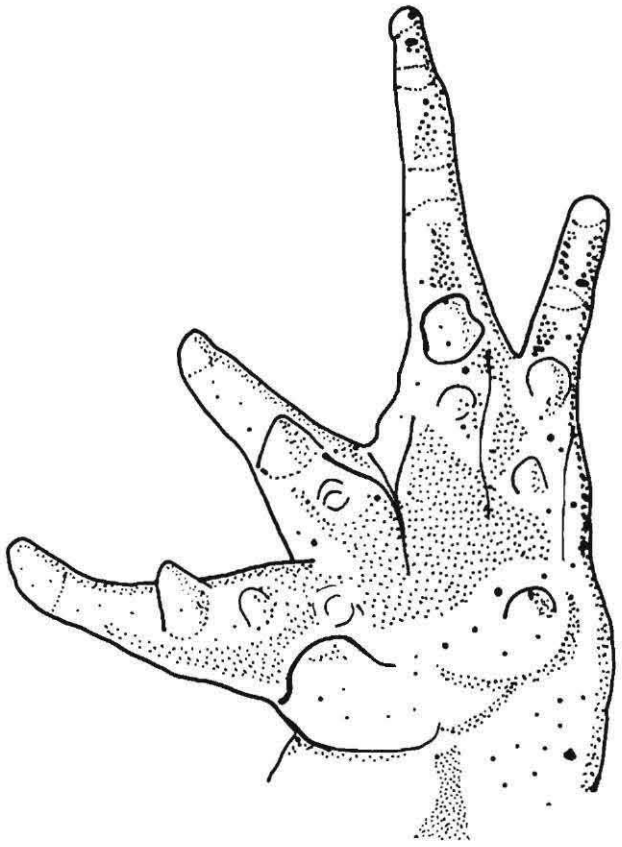

Figure 2 The hand of *Tomopterna tandyi*. PEM A 2283. Hand length including outer metacarpal tubercle 10 mm.

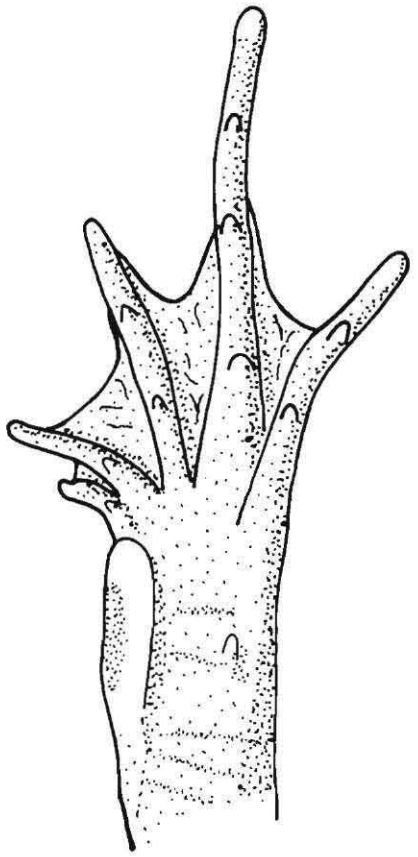

Figure 3 The foot of *Tomopterna tandyi*. PEM A 2283. Foot length including inner metatarsal tubercle 19 mm.

banded. The ventral surface is unpigmented except for the darkened throat.

Colour in life: The dorsal pattern consists of grey or olive patches on a lighter background. The dorsal warts are reddish-brown with black marks. A darker bar between the eyes is present in many specimens. This bar may be continuous in some animals. The common colour patterns in *T. tandyi* are shared by *T cryptotis* and *T. delalandii*. However, many *T. tandyi* showed a series of white flecks on the dorsum, a pattern not seen in the adjacent species. These may form a dorso-lateral row, starting at the posterior corner of the eye. Many individuals are indistinguishable from *T. cryptotis* or *T. delalandii* on the basis of colour pattern.

Etymology: This species is named for Mills Tandy, who collected the material first reported on to be tetraploid. The specific epithet is a genitive from *tandius*.

Karyotype

The karyotype of the material described here, and on which the molecular analyses were carried out, was confirmed to be tetraploid, with 4n = 52. Figure 4 shows a representative tetraploid mitotic metaphase spread from *Tomopterna tandyi*. This squash preparation was obtained from a small portion of the testis that was removed and fixed in 3:1 (ethanol:acetic acid) and squashed under a cover slip in 70% acetic acid. Mitotic cells are often found in the testis and are believed to be sertoli cell divisions. As a control, a squash preparation

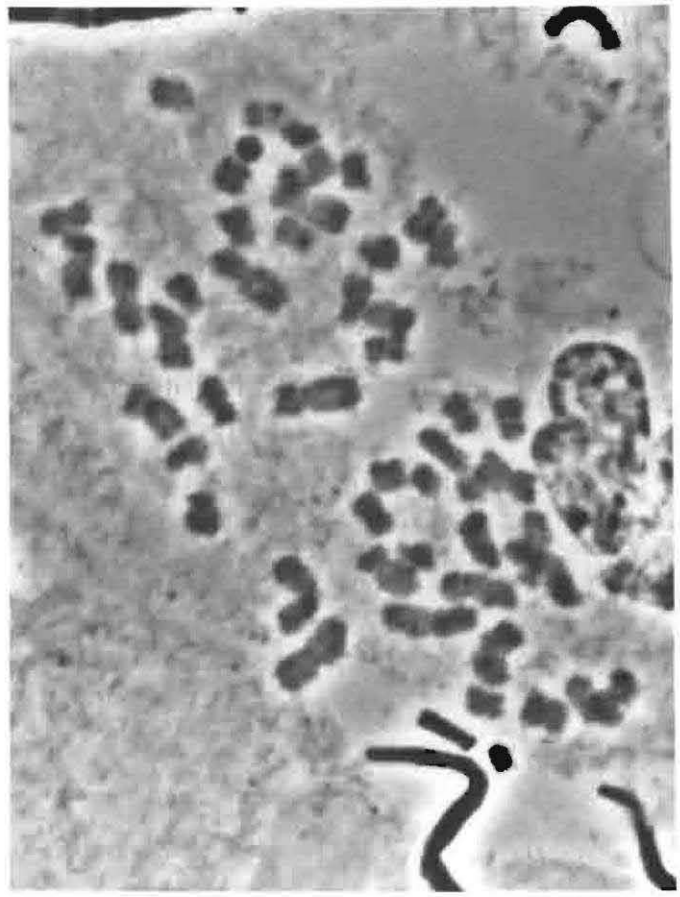

Figure 4 A representative tetraploid mitotic metaphase spread (4n = 52) from *Tomopterna tandyi*.

from fixed testis of one of the *T. cryptotis* specimens (2n = 26) shows 13 bivalents in the diplotene stage of first meiotic prophase (Figure 5).

Advertisement call

Males call from the edges of sandy pools, in the open or concealed against banks or under vegetation where this occurs. The call consists of a series of notes, repeated continuously in chorus. Each note is brief, 39–40 ms (n = 13). The note repetition rate is 7–8 per second at 18.5–18.8°C (n = 13). The emphasized frequency is 2.5–2.7 kHz (n = 13). There is slight frequency modulation, with an upward sweep sometimes present. Evidence from the calls of *T. delalandii* and *T. cryptotis* shows that the note repetition rate is temperature dependent, but that the emphasized frequency is constant.

The call data for the three species are compared in Table 1, and all three calls are illustrated in Figure 6. *Tomopterna tandyi* can be reliably distinguished from the adjacent species of *Tomopterna* by the emphasized frequency of the advertisement call. The call differences are not an artefact of differences in male size, as even our small sample shows overlap in male snout-vent length for all three species. The SVL of *T. cryptotis* males in our sample varies from 31–44 mm, *T. delalandii* ranges from 36–45 mm, and *T. tandyi* ranges from 31–44 mm.

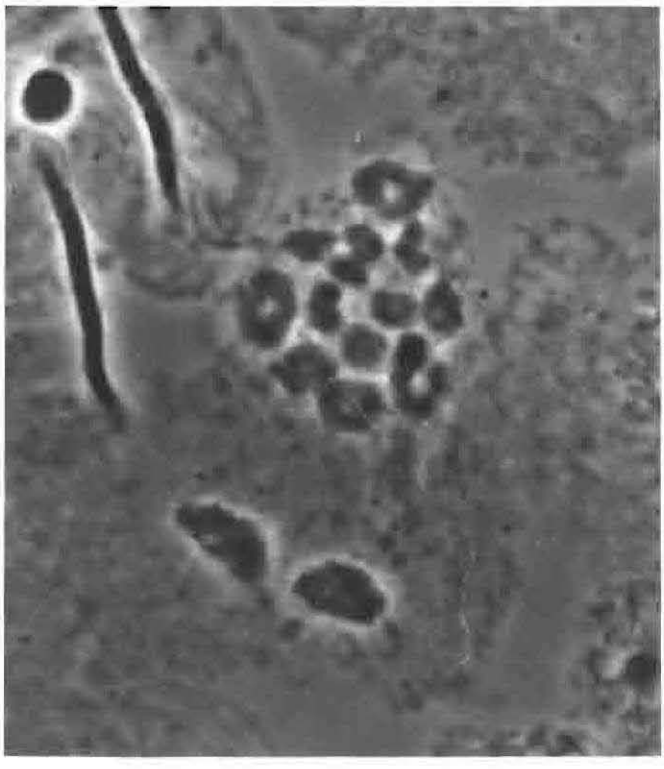

Figure 5 Squash preparation from fixed testis of *Tomopterna cryptotis* showing 13 bivalents in the diplotene stage of first meiotic prophase (2n = 26).

Eggs and tadpoles

Eggs and tadpoles have not been recorded. The eggs of *T. cryptotis* were described by Wager (1965), and those of *T. delalandii* by Rose (1929). Tadpoles of *T. cryptotis* were described by Power (1927), and those of *T. delalandii* by Wager (1965). The differences between the eggs and tadpoles of all the species in the genus are slight, and we expect that the eggs and tadpoles of this new species will prove to be indistinguishable from other African species.

Distribution

The distribution of *T. tandyi* is based on the tetraploid records (Bogart & Tandy 1976; and this study) and on specimens identified by field recordings of calls. The distribution is shown in Figure 7. The boundaries of the distribution of *T. tandyi* have not been determined. Presently it appears as if the new species is found in a broad band from the Eastern Cape coast between Port Elizabeth and the Kei River mouth, northwards to the highlands around the Vaal River.

The distribution of *T. cryptotis* and *T. delalandii* (Figure 7) is based on museum records, literature and fieldwork. *T. delalandii* is known from Namaqualand southward through sandy areas to the Western Cape, and eastwards along the coast to Cape St Francis. *Tomopterna cryptotis* occurs widely from the Karoo area of the Eastern Cape northwards.

The identity of individuals found at the common border of the ranges of *T. tandyi* and *T. cryptotis* needs to be confirmed based on field recordings of calls. Likewise the records intermediate between *T. delalandii* and *T. cryptotis* in the Karoo region need to be confirmed based on advertisement calls.

Table 1 Advertisement call analyses of nine populations of *Tomopterna*

Species	Air temp.	n	Locality	Call rate (per s)	Emphasized frequency (kHz)	Note length (ms)	Frequency modulation
T. tandyi	18.5	8	Adelaide	7	2.5–2.7	39	slight
	18.8	5	Adelaide	8	2.5–2.6	40	absent
	17.7	70	St Francis	7	1.8–2.2	39	upward sweep 200–250 Hz
T. delalandii	10.5	7	Stellenbosch	7	2.1–2.4	39	upward sweep 200 Hz
		11	Springbok	5	1.9–2.0	24	upward sweep 50 Hz
		9	Elandsberg	5	1.8–1.9	24	slight rise
	17.0	13	Bloemfontein	5	3.1–3.8	40	upward sweep 600 Hz
T. cryptotis	22.0	7	Bloemfontein	9	3.3–3.7	23	upward sweep 300 Hz
		8	Grootfontein	8	3.2–3.7	48	upward sweep 100 Hz

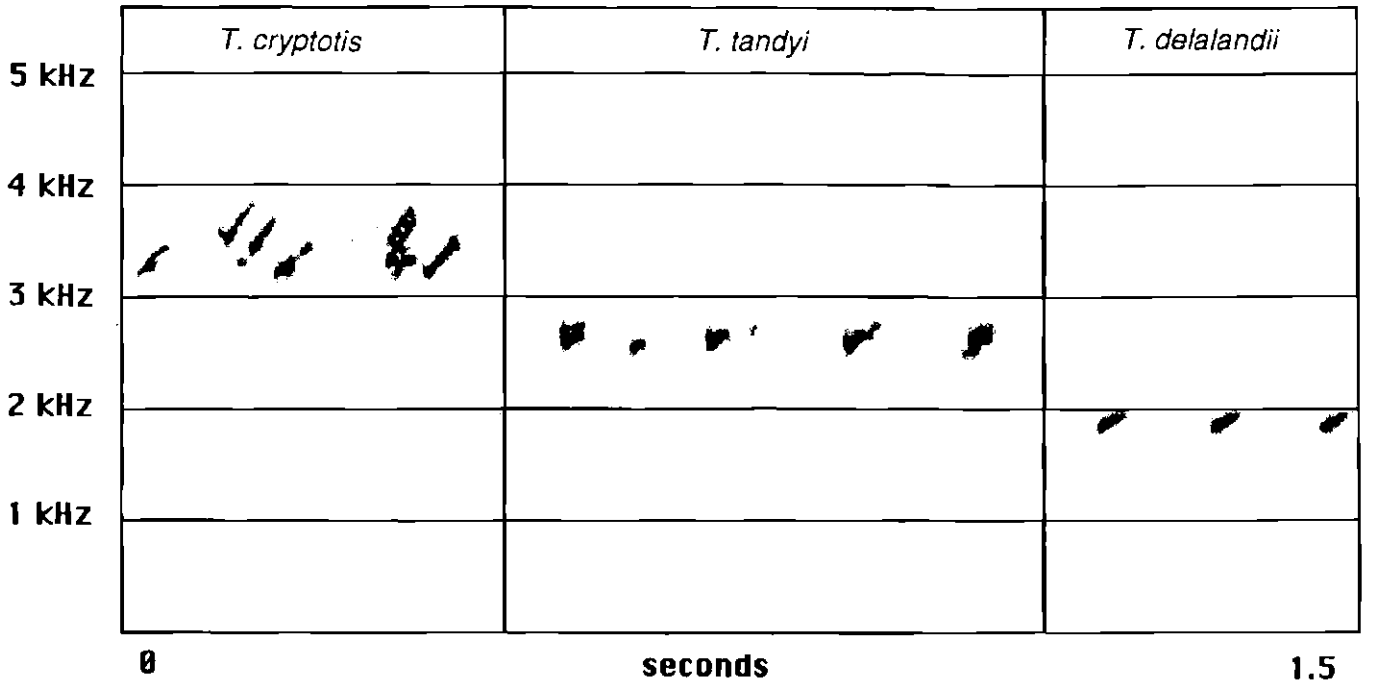

Figure 6 The advertisement calls of *Tomopterna cryptotis* (chorus), *T. tandyi* (two individuals), and *T. delalandii* (one individual). Only a few notes of each call are illustrated.

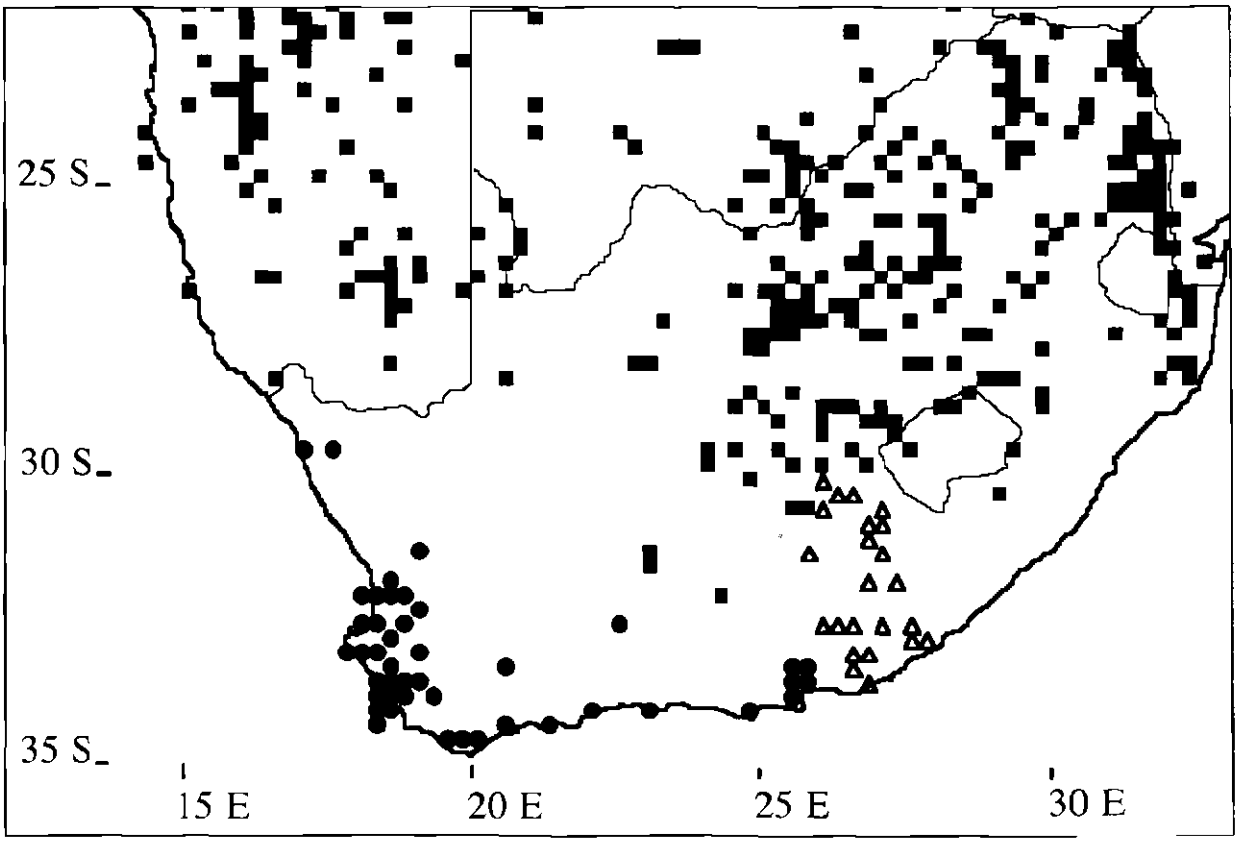

Figure 7 The distribution of *Tomopterna tandyi*, (open triangles) *T. cryptotis* (solid squares) and *T. delalandii* (solid circles). Each symbol occupies one quarter-degree.

Relationships to adjacent diploid populations

We investigated the relationships of the tetraploid to the two adjacent diploid species by means of an allozyme study and by sequencing mtDNA. Further studies are in progress investigating the genetic boundaries of the three species. The sequences and allozyme results will be presented in detail in

that report.

The molecular study was based on the following material: 7 specimens of *Tomopterna cryptotis* collected from Bloemfontein, 12 specimens of *T. delalandii* collected from Stellenbosch (9) and St Francis (3), and 8 specimens of *T. tandyi* collected from Bedford (6) and Adelaide (2). The allozyme analysis was carried out with liver and muscle tissue, using starch gel electrophoresis, and standard staining procedures (Murphy, Sites, Buth & Haufler 1990).

Sequencing was done manually using ^{35}S for the first segment and ^{32}P for the second. We sequenced two segments of the mitochondrial cyt b gene, using 30 cycles of PCR. For the first segment we used the primers 5'-CCA ACC CCA TCA AAC ATT TCA TCA TTA TGA AA-3', and 5'-ACT GTA CCC CTC AAA AAG ATA TTT GTC CTC A-3'. These amplify 307 bp corresponding to sites 14 842–15 148 of the human sequence (Hedges, Bogart & Maxson 1992). For the second segment we used the primers (H15547):5'-AAT AGG AAG TAT CAT TCG GGT TTG ATG-3' (Edwards, Arcander & Wilson 1991), and (L15162): 5'-GCA AGC TTC TAC CAT GAG GAC AAA TAT C-3' (Pääbo, unpublished). Together 450 bp were readable.

The allozyme study showed that all three species shared alleles for ICD, IDH, G6PDH, and AAT. *T. tandyi* and *T. cryptotis* shared alleles, different from *T. delalandii* for GPI and MDH. *T. tandyi* shared alleles with *T. delalandii*, different from *T. cryptotis*, for FUMH, GDH, and LDH. This is evidence of hybridization as the tetraploid shares alleles with both putative parental diploid species. The sequencing study showed that the difference between the diploid species was 16.5% of base pairs. A similar difference of 15.9% was found between *T. delalandii* and *T. tandyi*. In contrast, *T. tandyi* and *T. cryptotis* differ by only 5.8%. This is evidence that the allopolyploid derived from males of *T. delalandii* and females of *T. cryptotis*. Assuming that the rate of divergence for mtDNA in *Tomopterna* is clock-like and similar to the rate reported for lower vertebrates (Martin, Naylor & Palumbi 1992) that is calculated as approximately 2% per million years, the nearly 6% difference between the maternal mtDNA of *T. cryptotis* and that of *T. tandyi* suggests that the hybridization event might have taken place about 1.5 MYA.

Discussion

Polyploid salamanders and lizards have arisen in several lineages and have resulted in all-female populations of hybrid origin that are maintained by parthenogenesis, gynogenesis, or hybridogenesis (Bogart 1980; Dawley & Bogart 1989). With the exception of the hybridogenetic European *Rana esculenta* complex, polyploid frogs are even-ploid, normally reproducing, bisexual individuals and most are considered to have arisen from populations of extant, and similar, diploid populations. In fact, some extant species have been reported to have diploid and polyploid populations (Bogart & Wasserman 1972; Bogart & Tandy 1976; Bogart 1980). However, where diploid and polyploid populations are sympatric, the hybrids are expected to have an intermediate chromosome number and may be expected to be sterile. Such reproductive isolation would hasten speciation and serve as a selective force to strengthen premating isolating mechanisms. Further study of some diploid and polyploid populations (see Bogart 1980) has resulted in the recognition of these populations as being distinct species: *Ceratophrys cranwelli* (2n) was separated from *C. ornata* (8n) by Barrio (1980); the tetraploid '*Phyllomedusa burmeisteri*' (Bogart 1980) is now *P. tetraploidea* and produces triploid hybrids with *P. distincta* from which it is assumed to have arisen (Haddad, Pombal & Batistic 1994). Other than polyploid species of *Xenopus*, that are all believed to be allopolyploids (Tymouska 1991), *Tomopterna tandyi* is the only allotetraploid member of an advanced frog family that is known. Further fieldwork is required to investigate possible ongoing genetic interactions between the diploid and tetraploid species.

Although frogs in this genus are very abundant during the wet season, and are well represented in collections, it remains difficult to identify preserved material. Poynton (1964) separated *delalandii* from *cryptotis* on the basis of amount of webbing, size of tympanum relative to inner metatarsal tubercle, and the condition of the glandular ridge running from the upper lip to the arm insertion. Material from Grahamstown, Kei Road, East London, Port Alfred, Port Elizabeth and near Humansdorp, which he assigned to *T. cryptotis*, is here assigned to *T. delalandii* or *T. tandyi*. On the basis of advertisement calls, we assign all the *Tomopterna* along the south coast of South Africa from Cape Town to Port Elizabeth to *T. delalandii*. The sequence differences observed here of 15.9% between *T. tandyi* and *T. delalandii* and 16.5% between *T. delalandii* and *T. cryptotis* are similar to the differences found in cyt b between *Hyla chrysocelis* A and *H. arenicolor* (14.7%) and *H. femoralis* (14.7%) (Ptacek, Gerhardt & Sage 1994).

Material examined

All specimens described here are in the collection of the Port Elizabeth Museum. Other *T. tandyi* material examined includes: A male from Steynsburg (SE 3125Bd) PEM A 2256; two males and two females from KwaNcunca River (SE 3326Bb) PEM A 3063, 3066, 3073 and 3077.

Comparative material examined includes: *T. cryptotis* from Cedarville (SE 3029Ca), PEM A 1077, 1080; (SE 3029Cd) PEM A 991, 998, 1078, 1079; (SE 3029Ac) PEM A 1030–1040, 1076, 1081. *T. delalandii* from Goukamma (SE 3423Aa) PEM A 372–376; Addo (SE 3325Bd) PEM A 45–47, 49, 186; Port Elizabeth (SE 3325Dc) PEM A 39–44, 734–736; Lake Farm (SE 3325Cd) PEM A 194, 733; Alexandria (SE 3326Cb) PEM A 4826, 4828.

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