

Morphometric Differentiation between Two Closely Related Achatinid Snails (Gastropoda: Achatinidae) of West Africa and Implications for the Conservation of *Achatina togoensis* (Bequaert & Clench, 1934)

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Abstract

Achatina togoensis is a giant land snail taxa endemic to the northern sectors of the Monts Togo, which has been regarded as full species or subspecies of the highly variable, widespread *A. achatina* (L.). To address this issue, samples of snails were taken throughout the distribution range of the two taxa in the Dahomey Gap (West Africa) and separated into 112 *A. achatina* and 141 *A. togoensis* according to color of the columella and parietal wall. Radulae were compared and shell characters of these 2 species were evaluated through the traditional morphometric method. The results indicate that the species cannot be distinguished by overall shell size and radular morphology, but they exhibit significant differences in shell shape and meristic traits. The typical *A. achatina* with vinaceous-red columella has a low-spined shell and an expanded aperture (globose shape) whereas *A. togoensis* has a higher-spined and smaller aperture (a fusiform shape). We suggest that local environmental effects are probably the causes of morphological divergence between the two taxa. It is likely that peripheral populations of the typical form of *A. achatina* would have isolated and developed in the drier facies of the humid forest that individualized in the northern sector of the mountains after the arid or interpluvial period of Holocene. In addition, we find that *A. togoensis* meets the criteria B2ab (ii, iii) to categorize as “Endangered”. However, whether this latter should be considered as separate species cannot be decided at the current state of knowledge.

Keywords

Shell Morphometry, Radulae, *Achatina achatina*, *Achatina togoensis*, West Africa

1. Introduction

Members of the family Achatinidae are medium to very large terrestrial gastropods, native to continental Africa and its small adjacent coastal islands. Several species are important agriculturally, both as pests and as food resources. The broad, high-spired shells are readily identified to family but they present particular classification problems occasioned by wide variations within species tending to obscure a rather ill-defined interspecific differentiation which results in the large number of species described in the past [1]. Since the taxonomy of the Achatinidae has evolved considerably with the work of Bequaert [2], Mead [3] [4] Crowley and Pain [5] and Van Bruggen [6] [7]. The composition of different subfamilies and genera has largely been revised through the addition of new species, the removal of invalid species and the creation of new species groups. The family is actually believed to be made up of about 13 genera and approximately 200 species [8]. However, several species are poorly defined and many have been divided into subspecies whose links are even more controversial [9] [10] [11].

A number of giant land snail species have been described from West Africa [12] [13]. They included two very similar species namely the giant African land snail (*Achatina achatina* L.) and the giant Togolese land snail (*Achatina togoensis*). However, the separateness between these two taxa has a confused history that originated with Von Martens [14] when he identified samples of the giant African land snail collected in Bismarckburg (actually Yegue: 8°17'N et 0°36'E) as being a morphological variant of *Achatina hamillei* (= *A. fulica* Bowdich, 1822). Later, when the specimens were sent to them by the Berlin Museum for confirmation, Bequaert and Clench [15] suspected a new species, for which they give the specific name *Achatina togoensis*. According to these authors, the new taxa is rather close to typical *A. achatina* but it is distinct from the nominal species by having a smaller shell with a glazed bluish-white parietal wall (Figure 1(A) and Figure 1(B)). In contrast, *A. achatina* is characterized by a large, bulky shell and a vinaceous-red columella and parietal wall (Figure 1(C)). Later, considering the high degree of morphological variation reported for *A. achatina*. Bequaert [2] re-examined the type material and suggested that the Togolese giant snail be given at most a subspecific recognition. This reversal of position was not approved by [16] who, on the contrary, claimed that he found sufficient anatomical features and tangible shell characters that support full species status for this taxon. However, he did not provide any details. Recently, supporting the opinion of Bequaert [2], Afiademanyo [17] reported that the basal genital structures of the two taxa are remarkably similar and opined that they would interbreed in nature

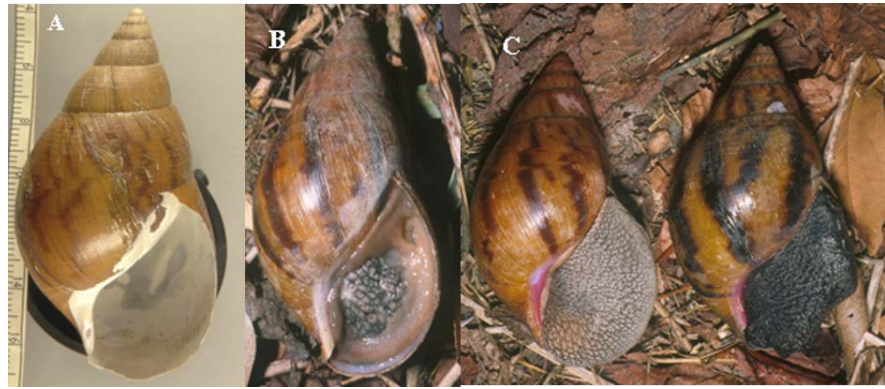


Figure 1. Representative specimens of *Achatina* spp. (A) Shell of *A. togoensis* holotype, ZMB, (B) Ventral view of *A. togoensis* and (C) Adults specimen of *A. achatina*.

if the opportunity arose. These contradictory views on the taxonomic status of *A. togoensis* deserved to be clarified using appropriate quantitative tools.

Morphometric techniques measure morphological similarities between organisms and capture variation of shape [18]. A number of previous studies showed that numerical quantification of shell characters may be a valuable tool for clustering and understanding the relationship between different shell forms, which may represent different taxa [19]. In many cases, analysis of the shell combined with studies of morphological traits of the radula has supported changes in the taxonomy of studied taxa [20] [21]. The radula has been used as an important instrument in the systematic studies of gastropods [22]. In Achatinidae and related taxa, taxonomic importance has been attached to differences in the structure of the radular teeth, especially to the shape and size of the mesocone cusps of rachidian and lateral tooth [23] [24] [25].

Improved knowledge of achatinid snails is desirable given their important role as a source of protein and revenue for the local communities and for their use in traditional medicine. In the present study, populations of *A. Achatina* and *A. togoensis* from Togo/Ghana were analyzed, using radular morphology, meristic traits, shell size and shape characteristics with traditional morphometrics. The ultimate objective is to determine whether any observed differences in radular and shell characteristics correspond to a polymorphism in *A. achatina* or rather confer a valid species identity to *A. togoensis*. As expected, proper taxonomic identification of giant African snails will assist in their management, including conservation and sustainable use of the resource.

2. Materials and Methods

2.1. Source of Data and Sample Collection

Fresh specimens of the two species were randomly collected by gatherers in different suitable habitats (woodland, agroforest and humid forest) covering most of the geographical distribution range of the snails in the Dahomey Gap (Figure 2). The collection method was qualitative visual searching, preferably after rain or very early in the morning. Prior to measurement, each shell was determined to

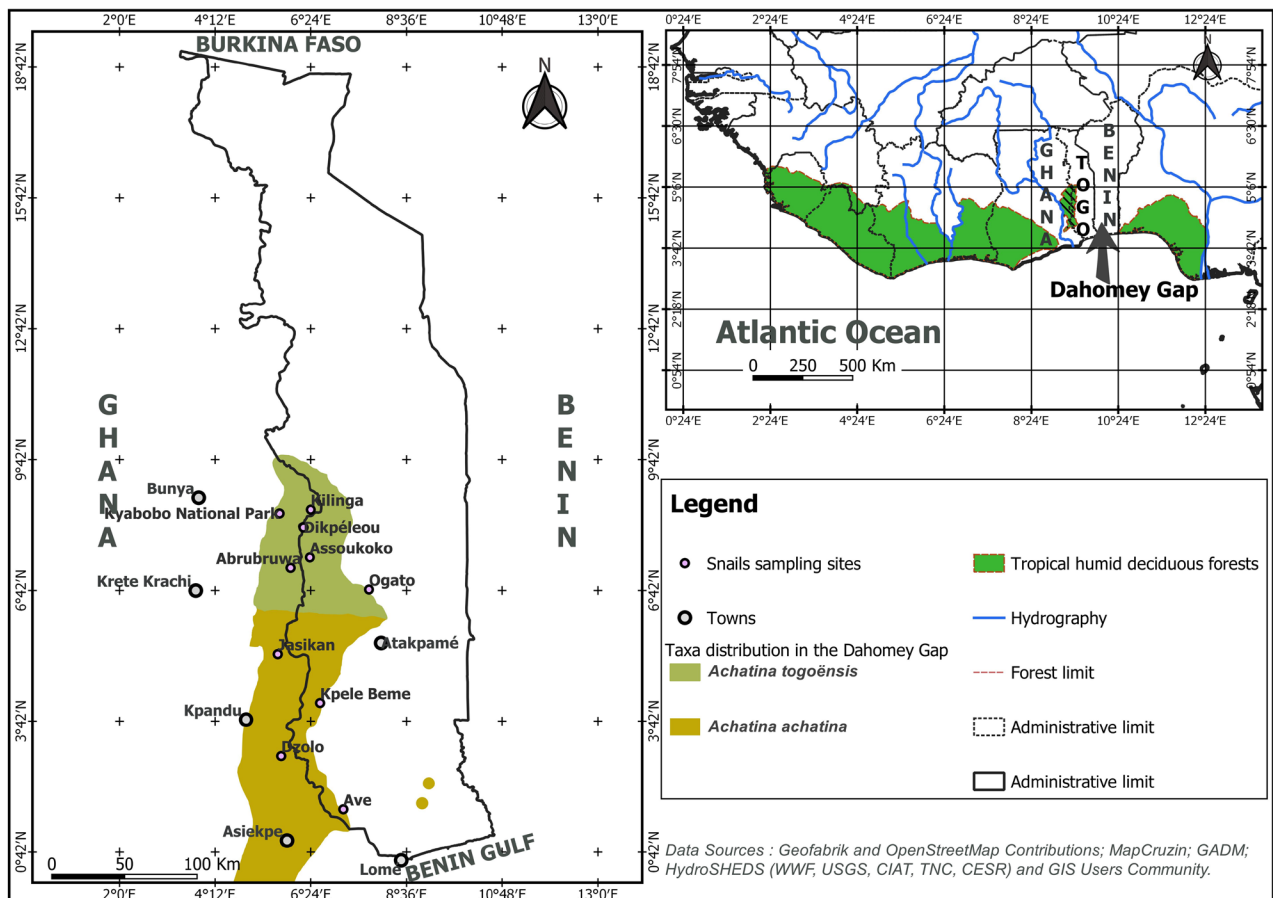


Figure 2. Map showing distribution of *Achatina* land snails in Dahomey Gap and the collection localities.

be *A. achatina* or *A. togoensis* according to color of columella, parietal wall and outer lip. In total, two hundred and fifty-three (253) individuals comprising one hundred and twelve (112) *A. Achatina* and one hundred and forty-one (141) *A. togoensis* were used in the present study. Specimens were sampled between July and September 2019.

2.2. Selection of Traits

The effectively useful traits for identification were selected based on the available taxonomic descriptions of the class Gastropoda from the literature [26] [27] [28]. A total of eight (8) traits were adopted for continuous measurements *i.e.* shell length (SL), shell width (SW), aperture length (AL), aperture width (AW), body whorl length (BWL), penultimate whorl length (PWL), maximum penultimate whorl width (PWW) and spire height (SpL). The number of whorls of the shell of each specimen was counted as meristic traits.

2.3. Selection of Traits

Specimens were identified and classified according to the columella colour and the parietal wall: Red for *A. achatina* and bluish-white for *A. togoensis*. The shells were imaged with the axis of the shell on the y-axis and the apertural later-

al axis in the same plane as the objective, *i.e.* the shell axis was placed parallel to the horizontal plane (Figure 3). A 20 cm scale bar was included within each shell image for size calibration. The morphological traits were measured at an accuracy of 0.01 mm. Shell length was measured along an axis passing through the apex (e) to the bottom (f) of the shell. Shell width is the maximum width perpendicular to the shell length distance ((b) and (c)). Aperture length is measured from the beginning of the 1st suture (i) to the bottom of the aperture (f). Aperture width is the maximum diameter perpendicular to the aperture length (k-j). Body whorl or last whorl length was measured from the beginning of the first suture (i'') to the bottom of the shell. Penultimate whorl length is the length between the beginning of the 2nd suture (i') and the beginning of the 3rd suture (h). Spire height was measured from the beginning of the 2nd suture (i') to the apex of the shell (e).

From the values obtained for each linear measurement, the following ratios representing different proportions of shell shape were calculated: shell length/shell width (SL/SW), Shell length/aperture length (SL/AL), aperture width/aperture length (AW/AL), penultimate whorl width/body whorl length (PWW/BWL) and spire height/body whorl height (SpL/BWL).

2.4. Dissection and Preparation of Radula

Radulae were prepared from at least 15 snails per sample. The buccal mass was dissected out of the head region of the soft body. The anterior portion of the proboscis was excised, the radula with adjoining tissues was removed from the proboscis and macerated in 8% - 10% sodium hydroxide solution for 24 hours at room temperature [29]. The radula was then washed with distilled water and

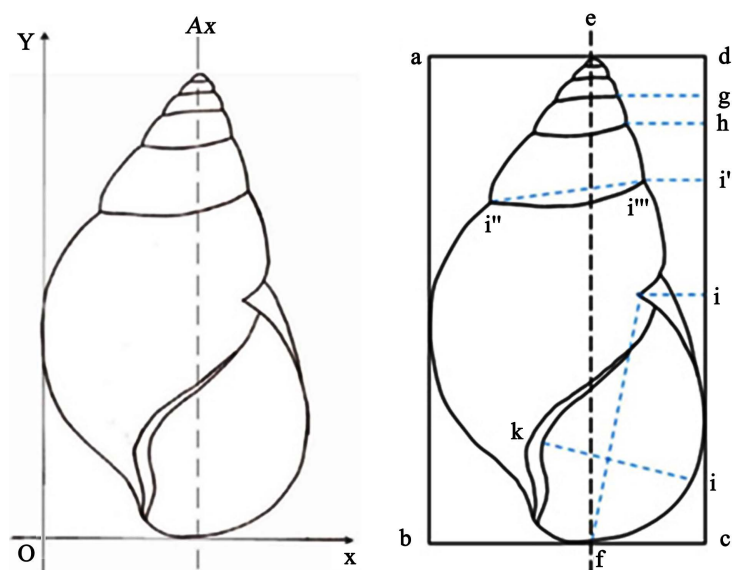


Figure 3. Characters measured for the morphological analyses of shell. Left-Shell arrangement. Orthonormal frame (Ox, Oy): The Oy axis is parallel to the columella axis and tangential to the left edge. The Ox axis is perpendicular to Oy axis and tangential to the lower edge of the aperture. Right-Measurements taken (see text for details).

dehydrated using alcohol. Dehydration was done by immersing the radula in increasing concentrations of alcohol (50%, 70%, 90%, 100%). Then it is ultrasonically blasted for about 10 seconds and stored in alcohol. The ultrasound expels the leftover tissues and impurities (grains of sand, food remains...) trapped between the teeth. The dehydrated radulae were prepared with a coating to make them suitable for scanning electron microscopy (SEM) observation. Coating the radula consists in placing the specimen in a vacuum evaporator, pumping the chamber down to a vacuum level equivalent to that in the SEM specimen chamber and heating the conductive metal (gold) to the point of vaporization. Continuous tilting and rotation of the specimen facilitated the metallic vapour to cover all the sides of the specimen. The preparations were finally examined using a JSM-840 scanning electron microscope.

2.5. Statistical Analysis

The data were analyzed using the statistical package Sigma Plot 14.0 (Systat Software, Inc) and PAST 3.32, and the programming software R 3.6.3 (R Core Team, 2020) along with packages FactoMineR [30], factoextra [31] and corrplot [32]. Significance of differences in mean between morphometric measurements was tested using Mann-Whitney U test which is appropriate for comparing differences between two independent groups when the dependent variable is not normally distributed. Results were considered significant at $p < 0.05$. An exploratory principal component analysis (PCA) based on correlation matrix extraction was performed to explore patterns of covariation in the data matrix and their relationships with species differentiation.

3. Results

3.1. Morphometric Analysis

Table 1 shows the morphometric data of the 253 shells examined. The shell of *A. achatina* ranged in total length (SL) from 94.8 to 186.5 mm while those *A. togoensis* measured 81.3 to 163 mm. It was determined in the field that *A. achatina*

Table 1. *Achatina achatina* and *A. togoensis*, conchometrics (mean, SD and observed range).

Species	Statistics	SL	SW	BWL	AL	AW	SpL	PWL	PWW
<i>A. achatina</i> (n = 112)	Mean	124.32	71.05	85.90	63.03	35	38.40	16.58	38.06
	SD of mean	21.81	12.57	14.911	11.028	5.76	7.55	3.41	6.61
	Minimum	94.80	53.20	63.9	50	27	26	11	27
	Maximum	186.50	107	133.5	97	51	56	26	57
<i>A. togoensis</i> (n = 141)	Mean	117.89	62.98	82.1	59.44	33.89	35.84	20.02	35.16
	SD of mean	18.86	10.56	12.819	9.12	5.54	6.91	3.85	5.79
	Minimum	81.3	43.7	59	41	23	22	19	25
	Maximum	163	88.5	116	89	52	51	29	50

usually grew larger than *A. togoensis* even if the observed means (124.32 mm and 117.89 mm respectively) were not statistically significant (Mann-Whitney U Statistic = 6182.500; $p = 0.151$). On the other hand, the differences in the median values of the total width (SW) between the two groups were greater than would be expected by chance ($p = 0.004$), *A. togoensis* being statistically narrower. Likewise, a very significant difference in proportional penultimate whorl width was also observed between the two species ($p \leq 0.001$).

The two snail species under study also differed significantly in most of the morphometric ratios except SL/AL. The ratio of the total length to the total width (SL/SW) clearly discriminates the two species (Table 2). *A. togoensis* had a higher ratio with a minimum value of 1.837 whereas the maximum ratio for *A. achatina* was 1.824. On the other hand, the ratio of the aperture width to the aperture length was statistically lower for the species *A. achatina* (0.56 ± 0.0016) and *A. togoensis* (0.57 ± 0.003) with a proportionally smaller aperture in the latter ($p < 0.001$). However, the two species overlap a little bit with regard to this character.

In the multivariate analysis, shell length (SL), maximum shell width (SW), aperture width (AW), penultimate whorl width (PWW) and body whorl length (BWL) were highly correlated with each other ($r > 0.9$) and whorls and penultimate whorl length were poorly correlated with them ($r < 0.85$). The values of these overall size measures overlapped usually within the size range that could be compared directly (SL > 90 mm), but a clear difference between the two species was evident in the relationships between SL and number of whorls (Figure 4). In general, at a given shell length, minimum whorl value for *A. togoensis* exceeds the maximum number for *A. achatina*. The latter completed as much as 0.5 of a whorl less than the maximum observed for *A. togoensis*.

Results of the PCA of morphological characterization show that the principal component 1 (PC1 or axis one or dimension 1) explained alone 82.1% of total variation and is therefore clearly the dominant pattern of morphometric divergence between the two taxa (Figure 5). While 82.1% of the total variation is explained by axis 1, 11.2% is explained by axis 2 and 6.7% by the remaining axes.

Table 2. Ratio of shell measurements of *A. achatina* and *A. togoensis* (mean \pm SD). Statistical method and p -value were provided.

Ratio	<i>A. achatina</i>	<i>A. togoensis</i>	Statistical difference
	(N = 112)	N = 141	
	Mean \pm SE	Mean \pm SE	
SL/SW	1.75 \pm 0.005	1.88 \pm 0.006	M. U = 415; $p = 4.867E-37$
SL/AL	1.98 \pm 0.011	1.99 \pm 0.008	M. U = 7146; $p = 0.4274$
BWL/SL	0.69 \pm 0.002	0.70 \pm 0.002	M. U = 6463; $p = 0.040$
AW/AL	0.56 \pm 0.0034	0.57 \pm 0.003	M. U = 5553; $p = 0.0046$
PWW/BWL	0.44 \pm 0.003	0.43 \pm 0.002	M. U = 5215; $p = 2.1E-5$

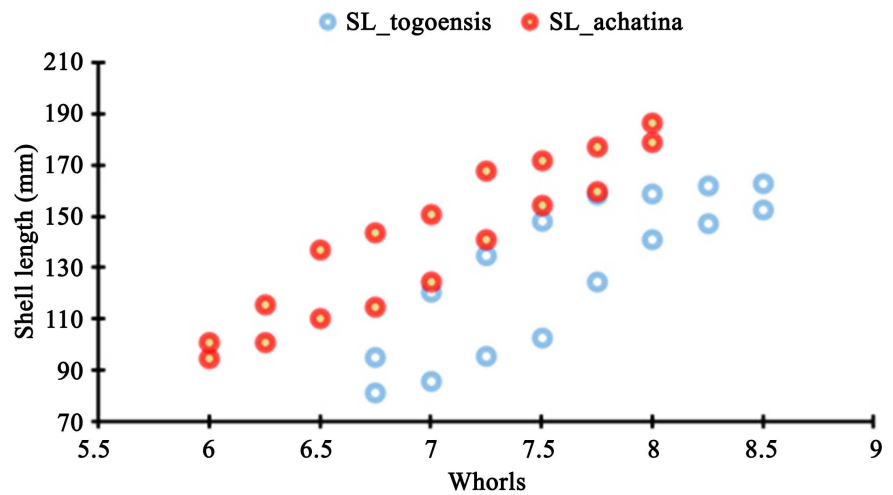


Figure 4. Relationship between number of whorls and shell length for all shells of *A. achatina* (n = 101) and for *A. togoënsis* (n = 151) in the directly comparable size range. Points were fairly distributed over areas of the dots.

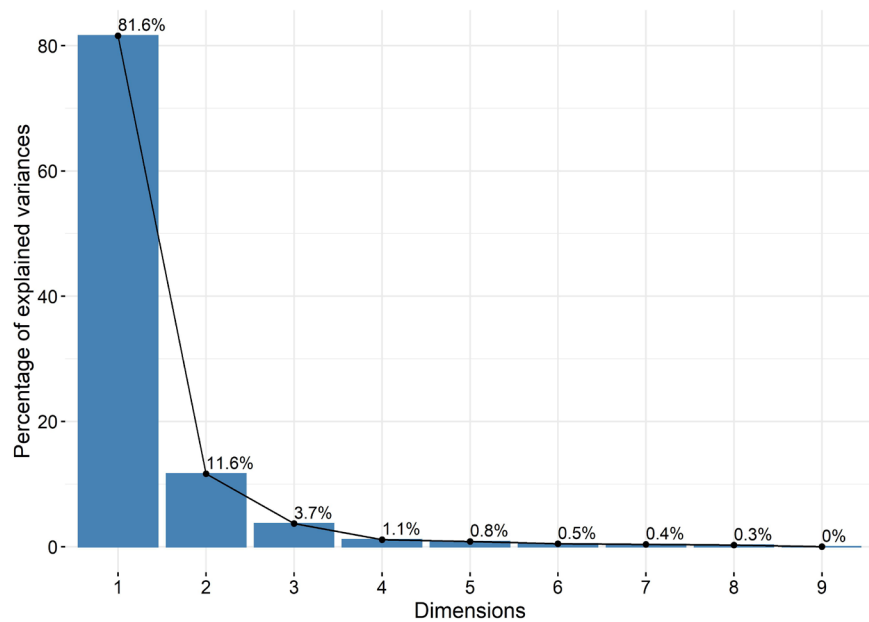


Figure 5. Scree plot produced by plotting the Eigenvalues with their respective components numbers.

The contribution of each variable to the 1st and the 2nd dimensions indicates that all variables contribute each to more than 12% of variation in PC1 except PWL and Wh which contributes each to more than 10% in PC2. All the shell dimensions record a high correlation in PC1 except penultimate length. The quality of the representation of each variable in different dimensions is in **Figure 6** using the squared cosine.

The first dimension was thus interpreted as a size factor. PC2 had the highest loading scores for the number of whorl. The scatter plot of the projection of individual snails of the two species in the first two dimensions (PC1 × PC2) is

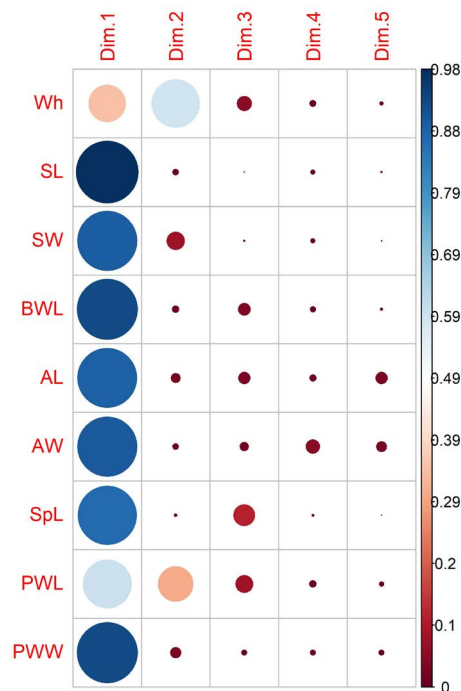


Figure 6. Correlation \cos^2 of variables on 5 dimensions.

presented in **Figure 7**. The 95% ellipses showed that individuals belonging to the two species were well distinguished and aggregated in separate clusters without overlap. These results show that *A. achatina* differs from *A. togoensis* by having a proportionally larger aperture width (SW), higher body whorl and spire lengths. On the other hand, specimens belonging to *A. togoensis* have a greater number of whorls and a higher penultimate whorl relative to the total height of the shell.

Hierarchical clustering (**Figure 8**) separated the two taxa similarly the results of the PCA. The resulting tree reflects their kinship but clearly shows that the two species are different from the morphological point of view, with *A. achatina* being the basal species.

3.2. Morphological Traits of the Radulae

The radular ribbon of the analyzed specimens of the achatinid species here studied consists of several tens of transverse teeth and around two tens of incipient teeth or rudiments (**Figure 9**). The radular formula is M + L + R + M. The entire radula in adult animals can be as much as 11 mm in its distal part and 20 mm long. The comparison of the radular morphology indicates that the radula of *A. togoensis* resembles that of *A. achatina* in every detail. Shape, proportions and relative position of teeth in the transversal row of the radular ribbon are basically stable traits. Moreover, no striking differences were observed in the radular mesocones. Teeth features in both species are as follows.

A very small essentially non-functional central tooth is visible in the center of the radula membrane on the longitudinal axis (**Figure 10**). On either side, in the

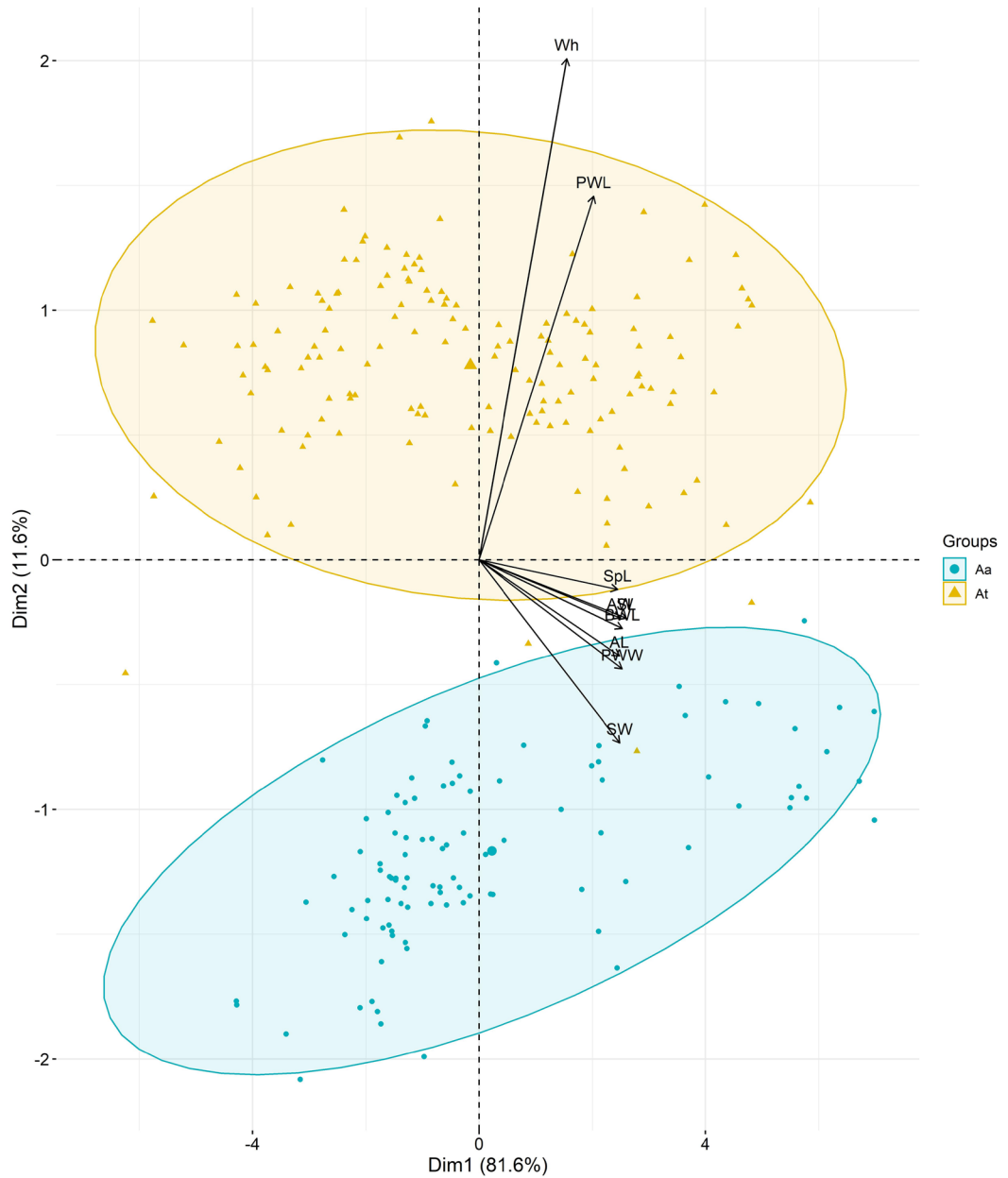


Figure 7. Scatter plot of PC1 vs PC2 scores and 95% confidence ellipses of the scores for PCA using morphometric characters. Data point represent individual discriminant function scores of the linear measurement data of 2 species of *Achatina*. Shells were identified on the basis of the colour of the columella and parietal wall. Wh, whorl number; PWL and PWW, penultimate whorl length and width; SW, total shell width; SpL, spire length.

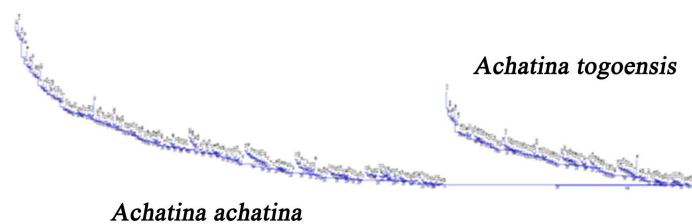


Figure 8. Neighbour-joining dendrogram of morphometric measurements.

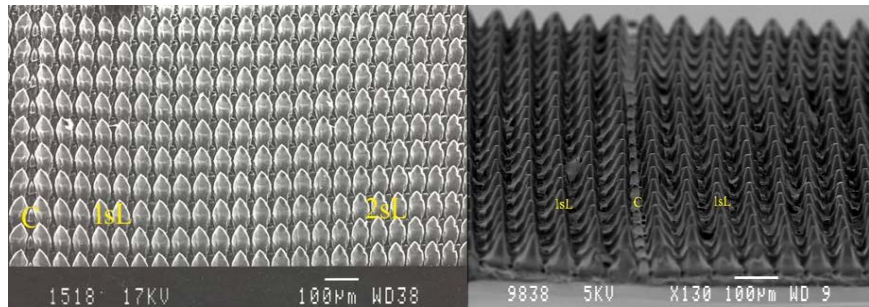


Figure 9. Scanning electron microscopy (SEM) images of the radulae dentition of *Achatina togoensis* and *A. achatina*. Left: Section of radular ribbon view of anterior part of *A. achatina*. Right: Dorsal view of central tooth and first lateral teeth of *A. togoensis*. Central tooth is indicated by “C” and the first and second sets of laterals are labelled 1sL and 2sL respectively.

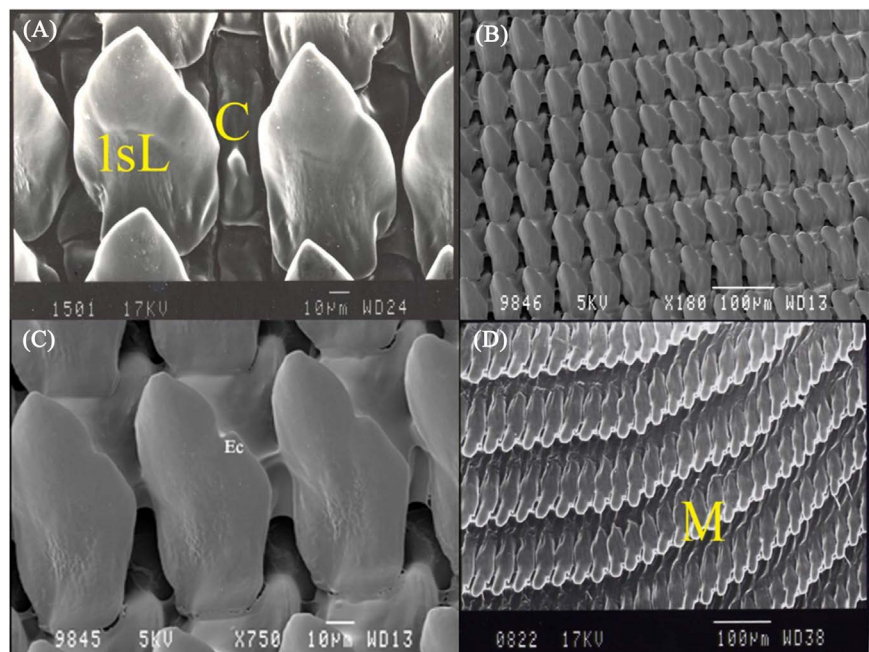


Figure 10. Scanning electron microscopy (SEM) images of the radula of *Achatina togoensis* and *A. achatina*. (A) Central region of radula illustrating central and adjoining lateral teeth; (B) First series of lateral teeth; note the are nearly monocuspid with bluntly pointed triangular or ovate mesocones; (C) Second series of lateral teeth; note that the ectocone becomes well individualized; (D) Outermost marginal teeth.

same transverse row, the central tooth is flanked by the first series of 14 to 20 nearly unicuspid (or weakly tricuspid) lateral teeth. Mesocone shape was commonly angular or rarely non-angular (dome-shape). The central cusp (*i.e.* the mesocone) is triangular or dome-shape. The mesocone column of each lateral tooth is in direct contact with the broad based basal plate of the tooth immediately posterior to it. From the 15-21th tooth, the mesocones become more and more obliquely truncated inwards and the ectocone gradually increases in size until the 26-39th tooth. This third type of teeth is in turn, flanked by a series of true tricuspid marginal teeth, which extend to the margin of the radula mem-

brane. The marginal teeth may be symmetrical but more often they are asymmetrical in shape. Right on the edge, there are some rudimentary teeth.

4. Discussion

4.1. Comparative Shell Morphology

This study demonstrates that *A. togoensis* and *A. achatina* are different from each other in both morphometric and meristic traits of the shell. This means that from a morphological point of view, our results support the conviction of [16] who considered them as distinct species. The traditional morphometric methods used here proved to be sufficiently powerful to allow differentiation of specimens of the two closely similar species. This finding agrees with previous studies, which suggest that although the achatinid shell shows high intraspecific morphological variation, traditional morphometrics may provide suitable taxonomic characters to discriminate between the species [19] [28].

Conchological comparisons between *A. achatina* and *A. togoensis* were first made by Bequaert [2] in his studies in Achatininae. He had found that the samples collected in Togo were roughly one third smaller than the average size of the nominate race. Indeed, the typical *A. achatina* is not only the largest, by also the most capacious extant land snails known. Our largest specimens (173 mm) comes close to the record length of 200 mm reported by Bequaert [2]. Accordingly, he had concluded that *A. togoensis* is a dwarf form of the typical *A. achatina*. Unfortunately, Bequaert and Clench's [15] original description of *A. togoensis* was based on very few sub adults specimens. The holotype is 89.5 mm. long, 48.6 mm wide, with the aperture 50 by 26 mm. and consists of 7¼ whorls; the corresponding measurements of the paratype are 89 mm, 49.2 mm, aperture 51.3 by 27.6 mm, 7¼ whorls. Since then, very large individuals of *A. togoensis* have been collected [17]. Full-grown specimens often reach 160 mm. or more. in length. Therefore, although we gained a clear impression in the field that *A. achatina* usually grew larger than *A. togoensis*, the present study established that both taxa are large and bulky snails when full-grown.

According to our results, *A. achatina* and *A. togoensis* do differ in ways that are independent of overall size. The clearest morphological differences between the two taxa lie in the spire index, aperture width and whorl number against shell length. *A. achatina* presents a significantly greater shell width which results in a shell height/shell width ratio significantly smaller compared to the ratios observed for *A. togoensis*. Consequently, the body whorl is wide and globular. Furthermore, the rates of whorls coiling in the two taxa are visibly distinct. Correspondingly, for a similar shell length, *A. togoensis* snails have more whorls and have proportionally higher spire. Other researchers have stated that very elongate snail's species tend to have more numerous whorls [2] [26], and this is consistent with the results of this study.

Bequaert [2] thought that morphological differentiations in achatinid snails were solely accidental byproducts of genetic drift in isolated populations.

Speaking of the subspecies *Achatina a. elegans*, he stated that “such dwarfs occur sporadically throughout the range of *A. achatina*, almost always together with the larger form, seemingly showing no preference either for a particular geographical section or for a special environment” (1950: 32). However, examination of the literature suggests that land snails are highly responsive to environmental conditions [33] [34], and many real species have evolved by divergent natural selection between environments, *i.e.* distinct in vegetation cover, dominant plant species, maximum altitude and soil type. Verdcourt [35] and Tattersfield *et al.* [36] reported that even fairly minor changes in rainfall and temperature have given repeated opportunities for vicariant speciation in East African land snails. This could be the case in our study.

The distribution range of the West African species of *Achatina* was provided by Hodasi [13] and modified by Afiademanyo [17] as regards the Dahomey Gap (Benin/Togo). Studies show that *A. achatina* and *A. togoensis* are allopatric and their populations occur in contrasting environments. Actually, *A. achatina* is widely distributed throughout the wet forest in West Africa [2] [13]. In contrast, *A. togoensis* is a restricted-ranged species centered on Mounts Adele, which constitutes the northern sectors of the Monts Togo which cross the country in the south-west towards Ghana, where they take the name of Akwapim. It was established that physical environments (in particular climatic parameters) differ substantially between the northern parts of the mountains and the rest of the humid semi-deciduous forest. The latter enjoys a subequatorial climate comprising a four-season cycle and a fairly high rainfall. In contrast, in the northern sectors, the climate is dry up because of the proximity of the Sudano-Guinean savannahs and especially because it is a zone directly influence by the harmattan. Diurnal temperature variation may reach 10°C to 15°C and humidity often falls below 50% in the hottest hours [37] [38]. Furthermore, the dense semi-deciduous forest described on these mountains is much drier because of lower rainfall (1200 mm) and their corollaries of long ecologically dry seasons and high water deficit [39].

We believe that the climatic regimes prevalent in the two geographic ranges are the most likely cause of the divergence in the shell morphologies of *A. achatina* and *A. togoensis*. Therefore, it could be hypothesized that striking distinctions in shell shape and certain meristic traits of the two land snails species are adaptive responses to different selective pressures specially to differences in the degree of exposure to drought. It was found out that even in the rain forest of Liberia, which is one of the most humid sections of West Africa, the snail buries for aestivation during the drier months in the superficial loose soil and litter. It then closes the aperture with a white, brittle calcareous epiphragm. Hence, the slender form and the smaller mean aperture height of the shell of *A. togoensis* may represent a strategy to prevent excessive water loss under water stress conditions [40] [41]. A review of the nature and causes of variation in size and form of land snail shells [40] noted that with many species, individuals living in moist conditions tend to be larger and have greater relative aperture areas than indi-

viduals in drier habitats. Likewise, analyzing the differentiation of the land snails *Eremina d. desertorum* and *E. d. irregularis* in the deserts of northern Egypt Ali *et al.* [42] stated that the shape differences between the two taxa are partly an indirect consequence of selection for body size, but are also directly affected by selection for reduction of aperture size. In contrast, our results conflict with expectations based on the assumption that large snails are superior in dry habitats, as they lose relatively less water due to their surface/area ratio lower volume.

4.2. Comparative Remarks on the Radulae

Detailed description of the radular teeth morphology of *Achatina togoensis* and *A. achatina* using scanning electron microscopy (SEM) were done for the first time in this work. In contrast to shell morphology, radular morphology presents a different picture. No obvious differences were found between the radulae of the two species. Assuming that the shapes and structures of molluscan radular teeth are often unique to a species or a genus, our result may suggest that the two taxa under study are conspecific and therefore *A. togoensis* [15] is synonymous with *A. Achatina* (L.). This assumption is supported by Odaibo and Olayinka [25] who showed that the absolute values of the rachidian tooth and the development of accessory cusps on the first laterals clearly indicate the existence of stable interspecific differences in the genus *Achatina* here studied.

As an alternative hypothesis, the observed similarity may be related to the plasticity in the feeding apparatus demonstrated in a wide array of taxa including molluscs [22] [43]. Indeed, radular tooth shape can be a labile trait and is sensitive to natural selection [44]. The reliability of radular characters in the taxonomy of the Achatinidae family remains uncertain and has not been adequately studied. Mead [23] had reported similar confusing pictures in the radulae of achatinid snails. These similarities may be attributable to convergent evolution among species subjected to similar environmental conditions for radula construction and functioning [45] [46].

4.3. Process of Differentiation and Taxonomic Status of “*A. togoensis*”

The restricted range and habitat specialization of *A. togoensis* compared to *A. achatina*, associated with the high degree of morphological differentiation that we observed in the present study, suggest vicariance-driven divergence model by colonization of new/marginal environments. Under the colonization scenario, one of the sister populations is founded by a very small number of individuals that come from a larger source population at the time of colonization, with subsequent isolation [47].

This suggestion partly supports Mead [4] assumption that *A. togoensis* and *A. achatina* are two distinct vicariants species, the former just about east, the later only west of the savanna corridor interrupting the zonal West African rain forest. However, he mistakenly suggested that the Chaîne du Togo-Volta River-Accra Plains acted as a biogeographical barrier. Later, it was shown that this

assumption was based on erroneous distribution records of the two taxa [17]: *A. achatina* overcame the Dahomey Gap (DG). The species has been definitively observed from Guinea through Ghana and Benin to Southern Nigeria. On the other hand, Van Bruggen [48] recognized four sub-Saharan centers of endemism among African terrestrial gastropod including one in Central/West Africa. Each center was assumed to have function as an important refugium during periods of forest contraction in Holocene. Therefore, considering this information and the results obtained in this study, it is likely that the typical *A. achatina* has dispersed westward from Lower Guinea of Cameroon and Gabon refugium after the periods of forest contraction and the extension of the savannas. Towards the North, this extension of the species would have been stopped by the Guinean savannah subjected to Harmattan winds. Subsequently, peripheral population of the typical form would have isolated and developed in the drier facies of the humid forest that is individualized in the northern part of Monts Togo. The small colonizing population (*i.e.* of *A. togoensis*), then grows “exponentially” until it reaches its present effective size.

Janson and Sundberg [49] argued that the only way to state with certainty the occurrence of two distinct species would be to show a morphological divergence where the two populations overlap, *i.e.* in a truly sympatric area. In allopatry, however, it is harder to assess the significance of observed differences in terms of the conventional biological species concept [50]. Moreover, although the isolation of populations suggests a potential for speciation, it has been shown that ecological induced population differentiation need not necessarily result in complete reproductive isolation and the formation of new species [51]; some morphological differences may reflect an incipient stage of speciation [33].

Nevertheless, allopatric populations often prove, when tested, to be reproductively isolated; therefore, it is possible to state that some allopatric populations have indeed diverged to species status [52]. Here, although the distinctness of *A. achatina* (L.) and *A. togoensis* [15] is well established on the basis of the conchological differentiation, the discrepancies of shell and radular morphology data do not provide strong arguments to warrant specific recognition for both taxa. Furthermore, the existence of reproductive isolation of the two taxa is not yet attested, there is nothing to indicate that they would not cross to give fertile offspring if the opportunity arose. Therefore, whether the 2 morphotypes documented in the present study represent 2 different subspecies of *A. achatina*, or 2 species of *Achatina* is worthy of further investigations. It will be necessary 1) To study the genetic structure of both taxa across the whole distribution range; 2) To evaluate variations in shell and genital morphologies to determine whether such variability correlates with genetic differentiation; 3) To test the potential for interbreeding (anatomical, cross breeding) before a final decision can be made about the taxonomic status of *A. togoensis*.

4.4. Conservations Implications

There is increasing evidence that human impacts over the past 10 millennia have

profoundly and permanently altered biodiversity on land, especially of non-marine molluscs [53]. Endemic species, especially those with restricted ranges seem to be at particular risk of extinction [54]. Indeed, species with naturally small population sizes are more vulnerable to habitat degradation simply because loss of even a small amount of habitat for a geographically restricted species could reduce numbers below sustainable levels.

As mentioned above, *A. togoensis* is endemic to the dry facies of the semi-evergreen forests, which covers a tiny territory (<500 km²) in the northern part of the Monts Togo. The present study assumes it represents a distinct evolutionary unit. Yet anecdotal evidence from local snail harvesters suggested that there has been a marked decline in the species abundance in the past 20 - 30 years [55].

Threats to the species assessed within one of the three threatened categories can be classified into three main causal groups: 1) Overexploitation, mostly for human consumption. The natives consider it an economic asset, as it is a favorite article in the diet of different tribes across the country. Snail consumption is also associated with certain healing practices [17]; 2) The environmental changes provoked recently by human settlement, including deforestation for agricultural ends and indiscriminate application of agricultural poisons and chemical fertilizers [56]; 3) Potentially, climatic change.

A. togoensis had never been evaluated under the Endangered Species Act and thus is not listed. For the purpose of this study, historical localities have been re-sampled. Despite an intensive search involving several prospectors, the result was that more accessible parts of the distribution range have lost almost all their entire snail populations. Further, areas deep in the forest, where snails were once abundant, now have markedly smaller populations. It is therefore likely that population reduction has reached over 50% during the 20th century. Based on these considerations, we suggest that *A. togoensis* meets the criteria B2ab (ii, iii) to categorize as “Endangered”.

The following conservation tips and recommendations are suggested, which attempt to address the major threats identified:

- 1) Collection of snails from protected areas (e.g. Community and Classified Forests such as Assoukoko forest, Koué) must be banned, although enforcing the ban is likely to be difficult;
- 2) Smallholder snail-farming should be encouraged among rural and urban dwellers to de-emphasize its collection from the optimal wild source;
- 3) An awareness campaign should be undertaken to educate collectors to harvest large individuals only;
- 4) Finally, ecological studies must be carried out to obtain more precise knowledge of the species’ range, a good understanding of population dynamics and a definition of sustainable harvesting rates.

5. Conclusion

In conclusion, the present study shows that the two individual forms, *i.e.* the

typical *A. achatina* and *A. togoensis* which lacks all traces of violaceous on the columella and parietal wall, differ in several morphometric and meristic traits, consistent with their initial treatment as distinct species. These morphological differences essentially associated with body shape, may be explained by divergent pressures associated with variation in environmental factors on either side of Togo Mountains/Akwapim range. In contrast, both forms had similar radula morphology suggesting that there is no distinction between *A. togoensis* and *A. achatina*, on the basis of differences in the shape of teeth, form, position and number of cups. This conflicting information, from shell morphology and radular features, denies drawing any firm taxonomic conclusions from the results obtained. Whether *A. togoensis* have arrived at species status cannot be decided at the current state of knowledge. An integrative taxonomy, *i.e.*, a combination of morphological, anatomical and genetic data would be employed in future works to conclusively clarify the delimitation between these two closely related species and understand relationships among taxa.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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