UNIVERSIDADE DE LISBOA
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A review of *Amietia angolensis* (Bocage, 1866) and *Amietia fuscigula* (Duméril and Bibron, 1841) (Anura: Pyxicephalidae), using morphology and advertisement calls

Ninda Lara Baptista

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Dissertação orientada por:

Professor Alan Channing
(University of the Western Cape)

Professor Rui Rebelo
(Universidade de Lisboa)

Ninda Lara Baptista

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“*As coisas têm peso, massa, volume,*
tamanho, tempo, forma, cor,
posição, textura, duração,
densidade, cheiro, valor,
consistência, profundidade, contorno,
temperatura, função, aparência,
preço, destino, idade, sentido.

As coisas não têm paz.”

Arnaldo Antunes

“*Things have weight, mass, volume,*
size, time, shape, colour,
position, texture, length,
density, smell, value,
consistency, depth, contour,
temperature, function, appearance,
price, destination, age, direction.

Things do not have peace.”

Arnaldo Antunes

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Mãe, Pai, Irmã'sss… palavras para quê?! **Muito obrigada** por **tudo**, **sempre**!

SUMMARY

The ability of identifying a species is vital for its effective conservation. River frogs from the genus *Amietia* are widespread from Southern to north-Eastern Africa, but delineation of species within this genus is at an early stage. The widespread distribution of two species, *A. angolensis* and *A. fuscigula* suggests their unresolved taxonomy.

A new phylogeny for the genus based on genetic data is under construction. It resulted in a new arrangement for some species, and proposed provisional names for some clades. The present work comprised seven clades from the referred phylogeny, five included in "*A. angolensis* group", from Southern and Eastern Africa, and two included in "*A. fuscigula* group", from Southern Africa.

This study used an integrative approach, based on morphological data from all seven clades; and acoustic data from two clades from Southern Africa, in order to corroborate the clades which delineation was based in genetic data. Morphological data were obtained from a total of 110 frogs and 31 tadpoles from museums and personal collections. Morphometric data were obtained from 76 adults belonging to seven clades, and were used for discriminant function analyses. The results were used to assess the appropriateness of the proposed working names for the clades and to try to identify the most useful features for distinguishing species within this genus.

The main results generally support the clades proposed on the new phylogeny. The provisional working names were, in most cases, supported by this work, except for two clades from Eastern Africa, for which there was neither support nor rebuttal. No observed character alone was enough for distinguishing all seven clades. Throat coloration pattern alone identified unequivocally adults from Southern Africa. Body measurements were useful for distinguishing groups of clades. Clades discovered through genetics show consistent morphological differences that seem to have been confused with a high intra-specific morphological variability.

Key words: Anura, Pyxicephalidae, *Amietia angolensis*, *Amietia fuscigula,* morphometrics, coloration pattern, bioacoustics

RESUMO

A capacidade de identificar uma espécie é crucial para a efectiva conservação da mesma. Em regiões pouco conhecidas como Angola e em "hotspots" de biodiversidade como as Eastern Arc Mountains na África Oriental, a Região Florística do Cabo Ocidental na África do Sul são prioritárias para a conservação, e, consequentemente, para a documentação da biodiversidade existente.

O género *Amietia* (Anura: Pyxicephalidae) ocorre desde o Sul da África Austral (África do Sul) ao Norte da África Oriental (Etiópia). Este género inclui actualmente quinze espécies. No entanto, o estudo da sua taxonomia encontra-se ainda num estádio inicial. A sugerir isto está o facto de duas das espécies terem amplas áreas de distribuição – *Amietia angolensis* ocorre desde a África do Sul à Etiópia, e *Amietia fuscigula* em quase toda a África do Sul e na Namíbia – contrastando com outras espécies, que têm distribuições muito limitadas – como por exemplo *Amietia vandijki*, conhecida apenas das montanhas nos arredores da Cidade do Cabo, e *Amietia dracomontana*, conhecida apenas do planalto do Lesoto.

A distinção entre ambas as espécies foi alvo de muito debate durante mais de um século, tendo sido considerada como um dos problemas clássicos da herpetologia africana. Apesar de actualmente já não haver dúvidas sobre a sua distinção, as complexas listas de sinónimos atribuídos a ambas as espécies sugerem também que a posição taxonómica actualmente atribuída a cada uma delas ainda não é definitiva.

Uma nova filogenia baseada em sequências genéticas está a ser construída para o género *Amietia*, e inclui exemplares de grande parte da sua área de ocorrência. Esta filogenia reconhece um determinado número de clados, alguns dos quais são novos, outros dos quais corroboram o conhecimento já existente sobre algumas espécies.

Este trabalho focou-se num total de sete clados reconhecidos pela referida filogenia: cinco pertencentes ao "grupo *A. angolensis*" – incluindo exemplares da África do Sul, Angola, Lesoto, Malawi, Quénia, Ruanda, Tanzânia, Uganda, Zimbabué, e dois pertencentes ao "grupo *A. fuscigula*" – incluindo exemplares da África do Sul e da Namíbia.

O trabalho teve como objectivos:

- utilizar uma abordagem integrativa para complementar os resultados obtidos através de dados genéticos com dados morfológicos – disponíveis para os sete clados – e acústicos – disponíveis para dois clados da África Austral;

- discutir os nomes provisórios propostos para os clados, comparando os resultados obtidos com as descrições originais das espécies e fotografias dos holótipos, quando disponíveis;

- procurar características morfológicas que permitam a distinção das espécies no campo, dada a elevada variabilidade morfológica referida para este género.

Para tal foram utilizados ao todo 110 exemplares adultos, juvenis, e recém-metamorfoseados, e 31 girinos depositados em colecções pessoais e museológicas. Os dados morfométricos foram recolhidos de 76 exemplares adultos, enquanto que os dados relativos ao padrão de coloração e à forma do corpo foram recolhidos de adultos, juvenis e recém-metamorfoseados. Para os girinos registou-se apenas a fórmula dentária.

Os dados morfométricos foram analisados através de análises exploratórias, testes de diferenças de médias entre as variáveis mais importantes para a rápida distinção dos clados no campo, e análises discriminantes entre várias combinações de clados. Os padrões de coloração e outros dados qualitativos relativos à textura da pele e à forma de partes do corpo foram, sempre que possível, transformados em categorias para facilitar a interpretação dos resultados obtidos. Estes dados foram analisados apenas descritivamente. Para analisar os dados bioacústicos foram determinadas as taxas de repetição dos pulsos por comboio de pulsos, taxas de repetição dos pulsos por nota, intervalo entre comboios de pulsos, intervalos entre notas, e frequência das vocalizações.

Os resultados morfométricos – nomeadamente o rácio entre a largura da cabeça e o comprimento da tíbia – permitiram uma separação entre o "grupo *A. angolensis*" e o "grupo *A. fuscigula*", característica já anteriormente referida na bibliografia como útil para distinguir as duas espécies. As análises discriminantes efectuadas com outros rácios entre os vários grupos mostraram capacidade de distinguir alguns clados, indicando que os clados descobertos têm diferenças morfométricas.

Os resultados dos dados qualitativos revelaram que os clados têm diferentes frequências para vários dos caracteres, e que existem poucos caracteres diagnosticantes de cada clado. Por exemplo, recorrendo apenas ao padrão de coloração da garganta, foi possível identificar correctamente todos os indivíduos adultos dos clados da África Austral. No entanto, nenhuma outra característica qualitativa se revelou tão útil para a distinção entre todas as combinações de clados, principalmente de clados da África Oriental.

Os dados acústicos revelaram diferentes frequências de vocalização, diferentes taxas de repetição dos pulsos e notas relativamente aos *taxa* com os quais foram comparados, corroborando a proposta revalidação de clados da África Austral.

A falta de caracteres exclusivos de cada clado revelou a grande variabilidade morfológica do género *Amietia*. Ainda assim, e apesar da elevada variabilidade, alguns dos resultados sugeriram que há padrões de variação consistentes em cada clado, e, por isso, também corroboram os clados propostos pela nova filogenia do género.

A discussão sobre a adequação dos sete nomes propostos para os clados sugeriu concordância entre cinco nomes [*Amietia angolensis* (Bocage, 1866) de Angola, *Amietia fuscigula* (Duméril & Bibron, 1841) do sudoeste da África do Sul, *Amietia quecketti* (Boulénger, 1845) do centro–norte da África à Sul e Namíbia, *Amietia tenuoplicata* (Pickersgill, 2007) da Tanzânia, *Amietia theileri* (Mocquard, 1906) para espécimens da África do Sul e Zimbabué], e nenhum suporte conclusivo para dois dos nomes [*Amietia desaegeri* (Laurent, 1972), do Uganda e Ruanda, e *Amietia viridireticulata* (Pickersgill, 2007), No Sul da Tanzania e Norte do Quénia].

Os padrões de variação morfológica consistentes de cada clado sugerem que, apesar da grande variabilidade morfológica relatada para várias espécies de *Amietia*, é possível que essa variabilidade possa ser devida não a uma variabilidade intra-específica extremamente elevada, mas sim ao facto de existirem várias espécies. No entanto, este estudo, por não ser exaustivo e por incluir amostras muito pequenas, pode ser considerado uma abordagem preliminar ao assunto.

Palavras-chave: Anura, Pyxicephalidae, *Amietia angolensis*, *Amietia fuscigula,* morfometria, padrão de coloração, bioacústica

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1. INTRODUCTION

This work is the result of a proposal of a new phylogeny for the genus *Amietia*, based on genetic data (Channing, *in prep.*). Data included is from seven clades from the referred phylogeny, five included in "*Amietia angolensis* group", and two included in "*Amietia fuscigula* group" (Channing, *pers. comm.*). These clades have been given a provisional working name and will probably be proposed as species in the new phylogeny (Channing, *pers. comm.*). Because of the complex historic and taxonomic confusion associated with the two main groups where the clades are included in, a brief description of these groups' history will be made, with reference to the original descriptions and the most relevant events concerning Channing's (*in prep.*) new findings.

1.1. Anuran taxonomy and conservation

To effectively conserve a species it is of course important to be able to identify it (Dayrat, 2005). The necessity of identifying species is even more important in biodiversity hotspots and in poorly known areas, where baseline information such as floral and faunal lists is still incomplete. For African anurans, clear examples of priority research areas are the Eastern Arc Mountains (Poynton *et al.,* 2007; Burgess *et al.,* 2007; Menegon *et al*., 2008) a biodiversity hotspot where new species of anurans are continuously being described (Channing & Schmitz, 2008; Poynton *et al.,* 2008; Blackburn, 2009), areas with a high degree of anuran endemics, such as the southwestern cape of South Africa (Seymour *et al.,* 2001); where new species are also still being discovered (Turner & Channing, 2008) and poorly studied areas such as Angola (Seymour *et al.,* 2001; Andreone *et al.,* 2008).

Currently, the importance of an integrative approach when delineating species is clearly recognized, as it allows more rigorous, informative and reliable results (Dayrat, 2005; Padial, 2010). Integrative approaches should include phylogeography, comparative anatomy, genetics, ecology and behavioural biology (Dayrat, 2005), including bioacoustical studies in anurans, which have species-specific advertisement calls (Padial, 2009).

1.2. River frogs

River frogs of the genus *Amietia* are widespread in the Afromontane region (Visser & Channing, 1997). There are 15 species presently recognised in this genus (Frost, 2011). Most of the species are listed as data deficient (DD) and some are listed as of least concern (LC), in the IUCN Red List (IUCN, 2011). Species of this genus were previously included in the genus *Rana* (Poynton, 1964). The generic name of these species was changed to *Afrana* Dubois, 1992, and in 2006 all species included in the genus *Afrana* were transferred to the so far monotypic genus *Amietia* (*sensu* Dubois 1987) (Frost *et al*., 2006).

This genus is included in the African anuran family Pyxicephalidae (Bonaparte 1850) (Frost *et al.,* 2006), which includes the "typical" frogs, with big eyes, pointed snouts, alert postures and long legs (Spawls *et al*., 2006). River frogs are associated to permanent bodies of water, usually large and flowing, from small streams to large rivers (Channing, 2001; 2004a). They spend a lot of time in the water, have extensive webbing and are excellent swimmers (Spawls *et al*., 2006). Species of *Amietia* are known for the high variability on coloration pattern (Channing 1979; 2001; Channing & Howell, 2006). They are both nocturnal and diurnal, and some species are reproductively active throughout the whole year (Channing, 1979; 2004b; 2004c).

As large frogs*, Amietia* play an important ecological role in the food webs. Members of this genus prey on flying and crawling arthropods, like Hemiptera, Coleoptera, Orthoptera, ants, spiders, caterpillars, millipedes, crabs, and snails (Channing, 1979; 2004a; Channing & Howell, 2006; Barbour & Loveridge, 1928), and even small vertebrates, such as mice and frogs (Rose, 1962 *in* Channing, 2004c). Small mammals like otters, mongooses and genets, as well as large birds, frogs, snakes and terrapins eat river frogs (Rowe-Rowe, 1977a, 1977b *in* Channing, 1979; Channing & Howell, 2006). Large specimens are even eaten by humans (Channing, 2004a). Many carnivorous insect larvae feed on *Amietia* tadpoles (Channing, 1979).

At least 11 *Amietia* species are known to occur in Southern and Eastern Africa: six species in South Africa and Lesotho: *A. angolensis, A. fuscigula, A. vandijki, A. dracomontana, A. vertebralis, A. umbraculata,* one single species in Angola – *A. angolensis*; another single species in Namibia – *A. fuscigula*; and five in Eastern Africa – *A. desaegeri, A. lubrica, A. ruwenzorica, A. tenuoplicata*, *A. viridireticulata*, *A. wittei*. Some species have limited distributions, such as *A. vandijki,* restricted to the mountains on the south of the Western Cape

Province, and *A. dracomontana*, restricted to the Leshoto plateau (Channing, 2004), but there are two widespread species: *A. angolensis*, from Southern to Northeastern Africa, and *A. fuscigula*, in Southern Africa, with overlapping distributions in some areas of Southern Africa (Figure 1).

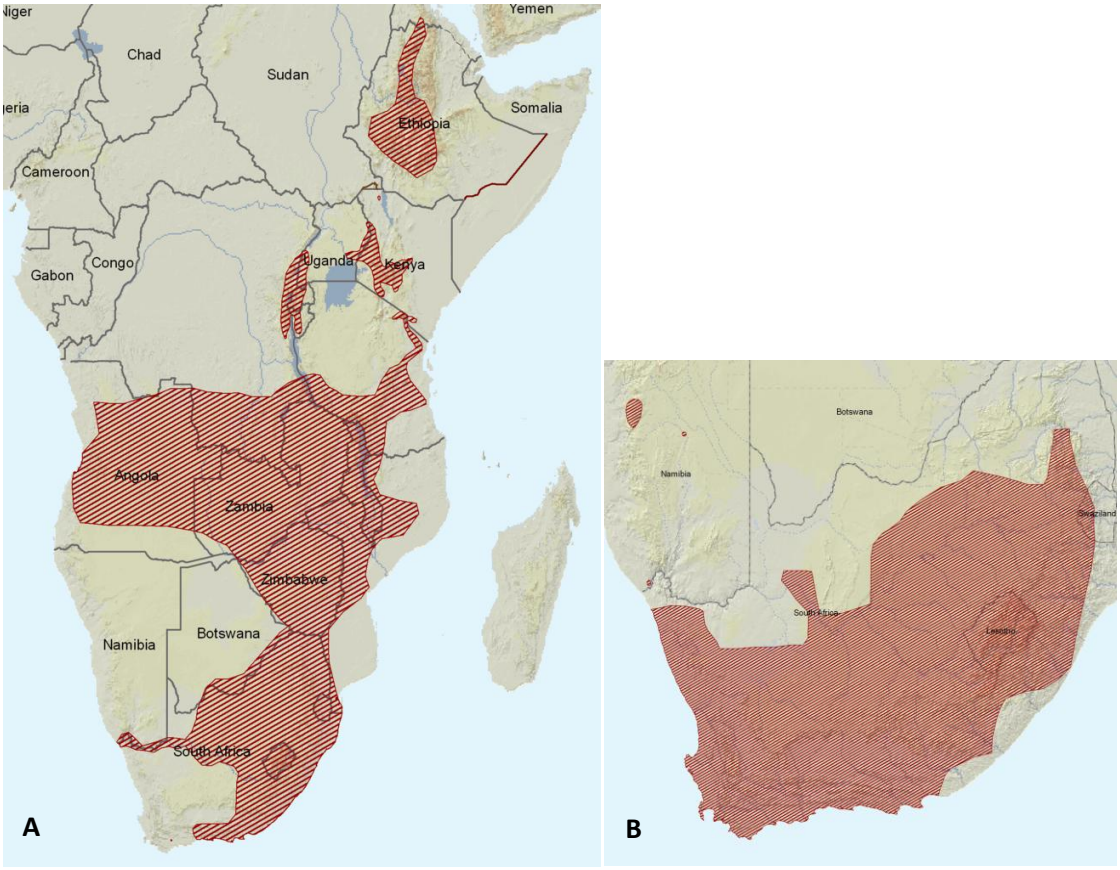

Figure 1 Currently known range of *A. angolensis* (A) and *A. fuscigula* (B). Source: Poynton *et al.,* 2011; Minter & Channing, 2004, *in* IUCN Red List of Threatened Species.

1.3. History of *Amietia angolensis* and *Amietia fuscigula*

Amietia angolensis (Bocage, 1866)

Duméril & Bibron (1841) described *Rana Delalandii*, allegedly from the type locality Cape of Good Hope, South Africa, a type locality that would later be considered as erroneous by Boulénger (1918). This taxon has been considered a synonym of *R. angolensis* Bocage, 1866 by Boulénger (1882), with no justification. Poynton (1964) argues that «...a certain amount of doubt must be attached to this synonymy», based on differences on coloration pattern and skin texture between specimens of Angola and South Africa.

Bocage (1866) described *Rana angolensis,* based on two male specimens from the type locality Duque de Brangança (now Calandula Waterfalls), in Angola. This species is currently known as *Amietia angolensis* (Bocage, 1866).

In 1906, Mocquard described *Rana Theileri***,** from the type locality Nelspruit (Transvaal), South Africa, based on one single specimen. This species has been considered a synonym of *R. angolensis* by Boulénger (1918), with no justification.

In a comparative work involving species of *Amietia* from Eastern Africa, Laurent (1972) used detailed descriptions of coloration pattern of adults and tadpoles, webbing, and a series of ratios. Based on these data, collected from hundreds of specimens, two new species were described: *Amietia ruwenzorica* and *Amietia desaegeri* (Laurent, 1972).

Several descriptions and synonymies of species of *Amietia* occurred in Eastern Africa (Frost, 2011), which will not be referred in the present work. It is thought that at least in Eastern Africa *A. angolensis* has been a "lump" taxon (Harper *et al*. 2010), and is almost certainly a complex of multiple cryptic species (Channing & Howell, 2006). These authors agree that more work is needed to clarify its status, including molecular studies and detailed advertisement call analysis, and that a full resolution of this species complex will probably result in the description of several new species.

Corroborating this, three species were recently split from *A. angolensis* by Pickersgill (2007) in Eastern Africa: *A. lubrica* from Lake Bunyoni in Southwestern Uganda; *A. tenuoplicata* from the Usambara Mountains in Northeastern Tanzania; and *A. viridireticulata* from the Udzungwa Mountains in Southern Tanzania and Nyika Plateau in northern Malawi.

Although Broadley *et al*. (2007) recognize that Pickersgill (2007) based this new classification by carefully comparing the specimens with other known species, they criticize the description of the new species based on such a few specimens – *A. tenuoplicata* description was based on 1 female and tadpoles from Amani, Tanzania, and *A. viridireticulata* was based on 2 females, 1 male and 1 froglet from Dabaga, Tanzania. Thus, it would be useful to corroborate the validity of these species examining further material.

***Amietia fuscigula* (Duméril & Bibron, 1841)**

Rana fuscigula was described in 1841 by Duméril and Bibron, from the type–locality Cape of Good Hope, South Africa. In 1895, Boulénger described *Rana quecketti* based on one single female specimen collected near Pietermaritzburg, Natal, South Africa. *Rana quecketti* Boulénger 1895 was considered a synonym of *R. fuscigula* by Boulénger (1910), with no justification. Later, Boulénger (1918) provisionally considered the holotype of *R. quecketti* as an «abnormal» *R. angolensis*, based on the similar shape of the head, the narrow interorbital space, and in the webbing of the toes of the holotype, highlighting, however, its shorter hindlimbs. Poynton (1964) synonymized it with *R. fuscigula,* based on its head width/tibia length ratio.

Several authors doubted that *Rana angolensis* and *Rana fuscigula* were different species, which as lead to comparative morphologic studies between these two species (Poynton, 1964). The distinction between these two species has been regarded as «… one of the classical problems of African herpetology» (Poynton, 1964). Boulénger (1918), analysing adults and tadpoles, and Poynton (1964), analysing only adults, both using material exclusive from Angola and South Africa, provided evidence showing that they are indeed different species. Poynton (1964) showed that the most useful feature to distinguish these species is the ratio head width/tibia length. Channing (1979), using specimens from the region of Natal, South Africa, provided acoustic, ecologic and morphologic data that distinguished the two species. Scott (2005) provided osteological data distinguishing both, and referred the potential of the distal subarticular tubercle on the third finger for this distinction. Presently there is no doubt about their specific status.

Nevertheless, the complex taxonomic history, reflected by their long lists of synonyms, and the widespread distribution of both *A. angolensis* and *A. fuscigula* suggest that the current knowledge of their specific status and distribution is not complete. In a review including species of this genus, Tarrant *et al.* (2008) note that there are still taxonomic difficulties associated with it, and that good delimitation of species still requires further taxonomic work.

1.4. Recent research

A recent study (Channing, *in prep.*) used genetic data to build a phylogeny of the genus *Amietia*, resulting in a new arrangement for some species. Among other results, this phylogeny

indicated the existence of several clades in Eastern Africa, apparently corroborating previously described species of this group. The phylogeny also revealed that the *A. angolensis* specimens from Angola are a different *taxa* than the *A. angolensis* specimens from South Africa, supporting the revalidation of *Amietia theileri* (Mocquard, 1906) (Channing, *in prep.*). Moreover, it showed that *A. fuscigula* specimens from South Africa and Namibia can in fact be divided in two clades: one that occurs only in Southern South Africa, and one that has a northernmost distribution, occurring in South Africa and Namibia, supporting the revalidation of *Amietia quecketti* (Boulénger, 1845) (Channing, *in prep.*).

1.5. Study aim

On the present study, seven clades from the phylogeny (Channing, *in prep.*) were used. Five clades are included on the "*A. angolensis* group", and two are included on the "*A. fuscigula* group" (Channing, *pers. comm.*). These clades include some regional variants that are in the process of being proposed as new or revalidated species (Channing, *in prep.*), and correspond to "*A. angolensis*" from Angola, "*A. fuscigula"* from South Africa and Namibia, "*A. angolensis"* from South Africa, Leshoto and Zimbabwe, and "*A. angolensis"* (including *A. desaegeri, A. tenuoplicata* and *A. viridireticulata*) from the Eastern Arc Mountains of Kenya and Tanzania, from Malawi, Rwanda and Uganda.

Channing (*in prep.*) proposed the following provisional working names for the clades included on the present study:

- Clade A: *Amietia angolensis* (Bocage, 1866) for the clades from the "*A. angolensis* group" with specimens from Angola;
- Clade B: *Amietia desaegeri* (Laurent, 1972) for the clades from the "*A. angolensis* group" with specimens from Kenya, Rwanda, Tanzania, Uganda,
- Clade C: *Amietia fuscigula* (Duméril & Bibron, 1841) for *A. fuscigula* occurring on the southwestern South Africa,
- Clade D: *Amietia quecketti* (Boulénger, 1845) for the northernmost clade from the "*A. fuscigula* group",
- Clade E: *Amietia tenuoplicata* (Pickersgill, 2007) for one of clades from the "*A. angolensis* group" occurring in Tanzania*;*

- Clade F: *Amietia theileri* (Mocquard, 1906) for the clade from the "*A. angolensis* group" with specimens from South Africa and Zimbabwe,
- Clade G: *Amietia viridireticulata* (Pickersgill, 2007) clade from the "*A. angolensis* group" from Tanzania and Malawi.

These names will probably be used in the overall taxonomic revision of the genus (Channing, *pers. comm*.). Fig. 2 depicts the collecting localities of the specimens and advertisement call with no voucher assigned to each clade on the present study.

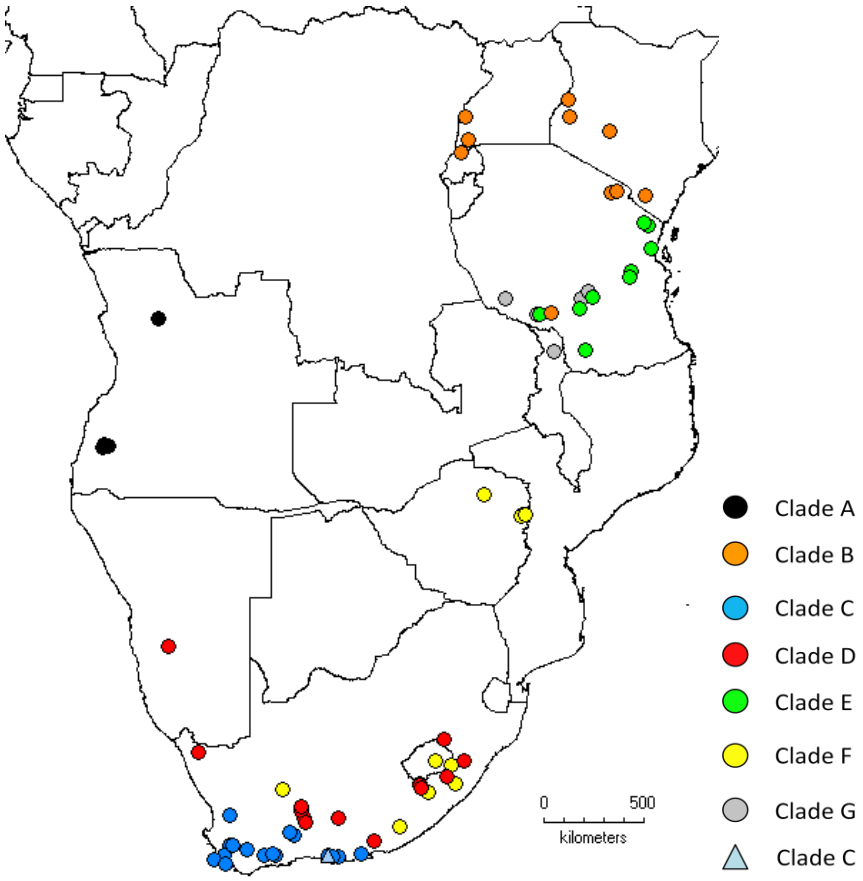

Figure 2 Map representing the collecting localities of the genetically and not genetically analysed specimens of each clade, with circles, and of the recording locality of the advertisement call with no voucher, with a triangle.

The main objectives of this study were:

- complement results obtained with molecular data with morphological – available for all seven clades – and bioacoustic data – available for two clades;
- discuss the provisional names proposed to the clades;
- look for characters useful for distinguishing species of *Amietia* in nature, giving the high morphological variability associated with this genus.

2. MATERIALS AND METHODS

2.1. Specimens

The analysed specimens belonged to Collection of Alan Channing (University of the Western Cape) – AC; Bayworld (Port Elizabeth Museum) – PEM; National Museums of Kenya – NMK; Collection of Stefan Lötters (Trier University) – SL; University of Texas at Arlington – UTA; Iziko (South African Museum) – SAM. Examined specimens and their collection locality is reported on Appendix 7, Table 15. Type specimens were analysed by observation of photographs provided by Alan Channing or available on the species' original descriptions. Abbreviations of museums to which type specimens belong to are as follows: Musée Royal de l'Afrique Centrale, Belgium – MRAC, Museum National d'Histoire Naturelle, Paris – MNHNP; Museum für Naturkunde, Berlin – ZMB.

First, the available specimens were separated into seven groups (A, B, C, D, E, F and G) according to the clades proposed by Channing *(in prep.)*, using only those from which molecular data were known. On a second step, non sequenced specimens were assigned to these clades, according to similarities with the sequenced specimens, and their collecting locality. All the observed tadpoles belonged to batches from which the molecular classification of at least one individual was known.

Sex was determined by observation of external secondary sexual characters – velvety nuptial excrescences on the thumbs present in breeding males, and absent in females (Scott, 2005). Adult age class was established by determining the SUL of the smallest male within all seven clades. Frogs with an SUL equal or longer than the smallest male (42,3 mm) were considered adults. Frogs with a SUL shorter than the smallest male were considered juveniles, and froglets with traces of tail were considered metamorphs. The total number of specimens analysed for each clade are listed on Table 1.

Table 1 Total number of specimens available for each clade.

Clade	Females	Males	Juveniles	Metamorphs	Tadpoles
A	3	3	10	1	7
B	10	4	1	-	-
C	12	9	5	1	1
D	3	5	6	-	12
E	8	3	4	-	-
F	7	2	4	1	11
G	5	5	-	-	-

Because of the preservation status of some specimens, it was not always possible to measure all characters on each specimen, which explains the sample size differences among analyses.

2.2. Morphometrics

Eighteen measurements (listed on Table 2) were taken from formalin–preserved specimens with a digital calliper to the nearest 0.01 mm. These were rounded to one decimal to avoid pseudo precision, following Hayek *et al.* (2001). To reduce the effects of allometry (Hayek *et al.* 2001), only data from adults were used. Measurements were taken on the right side of the specimen whenever possible.

Table 2 List of measurements, abbreviations and description.

Measurement	Description
Snout-urostyle length (SUL)	from tip of snout to posterior end of urostyle.
Snout-vent length (SVL)	from tip of snout to vent.
Femur length (Fe)	from tip of urostyle to knee on the bent hind limb.
Foot length (Fo)	from proximal edge of inner metatarsal tubercle to tip of fourth toe.
Tibia length (Ti)	on the bent hind limb.
Inner metatarsal tubercle length (IMT)	proximal to distal tips of internal metatarsal tubercle.
Head width (HW)	width at posterior end of jaw.
Head width at nostrils level (HWN)	
Head length (HL)	from tip of snout to posterior end of jaw.
Snout length (SL)	from anterior corner of eye to tip of snout.
Nostril-snout distance (NS)	from nostril to tip of snout.
Eye-nostril distance (EN)	from anterior corner of eye to nostril.
Inter-nostril distance (IN)	between the inner edges of the nostrils.
Upper eyelid (Ey)	from anterior to posterior corner of eye.
Distance between anterior corner of eyes (EE)	
Interorbital distance (IO)	shortest distance between orbits.
Eye-tympanum distance (ET)	shortest distance from posterior border of eye to anterior margin of tympanum.
Tympanum diameter (Ty)	horizontal tympanum diameter at widest point.

To assess the error associated to each measurement, one specimen was measured seven times during a period of ten days. The coefficient of variation of each measurement was determined and is available on Appendix 2, Table 14.

For two specimens from clade D (SAM 44658, SAM 44861) femur length, foot length, head length and snout length were not measured. The lack of these values would turn the number of measurements even smaller (given the 8–adult sample). Therefore, these values were estimated from linear regressions: femur length was estimated from a regression

between this and tibia length (R^2= 0.92; p<0.002), head length estimated from a regression between this and HW (R^2= 0.84; p<0.01), foot length estimated from a regression between this and tibia length (R^2= 0.88; p<0.005), snout length estimated from a regression between this and the sum of EN and NS (R^2= 0.75; p<0.03). Regressions included only the specimens from clade D. In all cases residuals showed normal distribution and homocedasticity. The error (95% confidence limit) of each estimative is provided in Appendix 2, Table 13.

To control for the effect of body size on morphometric variables, ratios were determined between body measurements and SUL, between head measurements and head width, and between the inner metatarsal tubercle length and foot length. Other ratios used for distinguishing species of *Amietia* were also determined: head width/tibia length (Poynton, 1964), foot length/head width (Channing, 1978; 1979), interorbital distance/upper eyelid and intra-nostril distance/eye-nostril distance (Laurent, 1972). Measurements were also used to determine the snout angle (SA= [arcsine ((head width / 2) / head length)] x 2).

2.3. Other morphological traits

Coloration features

Coloration features were described from preserved specimens. Each specimen was photographed from several angles. Colour photos of live specimens were available for some clades and used as a complement for colour diagnosis. Dark markings on the dorsum of the body were called blotches – roundish markings with variable size, dark highlight of the dorsolateral ridges was called ridge delineation, and dark markings along the anterior part of the thighs were called bars.

The term vermiculation was used referring to any kind of elongate wormlike dark marking, present on the flanks, ventral part of the body, and posterior surface of the thighs. Vermiculations were classified as follows: marbled – elongated vermiculations not forming a network; reticulate – wormlike markings forming a network in which elongated pale markings are closed; lacy – a network delimiting pale oval or round dots. Spots and vermiculations were further classified as thin or coarse, and as diffuse or conspicuous.

Skin texture

Ridges on dorsum were defined as dorsolateral (DL) – starting behind the eyes; dorsal – on dorsum; and lateral – on the space between the dorsolateral ridges and the ventral region. Ridges were classified according to shape (straight or wavy) and length: maximum detectable size reached by the ridge before any interruption. When the ridge continued beyond the scapular region, its classification was relative to space between armpit and groin.

Foot webbing

The amount of foot webbing was registered and included the number of phalanges free of web in each toe and depth of webbing notches, on the left foot whenever possible. Foot webbing formulas followed Savage & Heyer (1997). Depth of the notches was expressed relatively to the subarticular tubercles of fingers II, II, III and IV (see Fig. 2). In the formula, depth is written in brackets in Arabic numerals between the Roman numerals that represent the toes.

Vomerine odontophores

Vomerine odontophore shape, size, position and number of vomerine teeth were registered. Vomerine odontophore size (OS) was visually compared to the distance from each odontophore to the nostril (DN), and with the shortest distance between odontophores (DO), as shown in Fig. 3.

Other traits

General shape of the head and body, shape of snout from ventral view, presence of distal subarticular tubercle on the third finger of the third hand were also observed and registered.

Figure 2 Webbing of the left foot of a specimen (AC 1789, Clade B). Numerals represent the toe that contains the sub–articular tubercle used to express the depth of each webbing notch.

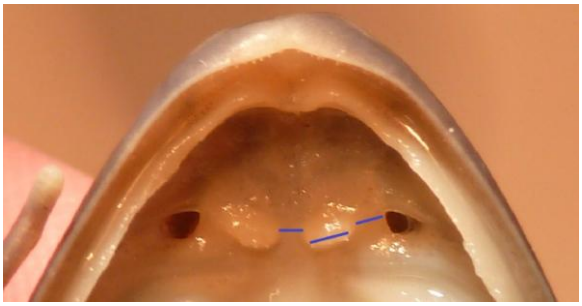

Figure 3 Palate of a specimen (AC 1953, Clade E), showing vomerine odontophores. Traces from left to right represent respectively the shortest distance between vomerine odontophores (DO); odontophore size (OS); and distance from odontophore to nostril (DN).

Tadpole labial tooth row formula

Tadpole labial tooth row formula (LTRF) followed McDiarmid & Altig (1999) *in* Channing (2001), where the total number of the anterior and posterior rows of labial teeth are followed in brackets by the number(s) of the incomplete anterior and posterior rows. The numbering of the rows increases from anterior to posterior, and the rows anterior to the jaw sheath are separated from the posterior rows by a slash.

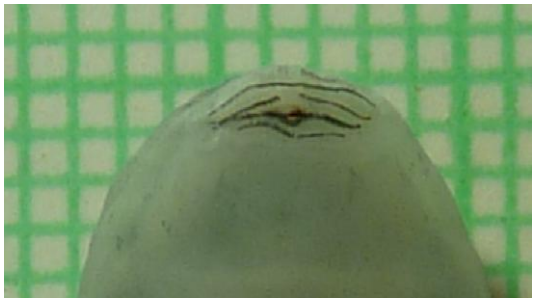

Figure 4 Mouthparts from a tadpole assigned to clade F. Not all of the labial tooth rows are visible.

Nineteen characters relative to coloration pattern, skin texture (relative only to ridges), and morphology were selected and their variation was classified in categories as described in Tables 3, 4 and 5. Photographs representing some of the characters and their states are provided on Appendix 6.

Table 3 Characters and states observed relative to body coloration pattern.

Characters	States
A Vertebral stripe:	(0) absent; (1) present.
B Dorsolateral ridges delineation:	(0) absent; (1) present.
C Dorsal blotches pattern:	(0) absence of blotches except for vertebral row , when present, and/or small blotches on the sacral region; (1) several blotches more or less organised in one or more longitudinal series, forming a relatively symmetrical pattern; (2) blotches confusingly scattered, usually with a diffuse pale outline, not organised in rows nor forming a symmetrical pattern; (3) plain dorsum; (4) other.
D Pale outline of bars on thighs:	(0) absent or diffuse; (1) evident.
E Dominant coloration pattern on posterior surface of thighs:	(0) nearly plain (coarse dark marking with very small pale dots); (1) lacy; (2) thin vermiculations (either reticulate or marbled); (3) coarse vermiculations (either reticulate or marbled).
F Dominant coloration pattern of flanks:	(0) irregular vermiculations (speckled or elongated, coarse or thin, conspicuous or diffuse); (1) apart from vermiculations, very conspicuous small white dots or stains scattered, usually coinciding with lateral ridges; (2) almost no other vermiculations except a conspicuous line with or without upwards and/or downwards projections, from the groin to the top of the forelimbs; (3) lacy.

Table 4 Characters and states observed relative to head coloration pattern.

Characters	States
G Interorbital blotch:	(0) faint; (1) evident.
H Blotch on frontoparietal region:	(0) absent; (1) present.
I Upward projection of pale facial stripe between eye and tympanum:	(0) absent; (1) slight wave; (2) pointed, sometimes contacting the eye.
J Dark line along upper lip:	(0) straight or nearly straight and plain (in the shape of a labial stripe); (1) with pale spots and/or upward projections.
K Line between nostril and tip of snout:	(0) absent; (1) present, half–complete or diffuse.
L Diffuse dark marking on the breeding males' throats:	(0) absent; (1) present only in the edges of the throat; (2) a coarse marking throughout the throat, more evident on the edges.
M Coloration pattern of throat:	(0) immaculate (with no markings); (1) speckled (only small conspicuous spots); (2) thin marbled; (3) coarse marbled; (4) thin reticulate; (5) coarse reticulate; (6) lacy; (7) diffuse speckled or diffuse marbled; (8) thin marbled more evident on the edges than on the centre, which is immaculate or has very pale vermiculations; (9) diffuse in the whole throat, darker on the edges; (10) other.

Table 5 Characters and states observed relative to skin texture and morphology.

Characters	States
N Dorsal skin texture:	(0) smooth; (1) with ridges.
O Shape of dorsolateral ridges:	(0) straight; (1) wavy.
P Continuous dorsolateral ridges reaching:	(0) up to scapular level; (1) between scapula and 1/2 body; (2) between 1/2 and 2/3 of body; (3) up to groin.
Q Lateral skin texture:	(0) smooth; (1) warts and/or short ridges; (2) short interrupted and/or long continuous ridges.
R Shape of snout tip from ventral view:	(0) not protruding; (1) protruding; (2) very protruding.
S Distal subarticular tubercle on the third finger of left hand, excluding basal subarticular tubercle:	(0) present; (1) absent.

2.4. Bioacoustic data

Analysed recordings were provided by Alan Channing:

a) from Humpata, Angola; one recording by Alan Channing on 22nd January 2009, at 21^{o}C, from a not genetically analysed voucher (AC 3120) assigned to the clade A;

b) from Northern Cederberg, South Africa; one recording by Alan Channing on the 6th February 2010, at 21h00, with a air temperature of 22^{o}C, from a genetically identified voucher (AC 3164) assigned to the clade C;

c) from Bloukrans River, South Africa; one recording by Harold Braack on 2009, at 19^{o}C, included calls of two individuals, and assigned to the clade C, based on the recording locality and the clade's distribution. This recording's location is shown on Fig. 2.

2.5. Data analysis

2.5.1. Morphometric data

Amietia species have sexual dimorphism: females are larger and have wider heads than males. A search for sexual dimorphism on the used ratios was performed, as suggested by Hayek *et al.* (2001).

Tests were conducted to look for significant differences among all clades relatively to the easier features to measure in the field: SUL and HW/Ti ratio. Normality and homocedasticity tests were performed for both variables. When these were normal and homocedastic (case of HW/Ti ratio), an ANOVA was conducted, and when not (case of SUL), Kruskal-Wallis test was. To find which clades showed significant differences in these features, *a posteriori* tests were conducted, and consisted in a Tukey honest significance differences test corrected for unequal sample sizes for HW/Ti, and multiple comparison of mean ranks for SUL. Box and whiskers plots were built for several body ratios, as these might be useful for identification of specimens.

Discriminant analyses

Body ratios were used for discriminant function analysis (DFA), to find the more useful ratios for distinguishing species (Quinn & Keough, 2002). Most of the ratios had normal distribution (*pers. obs.*). Tests to assess for homogeneity of variances and for outliers, recommended for discriminant analysis, were not performed. However, the main goal of this analysis was to find the most useful variables for distinguishing clades, and not to classify specimens. In this case the fulfilment of these assumptions is not as important (Quinn & Keough, 2002)

Separate DFAs were conducted using the clades belonging to:

- "*A. angolensis* group" (A, B, E, F and G);
- "*A. fuscigula* group" (C and D);
- *"A. angolensis*" from Southern Africa (A and F), discovered to be different *taxa* by Channing (*in prep.)*;
- *"A. angolensis*" from East Africa (B, E and G), more likely to occur in sympatry.

The best final subset of variables for each group was chosen by a forward stepwise selection procedure. Because of the high correlation between some ratios, not every ratios were included in the discriminant analyses. Ratios including measurements with the higher

coefficients of variation (eye–tympanum distance, interorbital distance, and nostril–snout distance) were not used. Ratios used to build the final model were the same for every models: HW/SUL, HL/SUL, HWN/HW, SL/HW, EN/HW, IN/HW, Ey/HW, EE/HW, Ty/HW, HW/Ti, Fo/HW.

Interpretation of the obtained models was based, on the value of Wilk's lambda (λ) for the model – varying between 1 (meaning no discrimination) and 0 (meaning complete discrimination) (StatSoft, 2001) –, on the percentage of discrimination explained by each variable (based on the partial Wilk's λ values, the lowest meaning the greatest discrimination ability of the variable), and on the percentage of correct classifications. All the analyses were carried out using Statistica (v. 7.0) software.

2.5.2. Qualitative data

Due to the small sample sizes, no statistical analyses were used for qualitative data. These were used on comparative tables and for the description of the clades.

2.5.3. Bioacoustic data

Nomenclature hereafter follows Ryan (2001). *Amietia* calls are biphasic, having a first clicking phase consisting in pulse trains, and a second phase consisting in rapidly pulsed notes (Channing, 1979; Visser & Channing, 1997).

Measured parameters for the first phase were: length, as the length of the pulse trains, pulse rate, length of the interval between the pulses, frequency of the pulses. The length of the interval between the two phases was also determined. All of these parameters were only determined for the call from clade A.

For the second phase, the determined parameters were length, as the length of each pulsed note, pulse rate per note, note repetition rate, length of the interval between notes, and notes frequency. These second phase parameters were determined for all the calls. Temporal measurements are given as range, followed by mean ± standard deviation and number of analysed units (notes, pulses, calls or intervals).

Calls were analysed with the acoustic software Adobe Audition 3.0. All the calls were resampled at 44.1 kHz and 16 bit resolution in the mono pattern and saved as uncompressed files. Frequency information was obtained through Fast Fourier Transformation (FFT, width 1024 points). The audiospectrograms were provided by Gonçalo M. Rosa and obtained using a Hanning window function with 256–band resolution.

3. RESULTS

A summary of the main results of qualitative features for all clades Appendix 8 (Table 16, Table 17 and Table 18). Box and whiskers plots for body ratios are available on Appendix 5 (Fig. 17, Fig. 18). Raw measurements for every adult specimens are provided on Appendix 9. Notes on the identification of each clade, and specimens assigned to each clade are available on Appendix 3. A dichotomous key proposed for distinguishing adult specimens belonging to the seven studied clades, is provided on Appendix 4.

Specimens belonging to clade D are currently – before Channing (*in prep.*) – assigned to *A. fuscigula* (Duméril & Bibron, 1841), and specimens belonging to clade F are currently assigned to *A. angolensis* (Bocage, 1866). In the present work, it is assumed that clade D is the same as *Rana fuscigula* (*sensu* Channing, 1979), and that clade F is the same as *Rana angolensis* (*sensu* Channing, 1979). These assumptions are based on distributional similarities.

3.1. Morphometrics

Although there seemed to be a tendency for females having larger body sizes and wider heads in most clades (*pers. obs.*), the few significant differences among sexes (*pers. obs.*), the small samples, and the main goal of the work – providing data to help distinguishing clades, regardless of the sex of the specimen – lead to the decision that sexes would not be separated for subsequent analysis.

For SUL, the Kruskal-Wallis test was significant (H=15.3; p=0.02), and *a posteriori* tests revealed that there were significant differences between body size of clades D and G (Appendix 1, Table 12). An ANOVA including all seven clades (p=0.000000) showed significant differences in HW/Ti, and *a posteriori* Tukey test revealed significant differences between the five clades from *"A. angolensis* group" and both clades from *"A. fuscigula* group" (Appendix 1, Table 11). Box and whiskers plots of the analysed samples are represented on Fig. 6. Despite the significant differences in both features, there are overlapping values among most of the clades.

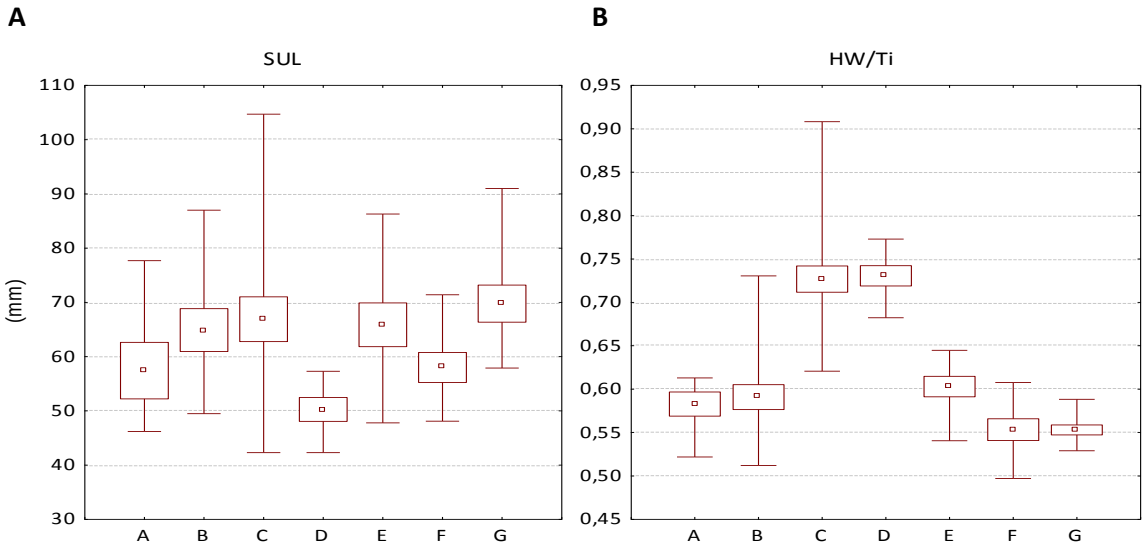

Figure 6 Box and whisker plots (central square=mean, boxes=mean±standard error, whiskers=maximum and minimum values) showing morphological variation among clades. **A**: snout-urostyle length (mm); **B**: head width/tibia length ratio.

Discriminant analyses

The results of the discriminant function analysis (DFA) are summarized on Table 6.

Table 6 Summary of the discriminant power of the models selected for each combination of clades. The first line depicts the ability of each model in discriminating clades. Partial Wilk's λ are presented for the variables present on the final models, a * depicts those that were significant, blank cells represent variables absent in the final model.

		"*A. fuscigula* group" (clades C, D)	"*A. angolensis* group" (clades A, B, E, F, G)	"*A. angolensis*" from Southern Africa (clades A, F)	"*A. angolensis*" from East Africa (clades B, E, G)
General Wilk's λ		0.52	0.15	0.20	0.19
Significance value		F (3.23)=7.1 p< 0.0015	F (32.134)=2.8 p< 0.0000	F (4.10)=9.99 p< 0.0016	F (14.48)=4.36 p< 0.0001
Model's overall correct classification		92.6%	72.9%	93.3%	88.2%
Partial Wilk's λ for each ratio	HW/SUL	0.92	–	–	–
	HL/SUL	0.93	0.81	0.65 *	0.80
	HWN/HW	–	0.90	–	0.91
	SL/HW	–	0.78	–	0.73 *
	EN/HW	0.64 *	0.84	–	0.87
	IN/HW	–	0.88	–	–
	Ey/HW	–	–	–	–
	EE/HW	–	–	0.45 *	–
	Ty/HW	–	0.89	–	0.90
	HW/Ti	–	0.55 *	0.37 *	0.56 *
	Fo/HW	–	0.60 *	0.73	0.60 *

The discrimination within all groups of clades was statistically significant, indicating that there are detectable differences among the clades, and reinforcing Channing's (*in prep.*) proposed clades. The percentage of correct classifications was above 70% in each group. Nevertheless, these results also show that using only morphometrics it is not possible to accurately identify all the specimens belonging to each clade.

"*A. fusciqula* group" (clades C and D)

The only significant ratio identified for the model for distinguishing the clades was EN/HW. Despite the statistical significance of the model ($p<0.0015$), the Wilks' lambda is above 0,5, showing that the discrimination between both clades is not evident. The model had an overall correct classification of 92.6%: 100% (19 / 19) for clade C, and 75% (6/8) for clade D. These values suggest that unequivocal distinction of theseclades based on morphometric data is not possible.

"*A. angolensis group*" (clades A, B, E, F, G)

The ratios with higher discriminant power for this group were, by order: HW/Ti and Fo/HW. Nevertheless, the overall correct classification of this model was 72.9% (50% (3/6) for clade A, 71.4% (10/14) for clade B, 66.7% (6/9) for clade E, 66.7% (6/9) for clade F, and 100% (10/10) for clade G), showing that, except for clade G, variables on the model do not enable accurate discrimination of the clades belonging to this group.

"*A. angolensis*" from Southern Africa (clades A and F)

This model had an overall correct classification of 93.3% (100% (6/6) for clade A, and 88.8% (8/9) for clade F. The ratios with higher discriminant power for this group were, by order, HW/Ti, EE/HW and HL/SUL. The variables on the model do not enable accurate discrimination of the clades A and F.

"*A. angolensis*" from Eastern Africa (clades B, E and G)

The ratios with higher discriminant power for this group were, by order HW/Ti, Fo/HW, SL/HW. This model had an overall correct classification of 88.2%, 92.8% (13/14) for clade B, 70% (7/10) for clade E, and 100% (10/10) for clade G, showing that using only these variables is not enough for accurate discrimination of clades belonging to this group, except for clade G.

3.2. Qualitative characters

The observed variation of all the registered qualitative characters and their states in each clade is summarized in the following tables. Table 7 depicts the body coloration pattern, Table 8 refers to head coloration pattern, and Table 9 to skin texture and morphology. Missing data were not included on the tables.

Table 7 Distributions (and percentage) of states relative to body coloration pattern among the seven clades. n=number of specimens for which data were available.

Vertebral stripe				**DL ridges delineation**			**Dorsal blotches pattern**					
Clade	*n*	A0	A1	*n*	B0	B1	*n*	C0	C1	C2	C3	C4
A	17	8 (47)	9 (53)	17	17 (100)		17		17 (100)			
B	14	13 (93)	1 (7)	14	12 (83)	2 (17)	14	1 (8)	3 (25)	8 (58)	1 (8)	1 (8)
C	27	22 (81)	5 (19)	24	24 (100)		24		13 (54)		11 (46)	
D	14	11 (79)	3 (21)	14	14 (100)		12		8 (67)		4 (33)	
E	12	12 (100)		12	2 (17)	10 (83)	12	10 (83)	2 (17)			
F	14	1 (7)	13 (93)	14	14 (100)		14		14 (100)			
G	10	8 (80)	2 (20)	10	10 (100)		10		7 (70)		3 (30)	

Thigh bars outline				**Posterior surface of thighs coloration pattern**					**Flanks coloration pattern**				
Clade	*n*	D0	D1	*n*	E0	E1	E2	E3	*n*	F0	F1	F2	F3
A	17		17 (100)	17	1 (6)	9 (53)	6 (35)	1 (6)	17	14 (82)			3 (18)
B	14	9 (64)	5 (36)	14	2 (14)		7 (50)	5 (36)	14	12 (86)		2 (14)	
C	26	17 (65)	9 (35)	23	2 (9)	4 (17)	4 (17)	13 (57)	15	15 (100)			
D	13	11 (85)	2 (15)	10			7 (70)	3 (30)	8	8 (100)			
E	12	10 (83)	2 (17)	12	8 (67)		3 (25)	1 (8)	9	2 (22)		7 (78)	
F	14		14 (100)	13		2 (15)	6 (46)	5 (38)	14	10 (71)	4 (29)		
G	10	8 (80)	2 (20)	10			10 (100)		10	10 (100)			

Table 8 Distributions and percentage (in parentheses) of states relative to head coloration pattern the seven clades. n=number of specimens for which data were available.

Interorbital blotch				**Frontoparietal blotch**			**Facial stripe projection**			
Clade	n	G0	G1	n	H0	H1	n	I0	I1	I2
A	17		17 (100)	17	6 (35)	11 (65)	17		1 (6)	16 (94)
B	15	9 (60)	6 (40)	15	14 (93)	1 (7)	14		6 (43)	8 (57)
C	23	9 (39)	14 (61)	24	24 (100)		22	2 (9)	10 (45)	10 (45)
D	9	3 (33)	6 (67)	11	11 (100)		10		1 (10)	9 (90)
E	13	9 (69)	4 (31)	15	15 (100)		12	11 (92)	1 (8)	
F	14	2 (14)	12 (86)	14	14 (100)		14	1 (7)	1 (7)	12 (86)
G	10	8 (80)	2 (20)	10	10 (100)		10	2 (20)	4 (40)	4 (40)
Marking along upper lip				**Nostril-snout line**			**Breeding males' throat**			
Clade	n	J0	J1	n	K0	K1	n	L0	L1	L2
A	17	10 (59)	7 (41)	17	16 (94)	1 (6)	3	3 (100)		
B	15	1 (7)	14 (93)	13	8 (62)	5 (38)	4	3 (75)		1 (25)
C	19	13 (68)	6 (32)	21	6 (29)	15 (71)	9	9 (100)		
D	10		10 (100)	6	1 (17)	5 (83)	5		5 (100)	
E	11	9 (82)	2 (18)	12	2 (17)	10 (83)	3	3 (100)		
F	14		14 (100)	13	4 (31)	9 (69)	2			2 (100)
G	10		10 (100)	10	8 (80)	2 (20)	5	4 (80)		1 (20)

Throat coloration pattern of adults												
Clade	n	M0	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
A	6							6 (100)				
B	13				1 (8)		3 (23)		7 (54)		1 (8)	1 (8)
C	15				2 (13)		13 (87)					
D	8		6 (75)	2 (25)								
E	10				1 (10)	1 (10)	3 (30)	1 (10)	2 (20)			2 (20)
F	9	1 (11)		1 (11)		1 (11)				4 (44)	2 (22)	
G	10			6 (60)	2 (20)		1 (10)					1 (10)

For most of the observed characters, frequencies of states differed among clades, but no states were diagnostic of any clade. However, some of the characters had states exclusive of certain clades: flanks coloration pattern, dorsal blotches pattern, throat coloration pattern of adults, breeding males' throat darkening, and these allowed an immediate and easy identification of the clade to which the specimen belonged.

Table 9 Distributions and percentage (in parentheses) of states relative to skin texture and morphology (among the seven clades. n=number of specimens for which data were available.

Dorsal ridges				**DL ridges shape**			**DL ridges size**				
Clade	n	N0	N1	n	O0	O1	n	P0	P1	P2	P3
A	5	2 (40)	3 (60)	17	16 (94)	1 (6)	17	1 (6)		3 (18)	13 (76)
B	12	6 (50)	6 (50)	15	13 (87)	2 (13)	15	3 (20)	7 (47)	5 (33)	
C	18	1 (5)	17 (95)	17		17 (100)	20	17 (85)	2 (10)	1 (5)	
D	5		5 (100)	8	2 (25)	6 (75)	9	8 (89)		1 (11)	
E	9	8 (89)	1 (11)	12	12 (100)		13		4 (31)	5 (38)	4 (31)
F	8		8 (100)	14	1 (7)	13 (93)	14	11 (79)	3 (21)		
G	10	6 (60)	4 (40)	10	9 (90)	1 (10)	10		2 (20)	8 (80)	

Lateral skin texture					**Snout shape from ventral view**				**DST on third finger**		
Clade	n	Q0	Q1	Q2	n	R0	R1	R2	n	S0	S1
A	17		12 (71)	5 (29)	17	3 (18)	12 (71)	2 (12)	16	15 (94)	1 (6)
B	11		11 (100)		14	8 (57)	6 (53)		13	13 (100)	
C	18		18 (100)		27	23 (85)	4 (15)		21	21 (100)	
D	11		10 (91)	1 (9)	14	12 (86)	2 (14)		12	12 (100)	
E	11	3 (27)	6 (55)	2 (18)	13	6 (46)	7 (54)		10	9 (90)	1 (10)
F	14		11 (79)	3 (21)	14		7 (50)	7 (50)	13	11 (85)	2 (15)
G	10		6 (60)	4 (40)	10		10 (100)		10	3 (30)	7 (70)

The distal subarticular tubercle on the third finger (DST) has been reported as a potentially diagnostic character for distinguishing *A. fuscigula* from *A. angolensis*, being present on the first, and absent on the late (Scott, 2005). On the present study, the DST was always present on both clades previously included in the "*A. fuscigula* group" (C and D), agreeing with this finding, but it was not always absent in the clades belonging to the "*A. angolensis* group". Therefore, this feature, if present, is not useful for distinguishing both groups. Specimens from clade G had a considerably high frequency of absence of DST, contrasting to every other clade.

The main obtained results relative to the number of phalanges free of webbing for every clades are presented in Fig. 8 and Fig. 9.

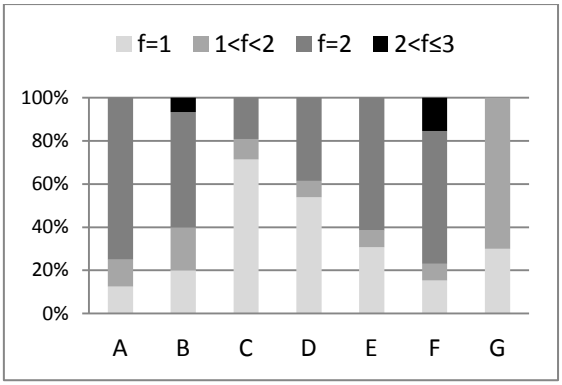

Figure 8 Frequencies of the number of phalanges free of webbing (f) on the outer 4th toe on each clade.

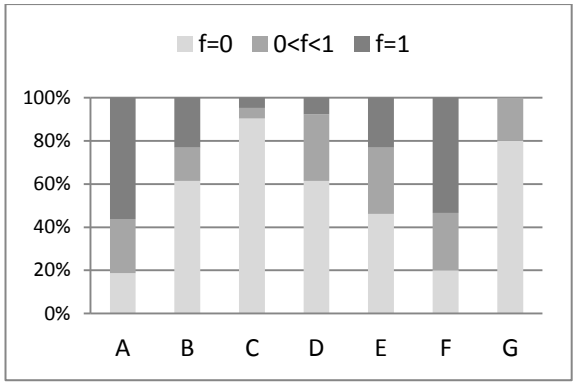

Figure 9 Frequencies of the number of phalanges free of webbing (f) on the inner 5th toe on each clade.

Results relative to number of vomerine odontophores, complete foot webbing formulae, depth of webbing notches, and tadpoles labial tooth row formulae are summarized in Appendix 8, Table 16. Tadpoles from reached clade A a higher number of labial tooth rows than the maximum described for *A. angolensis* (Boulénger, 1918; Channing, 2001). The small sample suggested that TLRF was variable and similar among clades, and that this is not a diagnostic feature for species of *Amietia*. Results relative to vomerine odontophores were partially in accordance with Boulénger (1918): there seemed to be a tendency of clades from the "*A. fuscigula* group" to having smaller odontophores (frequently with oval) and less vomerine teeth (maximum 6 per odontophore) than clades from "*A. angolensis* group", with longer odontophores (frequently elongate) and more vomerine teeth (reaching 11 per odontophore). Nevertheless, its usefulness for distinguishing clades was inconclusive.

3.3. Bioacoustic data

Clade A

Length of each pulse train in the initial phase was 430–493 ms (462±44.5, n=2). Each pulse train had eleven pulses (n=2), and the duration of each pulse varied between 2–4 ms (2.8±0.7, n=22), and the interval between pulses 29–61 ms (43.1±8, n=20). The pulse rate of the initial phase was 22.3–25.6 pulses/s (23.95±2.3, n=2). The frequency of the pulses ranged from 2.6 to 3.9 kHz, and the energy range of the first pulses of each pulse train (3.1–3.4 kHz) was smaller than the energy range of the last pulses (2.6–3.7 kHz). No harmonics were detected. The interval between the first and second phase was of 433 ms seconds (n=1).

On the second phase, note duration range was 32–52 ms (38.8±7.8, n=5), pulse rate ranged from 96.2–187.5 pulses/ms (145.6±34.8, n=5), and notes were vocalised at 2.6–3.7 kHz. The note repetition rate was 3.45 notes/s, and the interval between notes ranged from 230–363 ms (320±60; n=4). A sonogram and oscillogram of part of the analysed stretch is in Fig. 10.

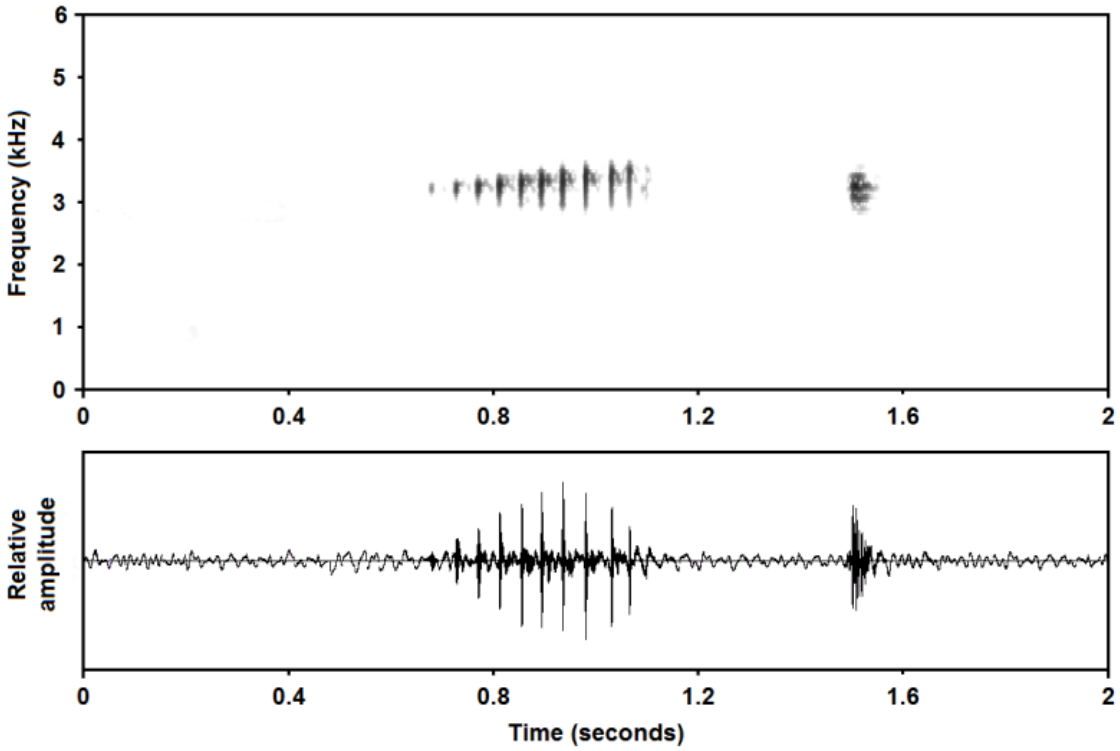

Figure 10 Sonogram (top) and oscillogram (bottom) of the advertisement call of clade A, recorded by Alan Channing, at Humpata, Angola (21°C, voucher specimen: AC 3120). Figure kindly provided by Gonçalo M. Rosa.

Clade C

Stretches of calls from 3 individuals were analysed for this clade. All calls consisted in series of pulsed notes vocalised in relatively regular intervals, a structure that resembled the second phase of the typical biphasic call of the genus *Amietia*. The sonogram and oscillogram of stretches of the analysed calls are depicted in Fig. 11 (representing the call of only one individual) and Fig. 12 (first two notes from one individual, and third note, with a broader frequency range, from a different individual).

The following parameters result from the combined data from all three individuals. In general, notes' duration ranged from 37 to 65 ms (48.7±7.4, n=23), pulse rate 30.8–133.3 pulses/s (91.4±27.4, n=23). Interval between notes varied from 1.3 s to 14.8 s (3.2±3.2, n=20). The note repetition rate was 0.14–0.59 notes/s (0.39±0.23, n=3), and frequency ranged from 0.1–3.1 kHz.

Call frequency varied highly among individuals. On the call from Northern Cederberg (Fig. 11), there was a more energetic band of frequencies from 0.7 to 2.4 kHz, another energetic band from 2.6–3.0 kHz, and there was appreciable energy up to 3.5 kHz. On the call from one of the individuals from Bloukrans River (Fig. 12, two first notes) the more energetic

call frequencies were at 0.6–1.1 kHz and 1.3–1.5 kHz, with considerable energy reaching 3.1 kHz. The other call from Bloukrans River (Fig. 12, third note) showed a broad range of frequencies, from 0.3–2.2 kHz lower frequencies, with considerable energy reaching 3.1 kHz.

Interval between notes also varied considerably: individuals calling at higher frequencies with intervals from 1.3 to 4.1 seconds, and the individual calling in lower frequencies with larger intervals, from 4.9 to 14.8 s. The considerable variation in frequency range among individuals might be due to different body sizes, which is known to be negatively correlated with the fundamental frequency of advertisement calls (Bee, 2002).

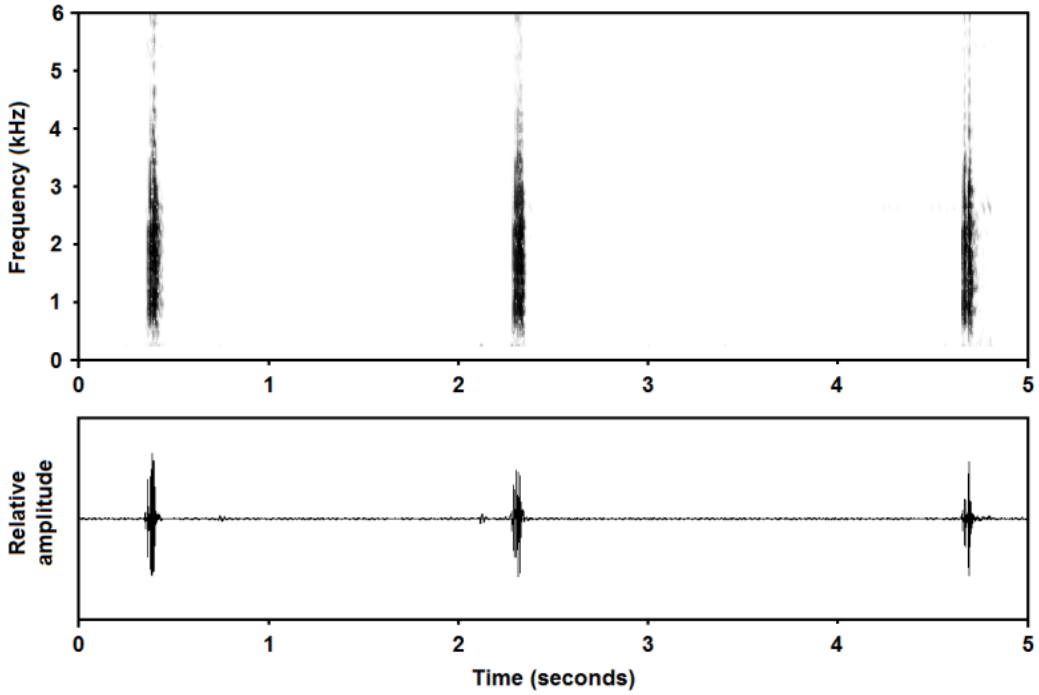

Figure 11 Sonogram (top) and oscillogram (bottom) of the advertisement call of clade C recorded by Alan Channing at Northern Cederberg, South Africa (22 °C, voucher specimen AC 3164). Figure kindly provided by Gonçalo M. Rosa.

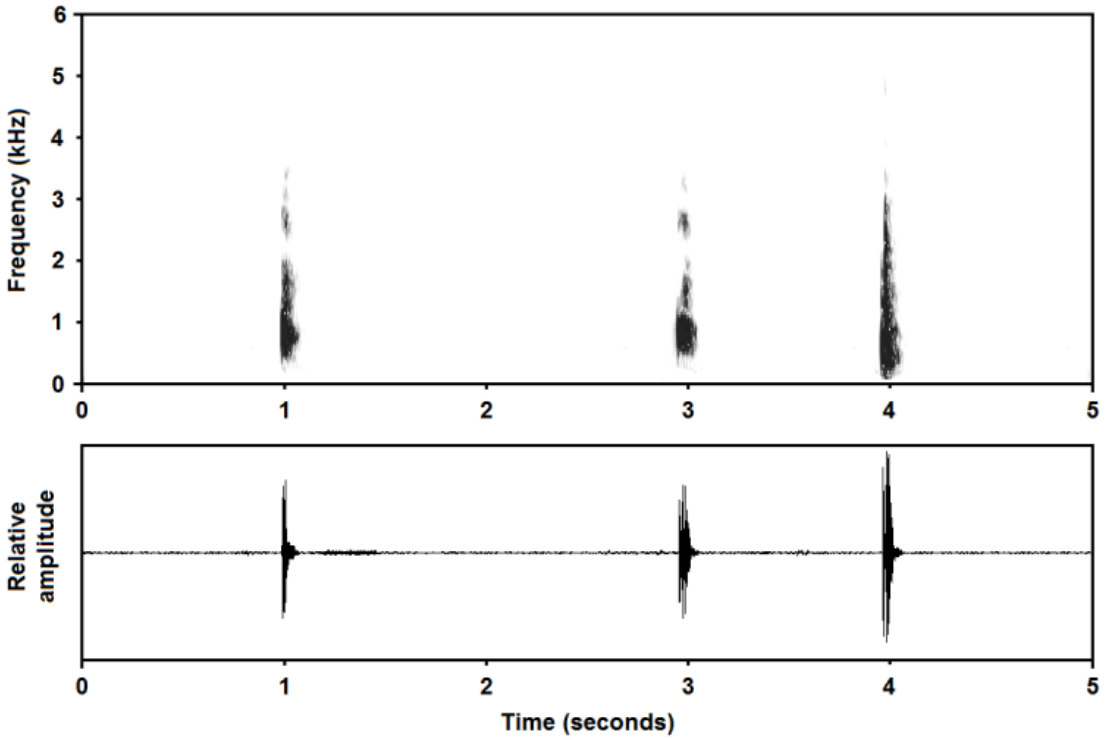

Figure 12 Sonogram (top) and oscillogram (bottom) of advertisement calls of two individuals of clade C recorded by Harold Braack at Bloukrans River, South Africa (19°C, no voucher specimens). Figure kindly provided by Gonçalo M. Rosa.

The obtained parameters for clade A are compared to the call parameters described for *A. angolensis* (*sensu* Frost 2006), from Channing (1979). The obtained parameters for clade C are compared to the call parameters described for *A. fuscigula* (*sensu* Frost 2006), from Channing. A summary of the compared values is presented in Table 10.

Table 10 Values [mean (range)] of pulse rates (pulses/s), duration (ms) and frequency (kHz) from calls described in the present work (clades A and C), and calls assigned to the species to which these clades were previously assigned to: *A. angolensis* and *A. fuscigula*, respectively. Values from Channing (1979).

	First phase				Second phase			
	pulse rate	**duration**	**frequency**	**n**	**pulse rate**	**duration**	**frequency**	**n**
Clade A	24 (22.3–25.6)	462 (430–493)	2.6–3.9	2	146 (96,2–187,5)	39 (32–52)	2.6–3.7	5
A. angolensis (*sensu* Frost 2006)	12.4 (8.9–16.6)	353 (159–868)	1.5–2.7 occasionally lower	91	112 (87–162)	540 (200–1300)	0.7–2.7 harmonics at 0.8 and 1.6	91
Clade C	–				91.4 (30.8–133.3)	49 (37– 65)	0.1–3.1	23
A. fuscigula (*sensu* Frost 2006)					174 (110–265)	110 (37–302)	0.3–1.6	83

Comparison between call parameters of Clade A and *A. angolensis* (*sensu* Frost 2006)

Clade A has a much higher pulse rate in the first phase (24 pulses/s) than *A. angolensis* (12.4 pulses/s), with no overlapping values in the ranges, and calls at considerably higher frequencies (2.6–3.9 kHz for Clade A, while 0.1–2.9 kHz for *A. angolensis* (*sensu* Frost 2006)). Average length of the first phase is higher on Clade A (462 ms) than in *A. angolensis* (353 ms), but the range of the second (159–868 ms) totally comprehends the range of the first (430–493 ms).

The average pulse rate on the second phase is also higher in clade A (146 pulses/s) than in *A. angolensis* (*sensu* Frost 2006) (112 pulses/s), but the whole range of *A. angolensis* is included on the range of clade A. The average note duration is considerably lower in the first (39 ms) than in the late (540 ms), with no overlapping values.

Comparison between call parameters of Clade C and *A. fuscigula* (*sensu* Frost 2006)

Clade C has a much lower average pulse rate in the second phase (91.4 pulses/s) than *A. fuscigula* (*sensu* Frost 2006) (174 pulses/s), although there are overlapping values (110–133 pulses/s). Notes have a lower average length in clade C (49 ms) than in *A. fuscigula* (*sensu* Frost 2006) (110 ms), but the range of the first (37–65 ms) is totally included the range of the late (37–302 ms). Although there is a narrow range of overlapping frequencies (0.1–1.6 kHz) between both taxa, clade C calls reach higher frequencies (maximum 3.1 kHz) than *A. fuscigula* (*sensu* Frost 2006) (maximum 1.5 kHz).

4. DISCUSSION

This study was an attempt to provide a morphological and acoustical diagnosis of the *Amietia* clades proposed by Channing (*in prep.*). Morphometric data allowed a distinction between "*A. angolensis* group" and "*A. fuscigula* group", and discriminant analysis showed significant discrimination among clade, corroborating the proposed clades. Despite there is considerable intra-specific variability in qualitative features such as coloration pattern, skin texture and morphology, and despite our small sample sizes, these characters showed consistent intra-clade variation patterns, suggesting that these have a good potential for distinguishing clades. Acoustic data, as expected, were helpful for distinguishing clades.

4.1. Bioacoustic data

The differences in advertisement call parameters of specimens from Angola (clade A) and specimens from South Africa (Channing, 1979) – higher pulse rate of both phases, higher note duration and higher frequencies on the first) – support Channing's (*in prep.*) split of "*A. angolensis*" from Southern Africa in more than one *taxon*.

Advertisement call of three specimens assigned to clade C showed that these reach higher frequencies than the call so far assigned to *A. fuscigula*, described by Channing (1979) from individuals from Natal. These differences support Channing's (*in prep.*) proposal of splitting what is presently considered as *A. fuscigula* in more than one *taxon*.

4.2. Methodology

Some aspects of the methodology might have affected the obtained results, and are therefore important to mention.

The criteria for assigning not genetically analysed specimens and advertisement calls with no vouchers to the clades were based on morphological and distributional similarities between these and the genetically identified specimens. This can be not totally reliable, and could have biased the data. It is important to stress though that there is also a "margin of error" even if considering the genetic results as the main criterion, because clade formation is

based on the interpretation of a dendogram and of genetic distances, and may not always be objective.

Some of the clades included in this study seemed to have different body sizes (see Fig. 6 A). Establishing the age class of all clades based on the size of the smallest male among all clades may not be the most accurate criterion. Nevertheless, no other information was available to make this decision.

The creation of categories for qualitative characters was not always exhaustive, because the distinction between states could sometimes be dubious. Therefore, some apparently consistent differences among clades were not reflected by the results. This was the case, for example, for the shape of the line on the upper lip, the flanks coloration pattern, and the shape of the upwards projection of the pale facial stripe. Thus, the creation of categories is not always the best approach for analyzing qualitative characters with ambiguous definition of the different states. In these cases, the mere description, or the use of images instead of textual descriptions, seem to be an easier and more practical approach.

The diagnosis of every clade was based on very small samples: the larger sample had only 20 adults. Therefore, it is expected that the variation obtained in this study does not reflect the complete intra-specific variation of each clade, and that characters that here were exclusive from clades, may prove to be non–exclusive using larger samples.

4.3. Provisional working names of clades

The provisional working names proposed by Channing (*in prep.*) for the clades included in this study are here discussed based on:

- original description of the species these are currently assigned to,
- original description of the species corresponding to the provisional names proposed by Channing (*in prep.*);
- reviews of *taxa* including these clades (not exhaustive);
- photographs of type specimens (when available).

4.3.1. Clade A – proposed working name: *Amietia angolensis* (Bocage, 1866)

Original description and other information

The original description of *Amietia angolensis* has few details, and most of the described characters could be applied to several species of *Amietia*, except for the ventral coloration pattern – dark throat scattered with roundish white spots, and chest and belly marbled (Bocage, 1866), which is similar to the lacy pattern always present on adults analysed on this study. Boulénger (1918) also referred to this particular pattern on throat coloration of specimens from Angola, and Poynton (1964) referred to the very pronounced mottling of the throat and bellies of the Angolan specimens.

Photographs of type specimen (*Rana angolensis* MNHN 1860)

The type specimen MNHN 1860 collected in Benguela, Angola, observed in photographs, has a lacy throat, dorsolateral ridges continuous up to groin and thicker than the dorsal ridges, short dorsal ridges, in two longitudinal rows on both sides of the vertebral stripe. All these features resemble the observations made on the analysed specimens of clade A, and are according to Bocage's (1866) description. The shape of the upward projection of the pale facial stripe, not linked to the main stripe, present in the type is similar to what was observed in some specimens from clade A.

Collecting locality

All specimens assigned to this clade were collected in Angola, and one was collected in Calandula Waterfalls – the type locality – which also supports Channing's proposal of the clade's name.

All the morphological results referred above support Channing's (*in prep.*) proposed working name for this clade, as they suggest that the clade corresponds to *Rana angolensis* Bocage, 1866.

4.3.2. Clade B – proposed working name: *Amietia desaegeri* (Laurent, 1972)

Original description and other information

The most obvious feature described by Laurent (1972) for identifying both *R. desaegeri* and *R. ruwenzorica* was an upward projection of the upper lip marking often reaching the dark

canthal line, a feature not observed in any analysed specimen. Many specimens had very irregular upward projections of the upper lip, giving the snout a very irregular coloration pattern, which resembled the diagnosis and the plate of *Rana angolensis chapini* Noble, 1924 available on Laurent (1972).

On *Rana desaegeri*, male throat coloration, if present, has two shadowy zones with a paler centre (found in one analysed specimen), and in females consists in separate spots, more concentrated on the edges than on the centre (found in one analysed specimen). The throat vermiculations of some specimens resembled the description and plate of *R. angolensis chapini* Noble, 1924 available on Laurent (1972).

The smooth skin with only dorsolateral ridges reaching the groin, described by Laurent (1972) for *A. desaegeri*, was observed in some specimens.

On SL 459, the nearly immaculate throat, the large dark blotches close to the groin, the ridges delineation until the mid back, and the contrasting more rounded and shorter snout, are all features that do not indicate that this specimen belongs to *A. desaegeri*, and are according to the description and plates available in Laurent (1972) for *R. ruwenzorica*. On SL 539, the obvious vertebral stripe and the number of phalanges free of webbing on the fourth toe reaching 3 are features from this specimen common to description and plates available in Laurent (1972) for *R. ruwenzorica.* Furthermore, *R. ruwenzorica* is more common between 1600 and 2500 m of altitude (and SL 539 was collected at 2500 m), also supporting the probability of this specimen belonging to *R. ruwenzorica.*

Photographs of holotypes (*Rana desaegeri* MRAC 74–018B5626; *Rana ruwenzorica* MRAC 74–18B5820)

The head and body shape of many specimens of the present study are similar to those of the *Amietia desaegeri* holotype. The shape of the holotype's dorsolateral ridges were found in many specimens from the clade. The presence of a frontoparietal blotch (partially hidden by a vertebral stripe) was also shown by one of the analysed specimens. The throat coloration pattern of the holotype does not resemble any of the analysed specimens.

The diffuse outline on the *Amietia ruwenzorica* holotype resembled the dorsal coloration pattern observed in many specimens assigned to this clade, but the throat coloration pattern does not resemble any of the observed patterns.

Collecting locality

The type locality of *Amietia desaegeri*: river Byangolo, Munsenene sector, 1300 m, and the type locality of *Amietia ruwenzorica*: Kikyo, Munsenene sector, 2080 m, both in Democratic Republic of Congo, is close to the border with Uganda and Rwuanda, and near the collecting locality of some specimens included in this study.

Some of the data obtained for this clade do not fit the description of *A. desaegeri*, and some data support the choice of this provisional working name. Moreover, for some specimens results indicate a probability of belonging to *A. ruwenzorica.* The inclusion of specimens morphologically very distinct, with a relatively widespread distribution (both in geographical range and in height) in the clade, may suggest that its phylogeny is not completely resolved yet. The correspondence between the clade data and the proposed working name is inconclusive.

4.3.3. Clade C – proposed working name: *Amietia fuscigula* (Duméril and Bibron, 1841)

Original description and other information

The following features present on the original description were also observed in the analysed specimens:

- the throat coloration, described as a marbling, over a white background, often extending to the chest and to the anterior part of the abdominal region;
- the lack of dorsolateral ridges (which might be interpreted as being the same of the short DL rigdes found in the studied specimens);
- the wide and rounded head;
- the short ridges irregularly scattered along the dorsum;
- foot webbing as long as the toes but with deep notches;
- dorsal coloration plain or with widespread blotches;
- vertebral stripe present in some individuals.

Photographs of holotype (*Rana fuscigula* MNHNP 4471)

It was not possible to assess the throat coloration pattern of the holotype, and its preservation status did not allow making any observation about head or dorsum coloration.

The specimen has short ridges irregularly scattered along dorsum, dorsolateral ridges continuous only until scapular level (maximum), belly with apparently coarse vermiculations, a rounded head from dorsal view, a not protruding snout from ventral view, posterior surface of thighs nearly plain, no delimitation of thighs semibars. All these features are in accordance with features observed in the specimens of the clade.

Collecting locality

All specimens were collected on Southern South Africa, some on the Cape of Good Hope – the type locality of *A. fuscigula*, which is a strong indication that this clade is the same as *Rana fuscigula* (*sensu* Duméril & Bibron, 1841).

All the discussed above suggest that specimens from clade C correspond to *Rana fuscigula* Duméril & Bibron 1841, supporting Channing's proposal of naming this clade as *Amietia fuscigula* (Duméril & Bibron, 1841). Curiously, the specimens' throat coloration (in accordance with the species name etymology, which means dark throat) is the most obvious feature that distinguishes this clade from clade D, also assigned to *A. fuscigula* so far.

4.3.4. Clade D – proposed working name: *Amietia quecketti* (Boulénger, 1895)

Original description and other information

Obtained data from specimens of this clade suggest that:

- They do not correspond to *A. fuscigula* (Duméril & Bibron 1841), because none of the specimens had throat coloration as described on the original description (see discussion of Clade C identification).
- Specimens of this clade have so far been identified as *A. fuscigula* probably because they have very similar body proportions, as shown in all the results, including the HW/Ti ratio, which has been used as a diagnostic feature for distinguishing *A. fuscigula* from *A. angolensis* (Poynton, 1964).
- The diagnostic feature observed in adults of clade D – speckled throat – is not clearly mentioned on the original description of *Rana quecketti* Boulénger 1895, where it is only stated «…lower parts white.» (Boulénger 1895).

- The short legs of the holotype of *Rana quecketti* referred by Boulénger (1918), the vomerine teeth in short transverse series (Boulénger, 1895), the small size for a female (SVL=48 mm), all resemble clade D.
- The frequencies of number of phalanges free of webbing on the fourth toe described for this what is assumed to be this taxon (*Rana fuscigula sensu* Channing, 1979), are similar to the observed frequencies.

Collecting locality

The type locality of *Rana quecketti* is near Pietermaritzburg, Natal, South Africa, which ison the same region (Northwestern South Africa) of the collecting localities of some specimens from this clade.

Although the collecting sites of specimens of this clade might not represent the entire range of the clade, no specimen assigned to this clade was collected close from the type locality of *A. fuscigula*. This might be an indication that this clade does not occur in that region.

Although the main feature of the clade – speckled throat – is not mentioned on the original description, distribution and other morphological data support Channing's (i*n prep.*) proposed name for this clade.

4.3.5. Clade E – proposed working name: *Amietia tenuoplicata* (Pickersgill, 2007)

Original description and other information

This clade corresponds to *A. tenuoplicata* (Pickersgill, 2007), as DNA from a specimen provided by M. Pickersgill was used to build the new phylogeny of the *Amietia* genus (Channing, *pers comm.*).

Although the original description of this species probably lacks intra-specific variability, as only one female and tadpoles were used (Pickersgill, 2007), the diagnosis of the analysed specimens is in accordance with the original description of the species in many aspects:

- almost uniform dorsal pattern with a few very small spots on the posterior dorsum;
- dorsolateral folds highlighted in brown (present in most of the specimens);
- sides of the face unmarked except for a straight dark edge to the upper lip;

- non–delimited semibars on the thighs;
- throat with a scattering of faint non–reticulate grey spots (present in some specimens from Amani);
- smooth skin.

Unmarked flanks on the holotype, are not in accordance with the general pattern observed on the specimens assigned to clade E.

Photographs of holotype (*Rana tenuoplicata* ZMB 66247)

Many of the analysed specimens were similar to the holotype in the following:

- head and body shape;
- no upwards projection of the pale facial stripe between the eye and nostril;

These features, as well as the very faint dorsal blotches and the smooth skin, were also evident on colour plates from *A. angolensis* present in Harper and Vonesh (2003), stated as being *A. tenuoplicata* by Pickersgill (2007).

Collecting locality

Some of analysed specimens assigned to clade E were collected on the type locality (Amani, Tanzania). Most of the referred above data corroborate Channing's (*in prep.*) provisional name for the clade.

4.3.6. Clade F – proposed working name: *Amietia theileri* (Mocquard, 1906)

Original description and other information

Observation of specimens assigned to this clade indicate that:

- they do not correspond to *Amietia angolensis* (Bocage, 1866) as all lack the ventral coloration pattern described by Bocage (1866), as well as the dorsolateral ridges continuous up to groin;
- they are in accordance with the description of *Rana Theileri* Mocquard, 1906 in the following features: a pale contour of the thighs bars, pale spots on the posterior part

of the thighs, white ventral coloration with very pale marblings on the throat and chest, vertebral stripe present,

- the description of this clade also agrees with the description of *Rana Delalandii* Duméril and Bibron, 1841, mainly because of the white ventral coloration some of its specimens.
- frequencies of number of phalanges free of webbing on the fourth toe for this *taxon* (Channing, 1979) are according to the results obtained for the specimens assigned to this clade.

Photographs of syntypes (*Rana Delalandii* MNHNP 4473–4*, Rana Theileri* MNHNP 1905.0473)

The photograph of the holotype of *Rana Theileri* MNHN 1905.0473, did not allow any detailed observation. Preservation status of MNHN 4473 did not allow any conclusions about coloration pattern, and it is not possible to see the size or size of dorsal and dorsolateral ridges. Head and body shape in both types is similar to the observed clade F specimens. MNHN 4474 has a vertebral stripe, immaculate belly, and reticulate posterior surface of thighs, all similar to the clades' specimens.

Collecting locality

Type locality of *R. Theileri* Mocquard 1906 is Nelspruit, locality that is on the same region (Northwestern South Africa) from the collecting locality of some of the clades' specimens.

The type locality of *R. Delalandii* Duméril & Bibron, 1841, is Cape of the Good Hope, and it is not according to the so far known distribution of clade F, but this type locality has been considered wrong by Boulénger (1918).

The original descriptions of *R. Delalandii* Duméril & Bibron, 1841, and *Rana Theileri* Mocquard, 1906 – especially the white throat and long legs referred in both – suggest that these probably correspond to the same taxonomic entity.

These findings, together with the findings about the specimens assigned to clade A (from Angola) support Poynton's (1964) doubts about Boulénger's (1882) synonymy of *R. Delalandii* Duméril & Bibron and *R. angolensis*, Bocage 1866. Poynton's (1964) doubts were based on the fact that «… types of *angolensis* show a prominent skin fold running dorsolaterally from behind each eye, and a very pronounced mottling of throat *and* belly. Very few specimens from Southern Africa have mottled bellies, and although there is a tendency for

more northern specimens to have a [dorsolateral] fold (…), it is not nearly as prominent as it is in most specimens in the *angolensis* type series. ».

All these data support Channing's (1978) suspicion that the African *Rana* (now *Amietia*) was a complex of species, and Channing's (*in prep.*) finding that *A. angolensis* from Southern Africa is not the same as *A. angolensis* from Angola.

Being in accordance with the original description of *Rana Theileri* Mocquard, 1906, these results support the working name proposed by Channing (*in prep.*) for this clade.

4.3.7. Clade G – proposed working name: *Amietia viridireticulata* (Pickersgill, 2007)

Original description and other information

Clade G corresponds to *Amietia viridireticulata* (Pickersgill, 2007), as DNA from a specimen provided by M. Pickersgill was used to build the new phylogeny (Channing, *pers. comm*.).

The following characters detected on the specimens assigned to the clade are in accordance with the species' original description:

- foot webbing formula;
- dorsolateral ridges, unbroken from eye to thighs, which is in accordance with the observed specimens.

Several features of the specimens assigned to clade G are not in accordance with the description of *A. viridireticulata*:

- some specimens had very evident ridges on the flanks and dorsum, as opposed to the almost smooth skin referred on the original description;
- no specimen had a dark delimitation of the DL ridges, as is described for *A. viridireticulata*;
- some specimens had a coarse marbling on the throat, but a coarse reticulum on the belly (on the original description) was never detected;
- specimens consistently showed a marbled pattern on the flanks, as opposed to a conspicuous reticulate pattern on the original description of *A. viridireticulata*;

- specimens in general had slender body shapes, as opposed to a wide head and sturdy body referred on the description. Nevertheless, he HW/SUL ratio was in accordance between specimens from the clade and Pickersgill's (2007) description.

The fact that this species was described based on a small sample (two females and one immature male), suggests that variation patterns not described on the original description are expected to occur.

Photographs of holotype (*Rana viridireticulata* ZMB 66248)

- The shape of the holotype's upper lip marking is similar to the observed in some specimens.
- Posterior surface of thighs is coarser and less intricate on the holotype than on specimens from clade G, where it was a very consistent feature.

Pickersgill (2007) assigns part of Stewart's (1967) description of *A. angolensis*, including the colour plate as a perfect representation of *A. viridireticulata.* The faint blotches in a vertebral row and the blotches disposed along the dorsolateral ridges of the individual on the plate resemble the coloration pattern of specimens assigned to this clade.

Collecting locality

Specimens assigned to this clade were collected on Northern Malawi and Southwestern Tanzania, regions where *A. viridireticulata* is known to occur.

Several morphological features were not in accordance between the specimens from the clade and the original description of the species. These might be related to the fact that the original description was based on a small number of specimens.

However, specimens collecting locality and fact that DNA from a specimen provided by M. Pickersgill was included in the phylogeny is strong evidence supporting that the clade corresponds to the provisional name suggested by Channing (*in prep.*). Nevertheless, it is not possible to conclude on the appropriateness of the suggested name for the present clade.

4.3. Final Considerations

HW/Ti ratio allowed the separation between "*A. angolensis*" and "*A. fuscigula*" groups. This was already expected, as this character has been identified as the more useful for

distinguishing both “species” (Poynton, 1964). Moreover, HW/Ti and Fo/HW, previously shown to be useful for distinguishing species of *Amietia,* were useful for distinguishing some of the studied clades. Nevertheless, no ratio alone allowed an obvious separation for distinguishing the clades included in the studied groups. Clade G could be reliably distinguished from all other clades from the “*A. angolensis* group”. However, all the discriminations needed a high number of measurements for total reliability. The low practicability to obtain several measurements in the field somehow limits the usefulness of this approach; however this can still be a very useful method, for instance, to detect which species is present in which region in a short visit and collecting relatively few individuals.

Among the clades occurring in Southern Africa (A, C, D, F), throat coloration alone was enough for identifying every adult specimens. It is important to stress that this study does not include every species of *Amietia,* and other species (*A. vandijki, A. dracomontana, A. umbraculata, A. vertebralis,* in Southern Africa*,* and *A. wittei, A. ruwenzorica* in Eastern Africa) may co–occur with the studied clades. Among Eastern African clades, consistent specific variation on coloration patterns were observed in the flanks, on facial markings and skin texture (clade E), and in the posterior surface of thighs (clade G), but no feature alone allowed a clear distinction among the three clades. These results reflect the high morphological and coloration pattern variability associated to the genus *Amietia* (Poynton, 1964; Channing, 1979; Channing & Howell, 2006).

The use of coloration pattern as a phylogenetic character in Anura is generally avoided with the justification that it is too variable to be informative (Scott, 2005). In some cases, though, cryptic lineages are discovered to be different species, and do show consistent morphological differences that have been confused with intra-specific variability (Dawood *et al.,* 2002; Channing & Schmitz, 2008; Blackburn, 2009). This seems to be the case for species of *Amietia* from Eastern Africa, which were previously considered to consist in cryptic populations not distinguishable using morphology (Channing & Howell, 2006), but for which there is data supporting consistent morphological differences among species (Laurent, 1972; Pickersgill, 2007).

The morphology results from the present work support this consistency of intra-specific variability, that corroborated Channing’s proposed clades. Nevertheless, the results suggest that the definite assignment of clades B and G, both from Eastern Africa, still requires further work. Advertisement calls showed considerable differences when compared with

previously described calls of the presumed same species, also supporting Channing's (*in prep.*) revalidation of Southern African species.

New species of *Amietia* are expected to be split from the *"A. angolensis* group", in East Africa (Channing & Howell, 2006), and particularly in the Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya (Harper *et al*., 2010). Detailed molecular and acoustical studies have been proposed to materialise these new descriptions (Channing & Howell, 2006). It might be expected that, as happened in the present study, the new discovered species show consistent intra-specific morphological variation.

Classifying species and providing tools to identify them are tasks of taxonomy (Dayrat, 2005). Species inventories are a key tool, providing baseline information for conservation studies (Dayrat, 2005), such as research projects, management plans, or environmental impact assessment studies. Amphibians are considered an indicator group for environmental quality (Waddle, 2006), and therefore, inventories of anurans are especially valuable for conservation, and these depend on the possibility of species identification.

The usefulness of morphology as an extra source of information for an integrative taxonomy is stressed (Dayrat, 2005). Despite the fact that genetic and acoustic data provide reliable and easier ways of discovering new species in anurans than morphology, the naturalist does not have easy access to this kind of data. The disclosure of what is known about biodiversity is one of the means of getting people interested and concerned about conservation. This is only possible if species are known and possible to identify by what is more accessible to everybody: morphology. Morphology is also an important feature as it is frequently the only information available from museum collections (Channing, 1999), which value for research is unquestionable.

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APPENDIX 1 – Statistics

Table 11 *p*–values results from the Tukey honest significant differences for unequal sample size test for the ratio HW/Ti among clades. * depicts significant values (α=0.05).

Clades	A	B	C	D	E	F
B	0.99	-	-	-	-	-
C	0.0002*	0.0001*	-	-	-	-
D	0.0001*	0.0001*	1	-	-	-
E	0.99	0.99	0.0001*	0.0001*	-	-
F	0.94	0.56	0.0001*	0.0001*	0.31	-
G	0.93	0.49	0.0001*	0.0001*	0.25	1

Table 12 *p*-values results from the multiple comparisons of mean ranks analysis for the Kruskal-Wallis test for SUL. * depicts significant values (α=0.05).

Clades	A	B	C	D	E	F
B	1	-	-	-	-	-
C	1	1	-	-	-	-
D	1	0.19	0.12	-	-	-
E	1	1	1	0.19	-	-
F	1	1	1	1	1	-
G	0.92	1	1	0.02*	1	1

APPENDIX 2 – Error estimates

Table 13 Error (95% confidence limit) associated to the estimated measurements. Abbreviations follow Table 2.

	SAM 44658		SAM 44861	
	Estimate value (mm)	Estimate error (±95% CL)	Estimate value (mm)	Estimate error (±95% CL)
Fe	22.3	± 2.46	18.9	± 3.67
Fo	22.0	± 3.21	24.4	± 2.15
HL	14.8	± 2.14	16.5	± 1.23
SL	7.9	± 1.25	8.3	± 0.98

Table 14 Descriptive statistics for 7 repeated measurements of AC 2757. Abbreviations follow Table 2.

	Minimum	Maximum	Mean	Standard Deviation	Coefficient of Variation
SUL	71.4	72.4	72.0	0.38	0.01
SVL	72.2	73.7	72.7	0.57	0.01
Fe	41.7	42.8	42.2	0.41	0.01
Fo	41.4	41.8	41.6	0.12	0.00
Ti	43.0	43.5	43.2	0.22	0.01
IMT	4.0	4.5	4.2	0.19	0.04
HW	25.1	25.9	25.6	0.27	0.01
HWN	12.7	13.4	13.0	0.29	0.02
HL	23.5	25.1	24.6	0.57	0.02
SL	13.2	13.7	13.4	0.17	0.01
NS	6.4	7.5	6.9	0.40	0.06
EN	6.6	7.5	6.8	0.36	0.05
IN	4.7	5.1	5.0	0.13	0.03
Ey	6.8	7.8	7.2	0.32	0.05
EE	11.2	11.6	11.4	0.16	0.01
IO	5.0	6.0	5.6	0.38	0.07
ET	2.2	2.8	2.4	0.27	0.11
Ty	5.5	6.0	5.7	0.19	0.03

APPENDIX 3 – Notes on the clades

Clade A – proposed working name: *Amietia angolensis* (Bocage, 1866)

Specimens assigned to the clade:

Confirmed by genetic data: from Calandula Waterfalls (1♂ AC 3016) and Lubango surroundings (1♀ AC 3101, 4 tadpoles AC 3078, 3 tadpoles AC 3108).
Not genetically analysed: from Lubango surroundings (2♀ AC 3094, PEM 9136; 2♂ AC 3100, AC 3120; 10 juveniles AC 3102, AC 3121, PEM 9136–43, PEM 9158; 1 metamorph AC 3099).

Diagnostic features:

- the lacy coloration pattern on the gular region of adults is exclusive from this clade (except for one specimen belonging to clade E) and turns them easily distinguishable from adults of all other clades.
- the blotch on the fronto–parietal region of the head is present in 65% (11 / 17) of the clade's specimens, and, except for one specimen from clade B, this character is also diagnostic.

Notes:

- slender body shape, with a usually pointed head.

Clade B – proposed working name: *Amietia desaegeri* (Laurent, 1972)

Specimens assigned to the clade:

Confirmed by genetic data: from Western Kilimanjaro (1♀ AC 1829), Elgon National Park (1♀ NMK/A/4364/3, 1♂ NMK /A/4364/2), Kakamega Forest (1♀ NMK/A/4706/2), Rwenzori National Park (1♀ SL 459), Semliki National Park (1♀ SL 539), Mount Meru (1♂ AC 2006), Rugezi Swamp (1♂ UTA A 58426).
Not genetically analysed: from Western Kilimanjaro (2♀ AC 1830 and AC 1837, 1♂ AC 1839, 1 juvenile AC 1838), Chimala River (1♀ AC 1789), Taita Hills (1♀ NMK/A/4329/1), Salient Aberdares (1♀ NMK/A/4727/3).

Specimens assigned to this clade had the most widespread distribution among the eastern african clades. Clade showing the highest morphological variation among all seven clades. Some of the most variable characters were throat coloration pattern, shape and size of dorsolateral ridges, flanks coloration pattern. The high variability resulted in a lack of diagnostic features.

This high variability could have been due to a wrong assigning of the non–genetically analysed individuals. Nevertheless, this clade had the highest number of genetically analysed adult specimens, and there was considerable morphological variation even among these.

SL 459 and SL 539 had obvious morphological differences distinguishing them from all other specimens from this clade, described below. Nevertheless, because they were genetically identified as belonging to clade B, both specimens were included on every analyses involving this clade.

Main features:

- Some of the specimens had a confused and sometimes asymmetrical disposition of dorsal blotches. This was exclusive from this clade, but was not present in all specimens.
- Most of the specimens had a sturdier head and body than all other clades.

Notes:

- Many specimens had a very irregular upper lip marking, and an irregular coloration on the snout (opposing to a plain coloration in most specimens from other clades).

Different features in SL 459 and SL 539

- Both specimens had stripes along the dorsolateral ridges;
- SL 459 had a very rounded snout, nostrils very apart from each other and very close to the tip of the snout, different from all other specimens assigned to this clade. (Fig. 13);
- SL 539 had a more slender body than other specimens of this clade, and was the only specimen of the clade having a vertebral stripe. It had straight dark line on the flanks, and the throat coloration was also very different from all specimens assigned to this clade (Fig. 14).

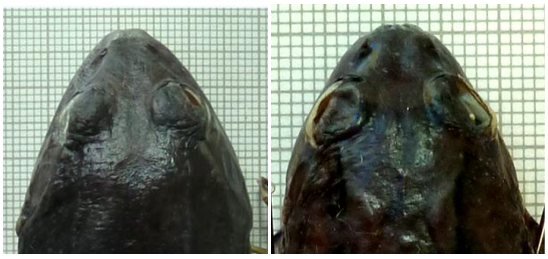


Figure 13 Common head shape of specimens assigned to clade B (left) and head shape of SL 459 (right).

Figure 14 Common throat coloration pattern of specimens assigned to clade B (left) and throat colour pattern of SL 539 (right).

Figure 15 Dorsal view of SL 459.

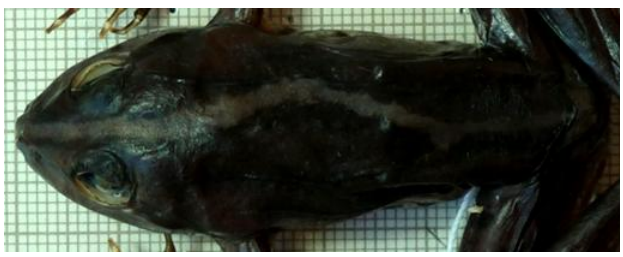


Figure 16 Dorsal view of SL 539.

Clade C – proposed working name: *Amietia fuscigula* (Duméril and Bibron, 1841)

Specimens assigned to the clade:

Confirmed by genetic data: from Longmore (1♀ AC 2671, 1 juvenile AC 2680), Garcias Pass (1 juvenile AC 2661), Bloukrans River (1 juvenile AC 2664), Witelsbos (1♂ AC 2666), Stellenbosch (1♂ AC 2687), Northern Cederberg (1♂ AC 3164), Bain's Kloof (1 metamorph AC 3167), Swartberg Pass (1 tadpole AC 3181).
Not genetically analysed: from Longmore (2♂ AC 2672, AC 2673), Stellenbosch (1♂ AC 2686), Bloukrans Pass (1♀ AC 2707), Bloukrans River (1♀ AC 2709 2♂ AC 2708, AC 2710), near Storms River (1♀ AC 2714), Tradouws Pass (1♀ AC 2725), Northern Cederberg (1♀ AC 3162, 1♂ AC 3163), Langeberg Mountains (2 juveniles SAM 45300, SAM 45305), Varkens Vlei, Ottery (2♀ SAM 46264–5), Muiskraal (1♀ SAM 50224), West Hangklip (1♀ SAM 50256), Swamp near Ritfonteinspruit (1♀ SAM 50338), Tulbagh (1♀ SAM 50375).

Diagnostic features:

- Coarse vermiculations on the throat among Southern African clades are exclusive from adults from this clade.

Notes:

- head broad and rounded.
- clade with higher frequency of specimens with plain dorsum.
- dorsal blotches, when present, often not very contrasting with background, differing from clade D, which usually had conspicuous blotches.
- differs from clade D on the coarse vermiculations on the throat (speckled in clade D), and on the smaller size of the last.

Clade D – proposed working name: *Amietia quecketti* (Boulénger, 1895)

Specimens assigned to the clade:

Confirmed by genetic data: from Naukluft (1♂ AC 2652), Riet River (1 juvenile AC 2833), Langfontein (1♂ AC 3136), Dargle (1♂ AC 3156), Rhodes (5 tadpoles AC 2764) Molteno Pass (6 tadpoles AC 2822), Little Switzerland (1 tadpoles AC 3034).
Not genetically analysed: from Anns Villa (1♂ AC 2742), Rhodes and surroundings (1♀ AC 2765, 1 juvenile AC 2762), Molteno Pass (1 juvenile AC 2829), Steinkopf–Vioolsdrift (1♀ SAM 46422), Naukluft (1♂ SAM 44658), Beaufort West (1♀ SAM 44861, 1 juvenile SAM 47405), Jakkalsdans, south of Loxton (2 juveniles SAM 45294–5).

Diagnostic features:

- A conspicuous speckled coloration pattern on the throat makes adults of this clade easily recognized. This pattern might be confused with the speckled coloration pattern sometimes present in clade B, but on the last it is usually diffuse, while on the first it is usually conspicuous.

- The throat darkening on the breeding males' throat as a diffuse pattern only on the throat edges is exclusive from this clade.

Notes:

- adults distinguishable from clade C by their speckled throat (throat has coarse vermiculations in clade C).
- shorter body size than clade C (see diagnosis of clade C)
- generally the dorsal blotches are evidently contrasting with background.

Clade E – proposed working name: *Amietia tenuoplicata* (Pickersgill, 2007)

Specimens assigned to the clade:

Confirmed by genetic data: from East Usambaras (1♀ AC 1896), West Usambaras (1♀ AC 2187), Luisenga (1♀ AC 1953), Tegetero, on the Ulugurus (1 juvenile AC 2054), Kitulo (1♀ AC 2059), Dabaga (1♀ AC 2151).
Not genetically analysed: from Mbeya (2 juveniles AC 1787, AC 2522), Dabaga (1♀ AC 1923), West Usambara Mountains (1 juvenile AC 2169), West Usambara Mountains (1♀ PEM 5279; 3♂ PEM 5280–81, PEM 5289), Uluguru Mountains (1♀ PEM 5277).

Considerable morphological variation was found on this clade. AC 2059 and AC 1787, from the same region, were both similar, but different from other specimens from this clade – both having a vertebral stripe, very large blotches and evident dorsal ridges. Genetic distances between AC 2059 and other members of the clade, revealed it was on the boundary between belonging to the clade or not (Channing *pers. comm.*), being high enough for being a considered as belonging to the same clade, but small enough for being hypothetically considered a beginning of divergence (Channing *pers. comm.*). Because their identity is uncertain even considering genetic results, their data (morphometrics and qualitative data) were not included in the analysis concerning the general descriptions of clade E. The aim of their mention is to document the possible variation that can be found. AC 2151, with a lacy throat, also looked different from most of the other specimens assigned to this clade.

Diagnostic features:

- The smooth skin on the dorsum and sometimes on the flanks allows to distinguish this clade from clades A, C, D, F and G.

Notes:

- Distinct from clade G on flanks coloration pattern (generally a oblique line on E; and irregular conspicuous vermiculations on G), of posterior surface of thighs (generally

nearly plain on E and thin intricate vermiculations on G), on pale facial stripe upward projection (generally absent or a small wave on E and pointed on G), and on line between nostril and snout (usually present on E and absent on G), on shape of snout from ventral view (more protruding on G than on E).

- Distinct from clade A and F on the thighs' bars conspicuous outline (evident on these clades and generally absent or diffuse on E), on the conspicuity of the dorsal blotches (conspicuous on A and F and very faint in clade E), on their arrangement (on clades A and F there is never a vertebral row of blotches, a arrangement present in clade E), on the delineation of the dorsolateral ridges (always absent in A and F and frequently present in E), vertebral stripe (frequent in clades A and F and absent in clade E).

Clade F – proposed working name: *Amietia theileri* (Mocquard, 1906)

Specimens assigned to the clade:

Confirmed by genetic data: from Ann's Villa (1♀ AC 2737), Hogsback River (1♀ AC 2757), Rhodes surroundings (1♀ AC 2761), Maclear surroundings (1♀ AC 2780), road to Matatiele (1 metamorph AC 2784), Drakensberg Gardens (1♀ AC 2813), Sani Top (1♀ AC 3036), Dargle (1♂ AC 3155), Nyanga (3 tadpoles AC 3143, 2 tadpoles AC 3148), World's View stream (3 AC 3150), Mt Nyangani (3 tadpoles AC 3182).
Not genetically analysed: from Hogsback (1♂ AC 2754), between Franklin and Kobstad (1 juvenile AC 2806), Drakensberg Gardens (1 juvenile AC 2812), Sani Top (1 juvenile AC 3035), Tiffindel (1♀ AC 3085), Mount Nyangani (1 juvenile AC 3175).

Diagnostic features:

- two throat coloration patterns of adults exclusive from this clade: immaculate, or with thin marblings on the edges and paler on the centre;
- sometimes white very conspicuous pale spots on the flanks.

Notes:

- slender body shape, with a usually pointed head.
- dorsal ridges usually elongate
- dorsal blotches in general conspicuous, very contrasting with background;
- phalanges free of webbing on the outer fourth toe sometimes reaching 3, which only happens in specimens from clade B.

Clade G – proposed working name: *Amietia viridireticulata* (Pickersgill, 2007)

Specimens assigned to the clade:

Confirmed by genetic data: from Itimba (1♀ AC 1757), Iringa (1♀ AC 1828), Mumba (1♂ AC 2029), Lukwe (1♂ AC 2639).
Not genetically analysed: from Itimba (3♀ AC 1755, AC 1756, AC 1758), Iringa (1♂ AC 1753), Mumba (2♂ AC 2310, AC 2311).

Notes:

- very intricate vermiculations on posterior surface of thighs,
- slender body, with an elongated head.
- dorsal blotches sometimes organized in rows coinciding with the dorsolateral ridges.
- vertebral row of blotches frequently present.
- clade with more extensive webbing, never having more than 2 phalanges free on the outer fourth toe, and never having more phalanges free on the 5th toe.
- see diagnosis of clade E for distinction between clade G and E.
- most specimens lack a DST on the third finger (opposing to all seven clades, where most or all specimens have the feature)
- except for vertebral stripe, which was present on clade G, all features referred for distinguishing clade E from clades A and F are also useful for distinguishing clade G from clades A and F.

APPENDIX 4 – Proposed dichotomous key

Proposed key to the identification of adult specimens of clades of the genus *Amietia* included in the present work

Note: the construction of this key was based on a small number of specimens, and has not been tested with a specimen not used for its construction. Therefore, its utility might be limited.

If the individual was collected in Southern Africa (Angola, Leshoto, Namibia, South Africa or Zimbabwe) or in an unknown locality, start the key on step 1. If the individual was collected in East Africa, start the key on step 5.

1a) Head width–tibia length ratio 62–1%……..2

1b) Head width–tibia length ratio 49–64%, exceptionally reaching 73% in large emales……..…3

2a) Throat heavily marked: with coarse vermiculations……**Clade C**

2b) Throat lightly marked: speckled, or, in breeding males, sometimes with small thin marblings, and a diffuse dark marking on the edges.....**Clade D**

3a) Throat lacy. Thigh bars always with a conspicuous pale outline. Frontoparietal blotch sometimes present……..**Clade A**

3b) Throat not lacy, of, if lacy, thigh bars without an outline……..4

4a) At least two of the following characters:

- dorsolateral ridges continuous maximum up to ½ of the body;
- throat immaculate, or with thin vermiculations in the whole throat or more evident in the edges, or diffuse with no evident pattern in breeding males;
- thigh bars with a conspicuous pale outline;
- flanks with conspicuous white spots;

……**Clade F**

4b) Only one or none of the characters referred in 4a)……5

5a) Absence of all the following features:

- 2 or more phalanges free on the outer fourth toe, one or more phalanges free on the 5th toe
- delimitation of dorsolateral ridges
- no dorsal blotches except for vertebral row, neither blotches forming an symmetrical pattern
- flanks with a conspicuous oblique vermiculation from groin to top of the forelimb

……**Clade G**

5b) Presence of any of the characters referred in 5a)……6

6a) Vertebral stripe absent, dorsal blotches pattern never asymmetric and blotches never with a diffuse outline, pale facial stripe never with a pointed projection between eye and tympanum, throat never thin marbled, never marbled more evident on the edges than on the middle, and never diffuse in the whole throat……**Clade E**

6b) presence of any of the characters referred in 6a)……**Clade B**

APPENDIX 5 – Body ratios

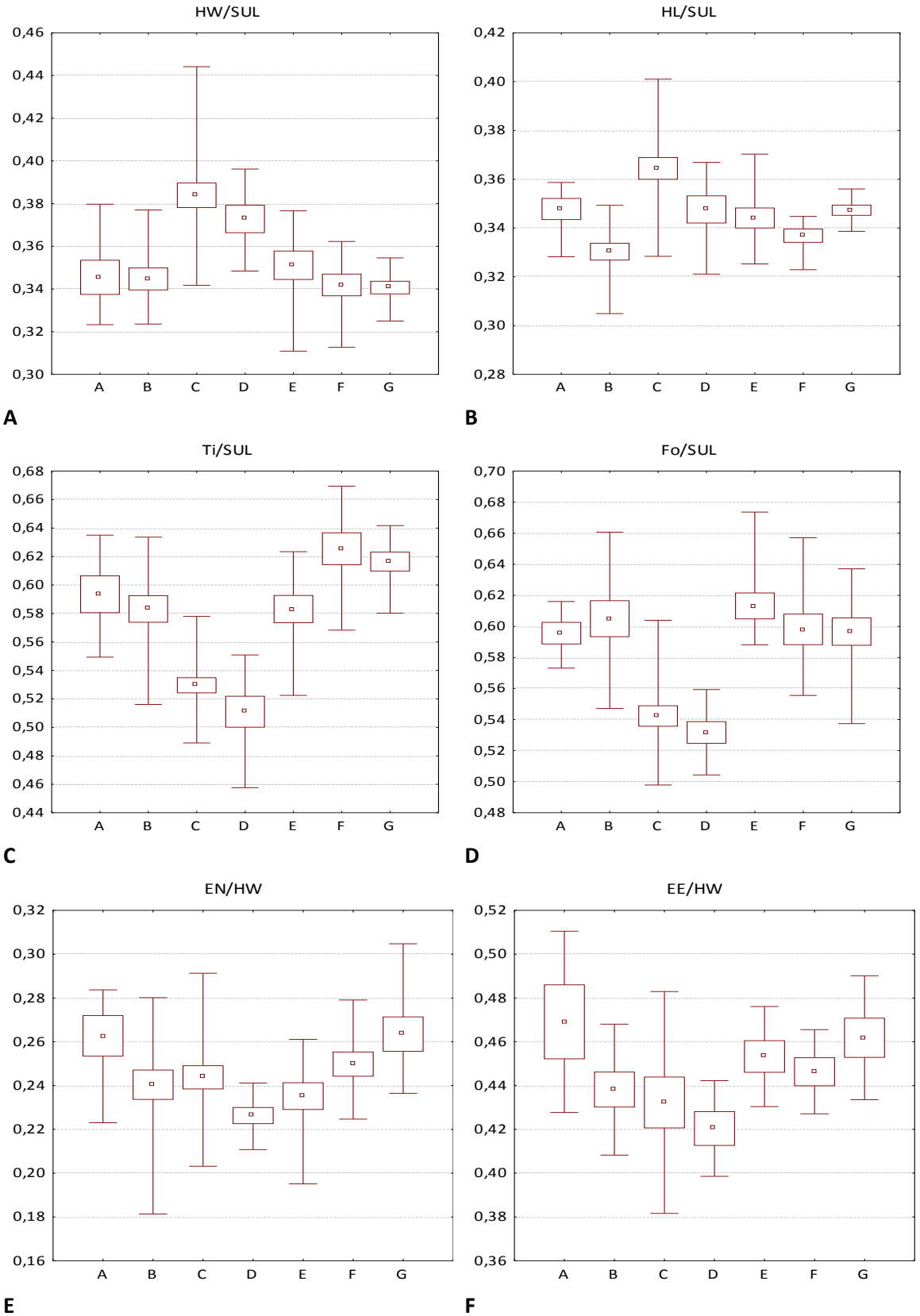


Figure 17 Box and whisker plots (central square = mean, boxes = mean ± standard error, whiskers = maximum and minimum) showing morphological variation among clades. **A**: head width/SUL; **B**: head length/SUL; **C**: tibia length/SUL; **D**: foot length/SUL; **E**: eye-nostril distance/head width; **F**: distance between anterior corner of the eyes/head width.

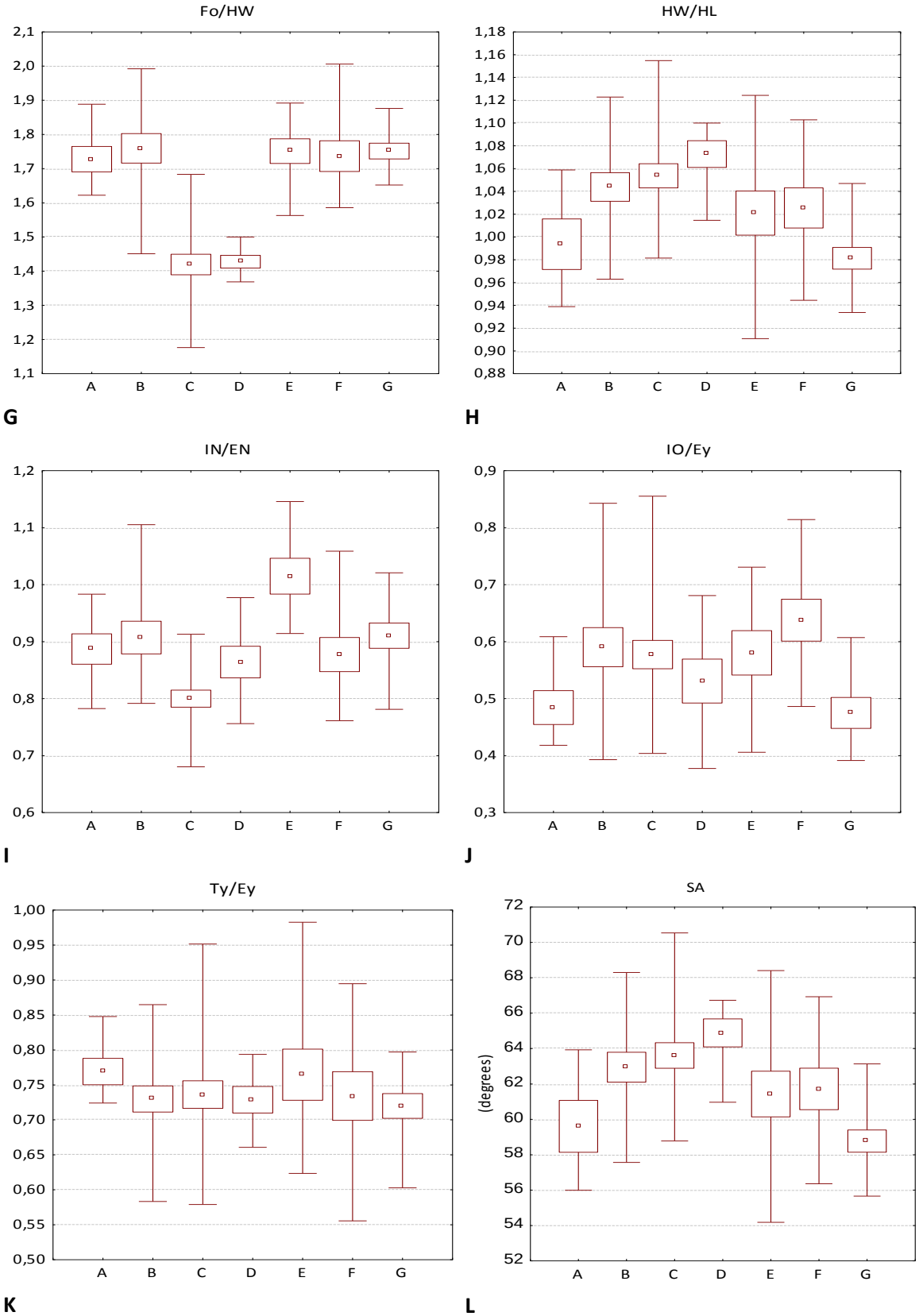
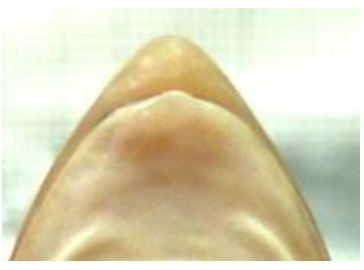


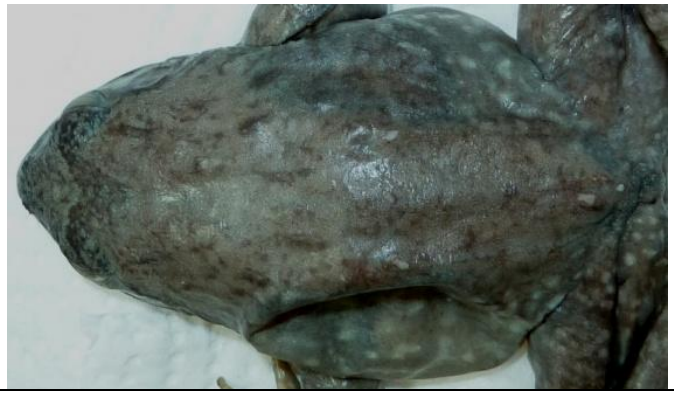
Figure 18 Box and whisker plots (central square = mean, boxes = mean ± standard error, whiskers = maximum and minimum) showing morphological variation among clades. **G**: foot length/head width; **H**: head width/head length; **I**: intra-nostril distance/ eye-nostril distance; **J**: interorbital distance/upper eyelid; **K**: tympanum/upper eyelid; **L**: snout angle.

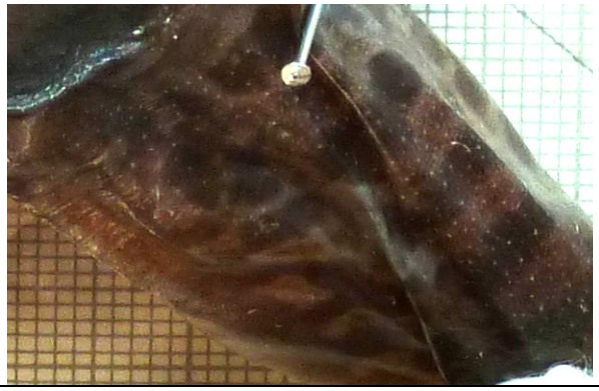
APPENDIX 6 – Representation of characters' states

Note: the representation on the states is indicative, not exhaustive.

AO: vertebral stripe absent **B1** dorsolateral ridges delineation present **D0**: faint interorbital blotch **G0**: outline of thighs bars absent 	**A1**: vertebral stripe present **B0**: dorsolateral ridges delineation absent **D1**: evident interorbital blotch **G1**: outline of thighs bars present 

R: Snout shape from ventral view:		
R0: not protruding 	**R1:** protruding 	**R2:** very protruding 

C: Dorsal blotches pattern:
C1: several blotches more or less organised in a relatively symmetrical pattern  
C2: blotches confusingly scattered, usually with a diffuse pale outline 
C3: plain dorsum 

: Posterior surface of thighs	
E0: nearly plain 	**E1**: lacy 
E2: thin vermiculations 	**E3**: coarse vermiculations 

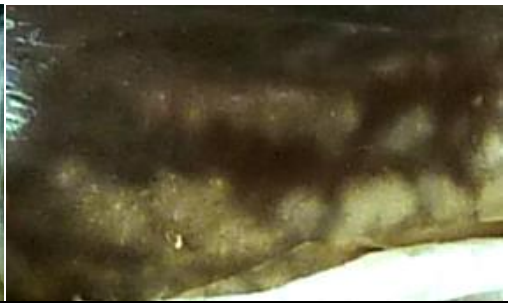
H: Blotch on frontoparietal region	
H0: absent. 	**H1**: present. 

I: Upward projection of pale facial stripe between eye and tympanum		
I0: absent 	**I1**: slight wave; 	**I2:** pointed, sometimes contacting the eye. 

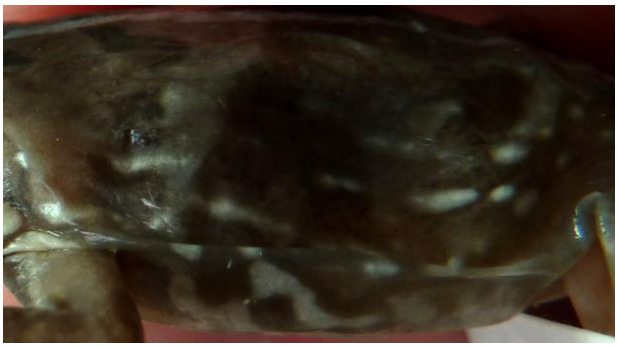
F: Flanks coloration pattern

F0: irregular vermiculations (speckled or elongated, coarse or thin, conspicuous or diffuse);



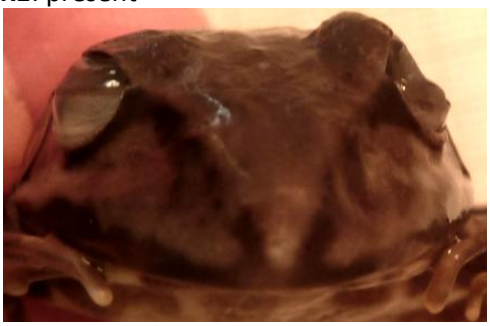

F1: apart from vermiculations, very conspicuous small white dots scattered

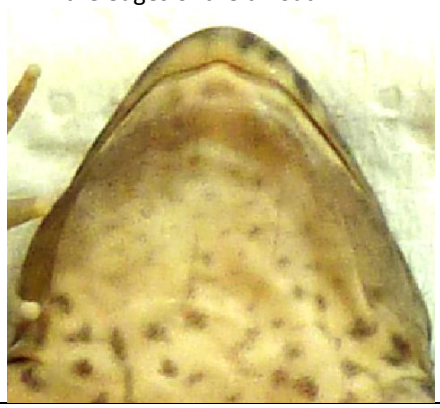


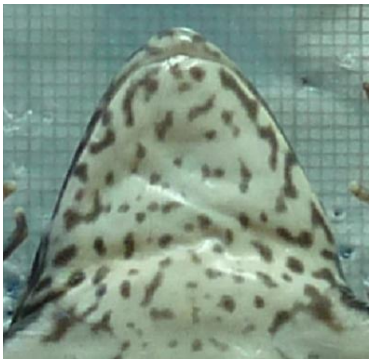
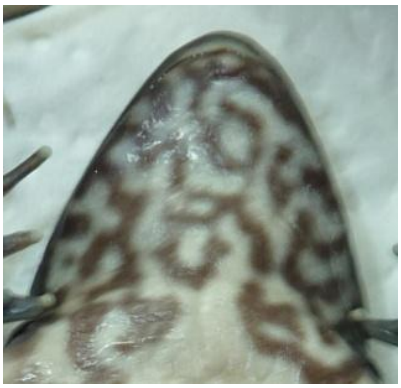
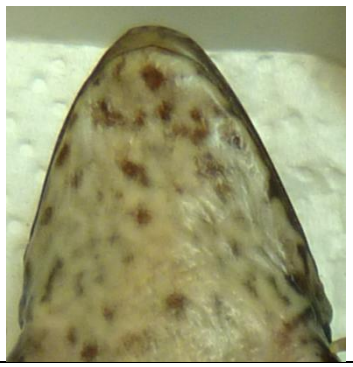
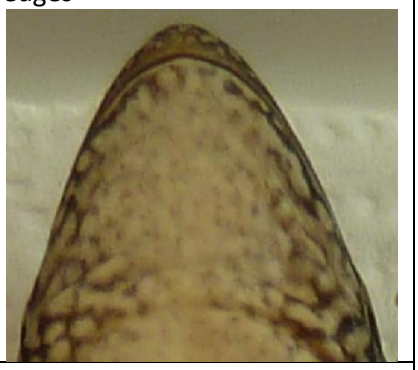
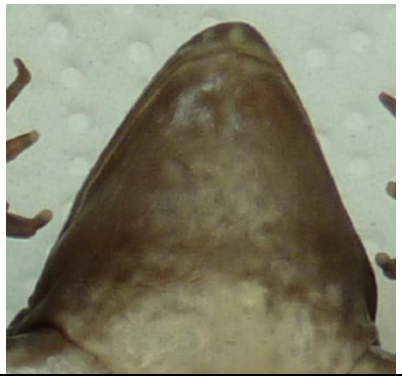
F2: almost no other vermiculations except a conspicuous line with or without upwards and/or downwards projections

F3: lacy.

J Dark line along upper lip:	
J0: straight and plain. 	**J1:** with pale spots and/or upward projections.   

K: Line between nostril and tip of snout:	
K0: absent 	**K1**: present 

L Diffuse dark marking on the breeding males' throats:		
L0 absent 	**L1** in the edges of the throat 	**L2** throughout the throat 

M Adults throat coloration pattern:		
M0: immaculaye 	**M1**: speckled 	**M2:** thin marbled 
M3: coarse marbled 	**M4:** thin reticulate 	**M5:** coarse reticulate 
M6: lacy 	**M7:** diffuse speckled/marbled 	**M8:** marbled more evident on edges 
M9: diffuse in the whole throat 		

APPENDIX 7 – List of specimens

Table 15 Specimens assigned to each clade, their collecting locality and coordinates in decimal degrees. S/A: sex or age class (T: tadpoles, m: metamorphs). M: specimens with known genetical identity. Lat: latitude, Long: longitude.

	Clade	Specimen	S/A	Country	Collecting locality	Lat	Long
M	A	AC 3101	♀	Angola	Zootechnica High Plateau	-14.92	13.26
M	A	AC 3016	♂	Angola	Calandula	-9.08	15.80
M	A	AC3108	T	Angola	Estação Zootechnica	-14.97	13.34
M	A	AC3078	T	Angola	Estação Zootechnica	-14.97	13.34
	A	AC 3094	♀	Angola	Zootechnica	-14.97	13.34
	A	PEM 9136	♀	Angola	Humpata	-14.92	13.26
	A	AC 3100	♂	Angola	Zootechnica High Plateau	-14.92	13.26
	A	AC 3120	♂	Angola	Humpata	-14.98	13.43
	A	AC 3102	J	Angola	Zootechnica High Plateau	-14.92	13.26
	A	AC 3121	J	Angola	Humpata	-14.97	13.34
	A	PEM 9137	J	Angola	between Humpata and Namibe	-15.04	13.20
	A	PEM 9138	J	Angola	Humpata	-15.00	13.33
	A	PEM 9139	J	Angola	between Humpata and Namibe	-15.04	13.20
	A	PEM 9140	J	Angola	Estação Zootechnica	-14.92	13.26
	A	PEM 9141	J	Angola	Leba Pass	-15.04	13.20
	A	PEM 9142	J	Angola	Leba Pass	-15.07	13.23
	A	PEM 9143	J	Angola	Zootechnica High Plateau	-14.92	13.26
	A	PEM 9158	J	Angola	Humpata	-14.92	13.26
	A	AC 3099	m	Angola	Zootechnica High Plateau	-14.91	13.30
M	B	AC 1829	♀	Tanzania	Western Kilimanjaro	-3.16	37.08
M	B	NMK A/4364/3	♀	Tanzania	Elgon National Park Western Kilimanjaro	1.03	34.78
M	B	NMK A/4706/2	♀	Kenya	Kakamega	0.27	34.88
M	B	SL 459	♀	Uganda	Ruwezori National Park, 2500 m	0.22	29.97
M	B	SL 539	♀	Uganda	Semliki National Park FR	-0.82	30.16
M	B	AC 2006	♂	Tanzania	West Kilimanjaro; Mt Meru, 2164 m	-3.25	36.81
M	B	NMK A/4364/2	♂	Tanzania	Elgon National Park Western Kilimanjaro	1.03	34.78
M	B	UTA A 58426	♂	Rwanda	Rugezi Swamp, Northern Province	-1.42	29.81
	B	AC 1789	♀	Tanzania	Chimala River	-8.85	34.02
	B	AC 1830	♀	Tanzania	Western Kilimanjaro	-3.16	37.08
	B	AC 1837	♀	Tanzania	Western Kilimanjaro	-3.16	37.08
	B	NMK A/4329/1	♀	Kenya	Wundanyi, Taita Hills	-3.40	38.37
	B	NMK A/4727/3	♀	Kenya	Salient Aberdares	-0.41	36.72
	B	AC 1839	♂	Tanzania	Western Kilimanjaro	-3.16	37.08
	B	AC 1838	J	Tanzania	Western Kilimanjaro	-3.16	37.08
M	C	AC 2671	♀	South Africa	Longmore FR	-33.85	25.19
M	C	AC 2666	♂	South Africa	Witelsbos	-34.00	24.10
M	C	AC 2687	♂	South Africa	Stellenbosch	-33.93	18.85
M	C	AC 3164	♂	South Africa	Northern Cederberg	-32.07	19.08
M	C	AC 2661	J	South Africa	Garcias Pass	-33.96	21.23
M	C	AC 2664	J	South Africa	Bloukrans River	-33.96	23.64
M	C	AC 2680	J	South Africa	Longmore FR	-33.85	25.19
M	C	AC 3167	m	South Africa	Bain's Kloof	-33.50	19.10
M	C	AC3181	T	South Africa	Swartberg Pass	-33.36	22.05
	C	AC 2707	♀	South Africa	Bloukrans River	-33.96	23.64
	C	AC 2709	♀	South Africa	Bloukrans River	-33.96	23.64
	C	AC 2714	♀	South Africa	Storms River	-33.97	23.88
	C	AC 2725	♀	South Africa	Tradouws Pass, 290 m	-33.97	20.70
	C	AC 3162	♀	South Africa	Northern Cederberg	-32.07	19.08

Table 15 *(continued)*

	Clade	Specimen	S/A	Country	Collecting locality	Lat	Long
	C	SAM 46264	♀	South Africa	Varkens Vlei, Ottery	-34.14	18.39
	C	SAM 46265	♀	South Africa	Varkens Vlei, Ottery	-34.14	18.39
	C	SAM 50224	♀	South Africa	Muiskraal, 400m	-33.89	21.11
	C	SAM 50256	♀	South Africa	Western Hangklip	-34.36	18.87
	C	SAM 50338	♀	South Africa	Near Ritfonteinspruit	-32.90	21.89
	C	SAM 50375	♀	South Africa	Tulbagh, 260m	-33.47	19.20
	C	AC 2672	♂	South Africa	Longmore FR	-33.85	25.19
	C	AC 2673	♂	South Africa	Longmore FR	-33.85	25.19
	C	AC 2686	♂	South Africa	Stellenbosch	-33.93	18.85
	C	AC 2708	♂	South Africa	Bloukrans River	-33.96	23.64
	C	AC 2710	♂	South Africa	Bloukrans River	-33.96	23.64
	C	AC 3163	♂	South Africa	Northern Cederberg	-32.07	19.08
	C	SAM 45300	J	South Africa	Langeberg Mountains	-33.66	19.89
	C	SAM 45305	J	South Africa	Langeberg Mountains	-33.66	19.89
M	D	AC 2652	♂	Namibia	Naukluft	-24.27	16.24
M	D	AC 3136	♂	South Africa	Langfontein, 965m	-32.20	24.16
M	D	AC 3156	♂	South Africa	Dargle	-29.54	29.97
M	D	AC 2833	J	South Africa	Riet River, 1519 m	-30.27	29.15
M	D	AC3034	T	South Africa	Little Switzerland	-28.57	29.07
M	D	AC2764	T	South Africa	Rhodes	-30.67	27.92
M	D	AC2822	T	South Africa	Molteno Pass, 1314 m	-32.21	22.56
	D	AC 2765	♀	South Africa	Rhodes	-30.80	27.97
	D	SAM 46422	♀	South Africa	Steinkopf–Vioolsdrift 610km N. Cape Town	-29.15	17.64
	D	SAM 44861	♀	South Africa	Beaufort West	-31.91	22.39
	D	AC 2742	♂	South Africa	Anns Villa	-33.25	25.77
	D	SAM 44658	♂	Namibia	Naukluft	-24.27	16.24
	D	AC 2762	J	South Africa	Rhodes	-30.80	27.97
	D	AC 2829	J	South Africa	Molteno Pass	-32.21	22.56
	D	SAM 45294	J	South Africa	Jakkalsdans	-31.65	22.39
	D	SAM 45295	J	South Africa	Jakkalsdans	-31.65	22.39
	D	SAM 47405	J	South Africa	Beaufort West	-32.41	22.64
M	E	AC 1896	♀	Tanzania	East Usambaras	-5.85	38.63
M	E	AC 1953	♀	Tanzania	Luisenga	-8.61	35.34
M	E	AC 2059	♀	Tanzania	Kitulo	-10.55	35.60
M	E	AC 2151	♀	Tanzania	Dabaga	-8.07	35.90
M	E	AC 2187	♀	Tanzania	Mazumbai, West Usambara Mountains	-4.80	38.50
M	E	AC 2054	J	Tanzania	Uluguru Mountains	-6.93	–37.72
	E	AC 1923	♀	Tanzania	Dabaga	-8.07	35.90
	E	PEM 5277	♀	Tanzania	Uluguru Mountains	-7.17	37.67
	E	PEM 5279	♀	Tanzania	Uluguru Mountains	-4.67	38.32
	E	PEM 5280	♂	Tanzania	Amani, Usambara Mountains	-5.85	38.63
	E	PEM 5281	♂	Tanzania	Amani, Usambara Mountains	-5.85	38.63
	E	PEM 5289	♂	Tanzania	Lushoto, Usambara Mountains	-4.67	38.32
	E	AC 1787	J	Tanzania	Poroto Mountains, 2600 m	-8.85	34.02
	E	AC 2169	J	Tanzania	Mazumbai, West Usambaras	-4.80	38.50
	E	AC 2522	J	Tanzania	Mbeiya Peak	-8.90	33.45
M	F	AC 2737	♀	South Africa	Ann's Villa	-33.25	25.77
M	F	AC 2757	♀	South Africa	Hogsback River	-32.61	26.97
M	F	AC 2761	♀	South Africa	Rhodes	-30.80	27.97
M	F	AC 2780	♀	South Africa	5 km North Maclear, 1250 m	-31.03	28.30
M	F	AC 2813	♀	South Africa	Drakensberg Gardens	-29.75	29.39
M	F	AC 3036	♀	Leshoto	Sani Top	-29.57	28.65

Table 15 *(continued)*

	Clade	Specimen	S/A	Country	Collecting locality	Lat	Long
M	F	AC 3155	♂	South Africa	Dargle	-29.54	29.97
M	F	AC 2784	m	South Africa	Road to Matatiele	-30.88	21.52
M	F	AC 3148	T	Zimbabwe	Nyanga	-18.20	32.63
M	F	AC 3143	T	Zimbabwe	Nyanga	-18.19	32.65
M	F	AC 3150	T	Zimbabwe	World's View stream	-18.15	32.78
M	F	AC 3182	T	Zimbabwe	Mount Nyangani	-17.23	30.87
	F	AC 3085	♀	South Africa	Tiffindel	-30.65	27.93
	F	AC 2754	♂	South Africa	Hogsback River	-32.61	26.97
	F	AC 2806	J	South Africa	Franklin to Kobstad	-30.61	29.56
	F	AC 2812	J	South Africa	Drakensberg Gardens road	-29.75	29.39
	F	AC 3035	J	Leshoto	Sani Top	-29.57	28.65
	F	AC 3175	J	Zimbabwe	Mount Nyangani	-17.23	30.87
M	G	AC 1757	♀	Tanzania	Itimba	-8.88	33.31
M	G	AC 1828	♀	Tanzania	Iringa	-8.14	35.40
M	G	AC 2029	♂	Tanzania	Mumba	-8.18	31.86
M	G	AC 2639	♂	Malawi	Lukwe	-10.59	34.13
	G	AC 1755	♀	Tanzania	Itimba	-8.88	33.31
	G	AC 1756	♀	Tanzania	Itimba	-8.88	33.31
	G	AC 1758	♀	Tanzania	Itimba	-8.88	33.31
	G	AC 1753	♂	Tanzania	Iringa	-7.80	35.76
	G	AC 2310	♂	Tanzania	Mumba, 1900m	-8.18	31.86
	G	AC 2311	♂	Tanzania	Mumba, 1900m	-8.18	31.86

APPENDIX 8 – Comparative tables

Table 16 Summary of results for morphological features. Terminology: generally: frequency over 75%, frequently: over 50% about half or more than half of the times, sometimes: less than half of the times; rarely: less than 25% of the times. Specimens from clade E with dubious identification were not included.

	Clade A	Clade B	Clade C	Clade D	Clade E	Clade F	Clade G
DL ridges **Shape**	Generally straight, rarely wavy. Sometimes thicker than other ridges.	Generally straight	Wavy immediately behind the eyes, after that sometimes straight.	Frequently wavy immediately behind the eyes, sometimes straight.	Straight.	Generally wavy, straight in only one specimen.	Generally straight. Sometimes thicker than other ridges.
Continuity	Continuous	Continuous or interrupted.	Interrupted	Interrupted	Continuous	Interrupted	Continuous
Length	A least up to ⅔ of body, generally up to groin.	Up to between scapular level and ⅔ of body.	Generally continuous up to scapular level, then interrupted or in stairways up to ½ of body, sometimes reaching groin.	Generally continuous up to scapular level, then interrupted until ⅓–⅔ of body or until groin.	Reach between ½ of body and groin.	Generally continuous up to scapular level or ½ of body, then usually in "stairway" with dorsal ridges.	Reach ½–⅔ of body.
Orientation	Parallel or slightly tapering.	Parallel or slightly hourglass shaped.	Parallel or slightly tapering.	Parallel or slightly tapering.	Parallel or slightly tapering or slightly hourglass shaped.	Parallel.	Parallel.
Dorsal ridges	Sometimes present. Straight, short, one or two longitudinal rows, sometimes placed along the edges of the vertebral stripe.	Sometimes present. Straight, short, not clearly organized in longitudinal rows.	Present. Short, not organized, sometimes oblique or transversal (in larger specimens).	Present. Short, interrupted, more or less organized in rows.	Generally absent.	Present, wavy, continuous or interrupted. Generally in two rows delineating vertebral stripe.	Sometimes present. Organized in rows or not. Interrupted, short, straight or wavy.
Lateral ridges	Generally present. Interrupted and/or continuous. Frequently continuous in breeding males.	Generally present. Short.	Generally present. Interrupted and/or continuous.	Present. Interrupted and/or continuous.	Sometimes absent. Interrupted and/or continuous.	Present. Interrupted and/or continuous.	Present. Interrupted and/or continuous.

Table 16 *(continued)*

	Clade A	Clade B	Clade C	Clade D	Clade E	Clade F	Clade G
Shape of snout from ventral view	Generally protruding, rarely not or very protruding.	Generally not protruding, rarely protruding.	Generally not protruding, rarely protruding.	Generally not protruding, rarely protruding.	Protruding and not protruding in similar frequencies.	Protruding and very protruding in similar frequencies, rarely not protruding.	Protruding.
DST on third finger	Generally present.	Present.	Present.	Present.	Generally present.	Generally present.	Generally absent.
More common foot webbing formulae	**I** 1–2; 2 **II** 1; 2 **III** 1; 2–2½ **IV** 2; 0–1 **V**	**I** 1; 1 ½ –2 **II** 0–1; 2 **III** ½–1; 2 **IV** 1–2; 0 **V**	**I** 1; 2 **II** 0–1; 2 **III** 0–1; 2 **IV** 1–2; 0 **V**	**I** ½–1; 1½–2 **II** 0–1; 2 **III** 0–1; 2 **IV** 1–2; 0 **V**	**I** 1; 2 **II** 0–1; 2 **III** 1; 2 **IV** 1–2; 0–1 **V**	**I** 1; 2 **II** ½–1; 2–2½ **III** ½–1½; 2–2½ **IV** 1–2 ½; 1 **V**	**I** ½–1; 1 ½–2 **II** 0–1; 2 **III** 0–1; 2 **IV** 1–1 ½; 0 **V**
Complete variation of foot webbing formulae	**I** 1–2; 2 **II** 1; 2 **III** 0–1; 2–2 ½ **IV** 2; 0–1 **V**	**I** 0–1; 1–2 **II** 0–1; 2 **III** 0–1 ½; 2–3 **IV** 1–2; 0–1 **V**	**I** 0–1 ½; 2 **II** 0–1; 2 **III** 0–1; 1–2 **IV** 1–2; 0–1 **V**	**I** ½–1; 1½–2 **II** 0–1; 2 **III** 0–1; 2 **IV** 1–2; 0–1 **V**	**I** 0–1; 1–2 **II** 0–1; 2 **III** 0–1; 2 **IV** 1–2; 0–1 **V**	**I** 0–1; 2 **II** ½–1; 2–2 ½ **III** ½–2; 2–3 **IV** 1–2 ½; 0–1 **V**	**I** ½–1; 1½–2 **II** 0–1; 2 **III** 0–1; 2 **IV** 1–1 ½; 0 **V**
Depth of webbing notches	**I** (1½–2) **II** (1–1½) **III** (1½–2) **IV** (3) **V**	**I** (2¯–2) **II** (1–1½) **III** (1½– 2¯) **IV** (2½–3½) **V**	**I** (2) **II** (1–1½) **III** (1½–2) **IV** (3) **V**	**I** (2) **II** (1–2) **III** 1–2 **IV** 2–3 **V**	**I** (2) **II** (1–1,5) **III** (1½–2) **IV** (3¯–3) **V**	**I** (2) **II** (1–⅗) **III** (1½–2) **IV** (3¯–3) **V**	**I** (2¯–2) **II** (1) **III** (1–1½⁺) **IV** (3¯) **V**
Vomerine odontophores shape and relative size	Oval or elongate ⅓ OS < DN < ½ OS ¼ OS < DO < ⅔ OS	Generally elongate 0 < DN < ½ OS ⅛ OS < DO < OS	Oval or elongate ⅕ OS < DN < ½ OS ⅙ < DO < ⅔ OS	Oval ½ OS < DN < OS ½ OS < DO < 1½ OS	Oval or elongate 0 < DN < ½ OS ¼ OS < DO < ¾ OS	Oval or elongate 0 < DN < OS ⅙ OS < DO < OS	Generally elongate 0 < DN < ⅕ OS ⅕ OS < DO < ⅓ OS
Min–max number of vomerine teeth per odontophore	0–7	2–11	2–6	2–4	3–10	2–9	3–8
Tadpoles labial tooth row formulae	3(2–3)/3(1) 4(2–4)/3(1) 4(2–4)/3(2) 5(2–5)/3(1)	–	4(2–4)/4(1)	3(2–3)/3(1) 4(2–4)/3(1) 4(1–4)/3(1)	–	3(2–3)/3(1) 4(2–4)/3(1)	–
Min–max SA (degrees) ♀	56,0–60,4	60,0–68,3	59,4–68,9	61,6–66,7	54,2–66,1	56,7–65,4	57,1–59,4
♂	57,1–63,9	57,6–63,2	58,8–70,5	61,0–66,1	57,9–68,4	56,4–66,9	57,7–63,1
Min–max SUL (mm) ♀	47,4–77,7	52,2–87	51,0–104,7	42,3–57,3	47,8–86,3	48,1–71,4	60,8–91
♂	46,2–66,5	49,5–56	42,3–61,6	44,6–56,8	55,4–58,1	53–54,2	69,1–57,9
HW/Ti average (min–max) ♀	0,61 (0,60–0,61)	0,60 (0,51–0,73)	0,75 (0,67–0,91)	0,74 (0,72–0,77)	0,61 (0,58–0,64)	0,55 (0,50–0,61)	0,56 (0,54–0,59)
♂	0,56 (0,52–0,59)	0,59 (0,52–0,62)	0,68 (0,62–0,75)	0,73 (0,68–0,76)	0,58 (0,54–0,64)	0,54 (0,52–0,56)	0,55 (0,53–0,57)
♀♂	0,58 (0,52–0,62)	0,59 (0,51–0,73)	0,73 (0,62–0,91)	0,73 (0,68–0,77)	0,60 (0,54–0,64)	0,55 (0,50–0,61)	0,55 (0,53–0,59)

Table 17 Summary of results for head coloration. Same terminology as in **Table I**.

	Clade A	Clade B	Clade C	Clade D	Clade E	Clade F	Clade G
Interorbital blotch	Evident.	Frequently faint.	Frequently evident.	Frequently evident.	Generally faint.	Generally evident.	Generally faint.
Frontoparietal blotch	Frequently present.	Generally absent. Present in one specimen.	Absent	Absent	Absent	Absent	Absent
Dark marking on the upper lip	Simple and straight or with small pale spots on the anterior part.	Generally a simple line on posterior part, with upward vermiculations or pale spots on anterior part. Rarely simple and straight line.	Frequently straight, sometimes with upward projections.	With upward projections, sometimes not forming a real uniform marking but a series of short upward projections.	Generally simple straight line, in the shape of a labial stripe. Rarely with upward projections or pale spots.	Always with upward projections and/or pale spots (reminding lacy pattern).	With upward projections and/or pale spots.
Marking between nostril and snout	Generally absent.	Frequently absent, sometimes diffuse in anterior part of snout.	Generally diffuse or incomplete.	Diffuse or incomplete.	Generally present, slightly diffuse and/or thicker than line between eye and nostril.	Frequently present; sometimes absent.	Generally absent, rarely diffuse or incomplete.
Upward projection of pale facial stripe between eye and tympanum	Generally pointed or touching the eye. Rarely slight wave.	Frequently pointed or touching the eye. Sometimes slight wave.	Slight wave and pointed or touching the eye in similar frequencies. Rarely absent.	Generally pointed or touching the eye. Rarely slight wave.	Generally absent. Rarely pointed or touching the eye or slight wave.	Generally pointed or touching the eye. Rarely slight wave or absent.	Slight wave and pointed or touching the eye in same proportion. Rarely absent.
Throat coloration pattern	Lacy in adults. Thin reticulate or thin marbled in juveniles. Present (reticulate or marbled) in all juveniles and metamorph.	Diffuse marbled/speckled; coarse marbled; coarse reticulate.	Coarse reticulate or coarse marbled. Immaculate in all juveniles.	Generally speckled. Very rarely thin marbled, only in breeding males. Immaculate or speckled in juveniles.	Frequently coarse reticulate, sometimes coarse marbled, thin reticulate, diffuse speckled/marbled. Immaculate in a juvenile.	On adult females: immaculate, or marbled on the edges and immaculate/ pale marbled on the centre. On breeding males: completely diffuse marking, darker on the edges, hiding a hard to see marbled pattern. Immaculate in all juveniles and metamorph.	Coarse marbled in every female, on males sometimes thin marbled or coarse marbled.

Table 18 Summary of results for body coloration features. Same terminology as in Table I.

	Clade A	Clade B	Clade C	Clade D	Clade E	Clade F	Clade G
Vertebral Stripe	Present and absent in similar frequencies.	Present in only one specimen.	Generally absent.	Generally absent.	Absent.	Absent in only one specimen.	Generally absent.
Dorsal coloration pattern	Symmetric. Blotches evident, scattered along dorsum. In juveniles, blotches fused with each other, forming big blotches.	Frequently asymmetric. Blotches sometimes with a diffuse pale outline. One plain specimen. Dark stripes delineating dorsolateral ridges in two specimens.	Symmetric when not plain. Occurrence of plain specimens. Blotches frequently not conspicuous.	Symmetric. Blotches frequently evident, scattered along dorsum.	Symmetric. Dark stripes delineating dorsolateral ridges frequently present. Blotches frequently faint, dorsum sometimes plain. Vertebral row of blotches sometimes present. Several small blotches irregularly scattered on the sacral region.	Symmetric. Blotches generally evident, scattered along dorsum. Blotches sometimes organized forming a circumference on the scapular area or mid–dorsum.	Symmetric, blotches frequently not conspicuous. Blotches sometimes organized in vertebral row and/or rows along the DL ridges. Several small blotches irregularly scattered on the sacral region.
Pale outline in thigh bars	Always present and evident.	Sometimes evident, frequently absent or diffuse.	Frequently absent or diffuse, sometimes evident.	Generally absent or diffuse, rarely evident.	Generally absent or diffuse.	Present and evident.	Generally absent or diffuse.
Chest and belly coloration pattern	Chest and belly sometimes lacy, reticulate or marbled in adults and juveniles.	Chest and lateral edges of belly sometimes with vermiculations.	Chest sometimes coarse marbled in adults. In large females, belly and sometimes ventral part of thighs coarse marbled.	Chest and lateral edges of belly sometimes speckled.	Chest and belly sometimes marbled, speckled, or reticulate.	Chest rarely marbled or speckled. Belly immaculate.	Chest and belly sometimes marbled or speckled.

(continued)

Table 18 *(continued)*

	Clade A	Clade B	Clade C	Clade D	Clade E	Clade F	Clade G
Dorsal posterior surface of thighs	Lacy / nearly plain / thin vermiculations/ coarse vermiculations.	Frequently thin vermiculations, sometimes nearly plain or coarse vermiculations	Frequently coarse vermiculations, sometimes nearly plain, lacy and thin vermiculations	Generally thin vermiculations, rarely coarse vermiculations or lacy	Generally nearly plain, rarely thin or coarse vermiculations	Generally thin or coarse vermiculations, rarely lacy	Thin vermiculations, very intricate.
Flanks	Frequently irregular vermiculations, sometimes lacy or coarse diffuse vermiculation.	Frequently irregular vermiculations, sometimes also coarse diffuse vermiculation, conspicuous oblique vermiculation, and vermiculations in a row.	Generally coarse diffuse vermiculation, rarely vermiculations in a row or conspicuous oblique vermiculation.	Frequently diffuse vermiculation, rarely irregular vermiculations, conspicuous oblique vermiculation, and vermiculations in a row.	Generally with an oblique dark conspicuous marking, from groin to the top of the insertion of the arm. Rarely with vermiculations in a row, irregular vermiculations or a coarse diffuse vermiculation.	Generally irregular vermiculations. Rarely coarse diffuse vermiculation, oblique conspicuous vermiculation, or vermiculations in a row.	Generally irregular vermiculations, rarely speckled.
Dorsal colour in life	Pale brown with dark brown blotches.	Brown with dark brown blotches.	Grey, pale brown, brown, pale green and olive background. Blotches dark green, brown, dark grey. Juveniles sometimes plain orange. Vertebral stripe cream–coloured, pale green, orange.	Olive or pale green with brown blotches.	–	In juveniles vertebral stripe bright to light green, basal colour beige or orange with brown blotches, sometimes red or orange stains. Adults green with dark blotches, bright green vertebral stripe.	–

APPENDIX 9 – Measurements

Table 19 Measurements spreadsheet. M depicts specimens with known molecular identity. * depict estimated measurements. Abbreviations follow Table 2.

Clade		Specimen		SVL	SUL	Fe	Fo	Ti	IMT	HW	HWN	HL	SL	NS	EN	IN	Ey	EE	IO	ET	Ty
A	M	**AC 3101**	♀	60 .1	58 .8	32 .3	33 .7	32 .3	3 .2	19 .8	10 .5	20 .7	10 .7	5 .9	5	4 .4	6 .1	8 .1	2 .7	1 .1	4 .5
A	M	**AC 3016**	♂	68 .1	66 .5	41 .6	40 .6	41 .2	3	21 .5	11 .7	22 .9	13	6 .6	6 .1	5 .6	7	11 .4	3	1 .9	5 .5
A		**AC 3094**	♀	48 .3	47 .4	28 .5	29 .2	30 .1	2 .2	18	9 .5	17	9 .7	5 .8	4 .9	4 .3	4 .6	8	2 .8	1 .4	3 .9
A		**PEM 9136**	♀	78 .8	77 .7	43 .4	45 .2	44 .2	3 .9	26 .9	14 .5	25 .5	13 .8	7 .8	6	5 .9	8	13	4 .2	1 .6	5 .9
A		**AC 3100**	♂	49 .1	47 .9	25 .5	28 .2	28 .2	2 .6	16	9 .4	16 .9	8 .2	4 .1	4 .2	3 .7	5 .5	7 .8	2 .3	1 .5	4 .3
A		**AC 3120**	♂	46 .1	46 .2	25 .9	27 .9	27 .7	2 .3	16 .3	9 .3	16 .2	7 .5	5 .5	4 .6	3 .6	5 .8	7 .5	2 .8	1	4 .2
B	M	**AC 1829**	♀	86 .4	85 .3	49 .5	50 .5	50 .4	4 .9	28 .3	15 .4	27 .1	14 .3	7 .2	7 .5	6 .6	7 .4	12 .8	3 .7	3 .4	6 .4
B	M	**NMK A/4364/3**	♀	76 .3	72 .8	39	42 .3	42 .1	3 .1	25 .1	12 .2	24	12 .8	6 .5	6	5 .5	7 .2	10 .7	4 .8	1 .6	5 .9
B	M	**NMK A/4706/2**	♀	79 .5	78 .6	47 .9	50 .8	49 .8	3 .3	25 .5	12 .5	25 .5	12 .6	6 .4	5 .9	5	7	10 .1	5 .9	2 .3	5 .3
B	M	**SL 459**	♀	59 .5	57 .4	32 .9	36 .6	34 .6	2 .8	19 .3	7 .5	17 .9	9 .5	5 .4	3 .5	5 .3	6	9 .2	4 .2	1 .5	3 .5
B	M	**SL 539**	♀	57 .8	54 .4	31 .1	32 .6	33 .1	2	19 .5	9 .7	18 .9	9 .5	5 .5	4 .8	4 .5	5 .9	8 .9	3 .3	1 .1	4 .1
B	M	**AC 2006**	♂	51 .4	50	30 .1	29 .7	29	2 .5	17 .4	10 .1	16 .6	8 .4	4 .8	4 .3	4 .1	5 .6	8	2 .2	1 .8	4 .1
B	M	**NMK A/4364/2**	♂	58 .3	56	33 .3	35 .3	34 .8	2 .9	18 .2	9 .1	18 .9	9	4 .7	4 .8	4 .2	5 .5	8 .6	4	1 .2	4 .3
B	M	**UTA A 58426**	♂	50 .6	50 .1	27 .3	33 .1	28 .5	1 .9	17 .8	8 .6	17 .5	8 .5	4 .9	3 .9	4	5 .4	7	2 .9	1 .8	4
B		**AC 1789**	♀	54 .9	55 .6	28 .7	30 .9	30 .3	2 .3	18 .5	9	18 .3	9	4 .6	4 .8	3 .8	5 .9	8 .4	3 .5	1 .7	4 .2
B		**AC 1830**	♀	74 .3	74 .1	40 .4	41 .2	41 .9	3 .4	25 .7	14	24 .2	12 .5	6 .6	7 .2	5 .9	6 .9	11 .4	4 .2	3 .3	4 .9
B		**AC 1837**	♀	53 .1	52 .2	30 .1	33 .8	31 .6	2 .4	19 .2	10 .8	17 .1	9 .5	5 .1	4 .4	4 .5	5 .4	8 .4	2 .7	2	3 .7
B		**NMK A/4329/1**	♀	91 .1	87	46 .6	47 .6	44 .9	4 .4	32 .8	14 .2	29 .7	15 .7	8 .4	7 .8	6 .4	9 .2	13 .2	5 .8	2 .3	7 .2
B		**NMK A/4727/3**	♀	85 .4	85 .6	51 .3	51 .9	51 .4	3 .7	27 .7	12 .9	26 .1	12 .8	6 .9	7	5 .8	9 .1	11	4 .6	1 .9	6 .1
B		**AC 1839**	♂	50 .6	49 .5	29 .3	31 .8	29 .3	2	17 .8	9 .7	17	8 .9	4 .9	3 .8	4 .2	5 .7	8 .3	3 .3	1 .8	3 .9
C	M	**AC 2671**	♀	66 .2	67 .3	35 .8	34 .7	34 .4	3 .7	23	12 .1	22 .1	12 .2	5 .6	6 .7	5	6 .2	10 .9	4	2 .8	4 .7
C	M	**AC 2666**	♂	58 .2	56 .2	30 .1	30 .6	29 .2	2 .9	20 .3	11 .7	19 .7	9 .5	5 .7	5 .6	4 .3	6 .7	10 .3	3 .7	1 .2	4 .2
C	M	**AC 2687**	♂	51 .5	49 .5	26 .6	25 .7	25 .6	2 .4	17 .1	9 .3	17 .1	7 .9	4 .1	4 .6	3 .5	5 .2	6 .7	2 .3	1 .9	4 .5
C	M	**AC 3164**	♂	45 .1	42 .3	22 .8	23 .3	22 .3	2 .1	15 .9	9 .4	16 .2	8 .3	4 .3	3 .8	3 .4	5 .2	7	2 .1	2	3 .8
C		**AC 2707**	♀	51 .9	51 .1	26 .5	28 .9	28 .3	2 .7	19 .4	10 .1	18 .1	9 .6	5	4 .6	4 .2	5 .7	9 .4	3 .2	2	3 .3
C		**AC 2709**	♀	85 .2	83 .6	43 .4	45 .2	44 .4	4 .3	31 .6	16 .4	31 .9	15 .5	8 .3	8 .1	6	8 .8	14 .3	6	2 .9	5 .9
C		**AC 2714**	♀	51 .8	51	26 .2	27 .1	26 .6	2 .5	19 .5	10	18 .1	10 .2	4 .9	4 .9	4 .3	4 .9	9 .3	3 .2	2 .1	3 .6
C		**AC 2725**	♀	89 .2	83 .8	43 .4	45 .2	47 .9	4 .4	33 .7	17 .7	31 .9	15 .5	8 .3	9	6 .4	7 .6	14 .6	6 .5	3 .5	5 .5
C		**AC 3162**	♀	73 .5	71 .3	36 .1	37 .2	37 .6	3 .6	28 .9	13 .7	26 .4	13 .2	7	6 .8	5 .6	6 .7	11 .5	3 .5	2 .8	5 .3
C		**SAM 46264**	♀	96	94 .4	51 .1	47	48 .1	4 .9	37 .8	21 .9	35 .4	16 .9	9 .4	8 .3	7	10	14 .4	6 .2	4	7 .4
C		**SAM 46265**	♀	95 .1	96 .9	57	49 .1	51 .9	4 .9	39 .9	22 .5	37 .1	18 .1	11 .3	8 .7	6 .3	10 .3	15 .2	4 .9	3 .2	9 .8
C		**SAM 50224**	♀	107 .6	104 .7	57 .8	54 .7	51 .2	5	46 .5	24 .1	41 .1	22 .1	12 .8	9 .7	6 .6	10 .8	14 .7	6 .3	4 .8	8 .1
C		**SAM 50256**	♀	69 .2	66 .9	35 .2	39 .5	36 .3	3 .5	25 .4	12 .8	24 .4	13 .1	6 .9	6 .1	4 .8	7 .2	11 .3	3 .8	2 .2	5

Table 19 *(continued)*

Clade		Specimen		SVL	SUL	Fe	Fo	Ti	IMT	HW	HWN	HL	SL	NS	EN	IN	Ey	EE	IO	ET	Ty
C		**SAM 50338**	♀	61 .6	61 .1	32	32 .1	30 .8	3 .5	25 .1	13	24 .5	11 .3	6 .6	5 .1	4 .5	6 .8	9 .3	3 .2	2 .6	5 .5
C		**SAM 50375**	♀	63	63 .7	32 .6	34 .5	32 .1	3 .3	24 .7	13 .4	22 .3	11 .4	6 .3	5 .7	4 .5	7	10 .4	3 .8	1 .9	4 .6
C		**AC 2686**	♂	60 .4	59 .9	33 .8	32	32 .2	3 .3	21 .7	11 .5	21 .2	10 .5	5 .6	5 .4	4 .3	6 .7	8 .9	3 .2	1 .4	5 .2
C		**AC 2708**	♂	63 .5	61 .6	35 .3	37 .2	35 .6	3 .2	22 .1	11 .6	21 .5	11 .8	5 .5	5 .7	4 .7	6 .4	11 .2	4 .6	2 .3	4 .3
C		**AC 2710**	♂	61 .6	60	33 .6	34 .9	33 .3	3 .2	22 .5	11 .6	22	11	5 .9	5 .7	4 .6	6 .5	10 .6	3 .9	1 .7	5 .1
C		**AC 3163**	♂	42 .3	45 .7	23 .7	26 .1	24	2 .1	17 .9	8 .8	15 .5	9 .3	4 .6	4 .1	3 .4	5 .2	8 .1	3 .3	1 .7	3 .5
D	M	**AC 2652**	♂	54 .3	53 .1	25 .1	27 .1	24 .3	2 .6	18 .5	10 .7	18	9 .1	5 .1	4 .4	4 .3	5 .8	7 .9	3 .2	1 .9	4 .4
D	M	**AC 3136**	♂	58 .7	56 .8	32 .1	31	30	3 .4	20 .8	10 .9	20 .5	10	5 .2	4 .6	3 .8	6 .3	9 .2	3	1 .6	5
D	M	**AC 3156**	♂	60 .6	56 .1	32	30 .6	29 .9	3 .2	20 .4	10 .9	18 .7	10 .1	5 .1	4 .3	4	5 .8	9 .3	3 .3	2	4 .2
D		**AC 2765**	♀	57 .5	57 .3	26 .9	28 .9	27 .8	2 .7	20 .2	11 .4	18 .4	9 .8	5 .8	4 .4	3 .7	5 .5	8 .1	3 .7	1 .4	4 .3
D		**SAM 44861**	♀	42 .7	43	18 .9*	22*	20 .7	2 .3	16	9 .1	14 .8*	7 .9*	4 .2	3 .7	3 .5	5 .3	6 .4	2	1 .3	3 .6
D		**SAM 46422**	♀	48 .2	47 .2	26 .8	26 .4	26	2 .5	18 .7	9 .4	17	9	4 .6	4 .3	3 .4	5 .1	7 .4	2 .5	1 .4	3 .4
D		**AC 2742**	♂	45	44 .6	21 .8	23 .5	23 .4	2 .5	17	9 .1	15 .8	8	4 .1	4 .1	3 .1	4 .7	7	3 .2	1 .3	3 .6
D		**SAM 44658**	♂	45 .2	45 .9	22 .3*	24 .4*	23 .2	2	17 .7	9 .6	16 .5*	8 .3*	4 .4	3 .9	3 .3	5 .6	7 .6	2 .4	1 .5	3 .7
E	M	**AC 1896**	♀	80 .5	75 .3	44 .8	46 .6	45 .3	3 .5	27 .1	13 .7	27 .1	14 .7	7	6 .9	6 .7	8 .5	12 .6	3 .8	3 .3	5 .3
E	M	**AC 1953**	♀	48 .8	47 .8	27 .8	32 .2	29 .8	2 .5	17 .3	9 .5	17 .7	8 .9	4 .9	4 .3	4 .9	5 .2	8	3 .8	1 .3	4 .2
E	M	**AC 2059**	♀	58 .7	58 .5	34	35 .5	34 .3	2 .9	18 .7	11 .2	18 .4	10 .1	5 .3	4 .3	4 .9	5 .9	8 .9	3 .1	2 .8	3 .4
E	M	**AC 2151**	♀	71 .5	68 .9	41	41 .9	38 .2	3 .1	24 .6	13 .3	23 .8	11 .4	6	4 .8	5 .5	6 .7	11 .3	4 .4	2 .8	5 .8
E	M	**AC 2187**	♀	90 .4	86 .3	48 .7	50 .8	50 .4	4 .3	32 .5	17 .3	29 .8	15 .8	8 .2	7 .4	–	8	13 .7	5 .3	3 .7	6 .9
E		**AC 1923**	♀	57 .4	62 .4	36 .1	36 .7	32 .6	2 .8	19 .4	10 .1	21 .3	10 .8	5 .8	4 .8	5 .3	6 .8	9 .3	3 .9	1 .7	4 .3
E		**PEM 5277**	♀	88 .9	84 .1	45 .3	49 .8	50 .5	3 .6	29 .9	15 .8	29 .1	14 .6	7 .7	7	6 .4	8 .3	13 .9	4 .4	3 .7	5 .6
E		**PEM 5279**	♀	65 .4	65 .1	34 .1	38 .8	36 .2	2 .3	22 .9	12 .9	22	11 .1	6 .3	5	4 .7	5 .8	9 .8	4	2 .1	5 .7
E		**PEM 5280**	♂	57	55 .4	31 .5	34 .7	32 .3	2 .4	20 .8	11 .2	18 .5	9 .9	5 .3	4 .9	4 .6	7 .2	8 .8	3	1 .9	5
E		**PEM 5281**	♂	58 .7	58 .1	33 .9	36 .8	35 .1	2 .3	19 .6	10 .4	18 .9	9 .9	4 .8	4 .5	4 .7	6 .9	9 .5	2 .8	2 .1	5 .1
E		**PEM 5289**	♂	55 .6	55 .4	32 .3	33 .6	33 .3	2 .6	18	10	18 .6	9 .5	4 .8	4 .7	4 .4	5 .5	8	3 .8	1 .4	4 .2
F	M	**AC 2737**	♀	72 .8	71 .4	41 .7	41 .5	43 .3	4	25 .1	13 .2	24 .6	13 .2	6 .8	6 .7	5 .1	7	11 .6	5 .7	2 .8	5 .6
F	M	**AC 2757**	♀	69 .3	66 .8	39 .1	40 .7	43 .3	3 .4	24 .2	12 .7	22 .9	11 .5	6	5 .7	4 .6	6 .9	10 .8	5 .4	2 .5	5 .1
F	M	**AC 2761**	♀	63 .2	61 .4	37 .9	37 .4	39 .4	3 .7	21 .2	11 .6	20 .8	11 .2	6 .2	5 .1	4 .5	5 .8	9 .5	3 .4	2 .6	4 .2
F	M	**AC 2780**	♀	49 .7	48 .1	27 .5	28 .8	32 .2	2 .4	16 .7	8 .9	16 .3	9 .5	4 .7	4 .1	3 .5	5 .4	7 .9	3 .3	2	3
F	M	**AC 2813**	♀	56 .7	52 .5	30 .9	34 .5	34 .6	2 .7	17 .2	9 .6	18 .1	9 .8	5 .3	4 .8	4 .2	5 .8	8 .1	3	1 .7	4 .7
F	M	**AC 3036**	♀	48 .4	49 .8	26 .8	28 .2	28 .3	2 .3	17 .2	8 .6	16 .7	9 .1	4 .9	4 .2	3 .8	4 .9	7 .2	3	1 .8	3 .7
F	M	**AC 3155**	♂	53 .6	53	28 .8	32 .3	32 .7	2 .5	17	8 .3	18	9 .3	5	4 .3	4 .1	5 .2	7 .4	3 .3	1 .9	3 .8
F		**AC 2812**	♀	42 .4	41 .9	24 .3	26 .7	26 .7	1 .9	13 .1	7 .1	13 .3	8 .2	4 .4	3 .8	3 .2	4 .7	6 .2	2 .7	1 .4	3
F		**AC 3085**	♀	66 .2	64 .8	35 .8	36	38 .1	3 .4	22 .7	12 .2	21	10 .7	6 .2	5 .1	5 .4	7 .2	9 .7	3 .5	2 .3	4 .3

Table 19 *(continued)*

Clade		Specimen		SVL	SUL	Fe	Fo	Ti	IMT	HW	HWN	HL	SL	NS	EN	IN	Ey	EE	IO	ET	Ty
F		**AC 2754**	♂	55	54 .2	33 .2	32 .3	34 .2	2 .8	19 .3	9 .6	17 .5	10 .6	5 .5	5	4	5 .7	8 .4	3 .9	1 .7	5 .1
G	M	**AC 1757**	♀	85 .7	84 .3	49 .4	51 .4	53 .3	4 .7	28 .8	14 .8	29 .2	14 .1	6 .7	7 .3	6 .7	8 .4	12 .6	5 .1	2 .7	6 .4
G	M	**AC 1828**	♀	77 .3	76 .4	41 .4	44 .5	44 .7	3 .8	26 .3	13 .6	27 .2	13 .7	7	6 .3	6 .1	7 .8	11 .7	3 .7	1 .8	5 .4
G	M	**AC 2029**	♂	59 .7	57 .9	34 .3	33 .8	36 .1	3	20 .2	10 .8	20 .5	10 .1	5	4 .8	4 .9	6 .9	8 .9	2 .7	1 .3	5
G	M	**AC 2639**	♂	70 .3	69 .1	40 .4	41 .3	42 .9	3 .6	24 .5	12 .4	23 .4	12 .7	6	6	5 .7	7 .3	10 .6	3 .1	1 .1	4 .9
G		**AC 1755**	♀	68 .3	67 .7	36	39 .1	40 .6	3	22	12 .2	23	12 .2	5 .9	6 .2	5 .5	6 .5	10 .3	3 .7	2 .5	5
G		**AC 1756**	♀	60 .6	60 .8	35 .8	37 .2	37 .9	2 .6	21	10 .7	21 .2	11 .8	6 .3	6 .4	5	7 .3	10 .4	3	1 .7	4 .4
G		**AC 1758**	♀	90 .8	91	51 .8	48 .9	52 .8	4 .8	29 .6	15 .4	31 .7	15 .6	8 .2	7	6 .7	8 .4	13	5 .1	2 .7	6 .2
G		**AC 1753**	♂	67 .3	64 .2	39 .9	40 .9	41 .2	3 .1	21 .8	12 .3	21 .8	12	6 .5	5 .8	5 .3	7 .3	10 .3	3	2 .3	5 .4
G		**AC 2310**	♂	65 .5	63 .5	34 .7	38 .7	39 .4	3 .1	21 .8	12	22 .6	11 .9	6 .2	6 .4	5 .3	6 .9	11 .3	2 .9	1 .7	5 .5
G		**AC 2311**	♂	62	62 .8	37 .6	38 .9	40	2 .8	21 .3	12	21 .7	12 .7	6 .5	5 .9	5 .2	6 .7	10	2 .9	2 .2	4 .7