Comparing Shell Size and Shape with Canonical Variate Analysis of Sympatric *Biomphalaria* Species within Lake Albert and Lake Victoria, Uganda

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Abstract:

The Great African Lakes in Uganda (Lake Albert and Lake Victoria) are known habitats to several sympatric species of *Biomphalaria*, intermediate snail hosts of the human parasite *Schistosoma mansoni*. Accurate identification of snails by morphology alone, however, can be problematic highlighting a need for robust, on-site identification methods, since only certain species have important roles in parasite transmission. This study investigates the conchological variation within *Biomphalaria* species collected from these two Great East African Lakes. We compared the shell morphologies of *Biomphalaria* species using landmark-based morphometric techniques and were able to distinguish *Biomphalaria* species through canonical variate analysis (CVA) of the apical and apertural shell angles. After identification with molecular methods, three *Biomphalaria* species (*B. pfeifferi*, *B. stanleyi* and *B. sudanica*), with heterogenous occurrences along the shoreline, were identified at Lake Albert that could be differentiated from one another using CVA of apical and apertural datasets; by contrast, a single *Biomphalaria* species was identified at Lake Victoria (*B. choanomphala*). When snails from both lakes were compared together, CVA was able to differentiate all four species using the apical dataset but not the apertural dataset. Of the *Biomphalaria* species identified, ecological phenotypic variation was only found in *B. choanomphala*, which exhibited two distinct ecological morphotypes. Furthermore, these two *B. choanomphala* morphotypes from Lake Victoria, overlapped upon analysis of the apical dataset yet were clearly separated upon analysis of the apertural dataset. Our study demonstrates that landmark-based morphometrics could play a future role in distinguishing sympatric *Biomphalaria* species in Uganda.

Keywords:

Gastropoda, Planorbidae, Conchology, Morphometrics, Schistosomiasis.

Introduction:

Freshwater snails of the genus *Biomphalaria* (Gastropoda: Planorbidae) are found in South and Central America, Africa, the Middle East and Madagascar (Brown, 1994; Dejong et al., 2001; Rollinson, 2011). They act as the obligatory intermediate hosts of *Schistosoma mansoni* (Trematoda: Schistosomatidae), a globally important trematode responsible for intestinal schistosomiasis (Brown, 1994; Colley et al., 2014). In Africa, a total of 15 species of *Biomphalaria* are recognised, Mandahl-Barth (1957) being the first to categorise them into four main taxonomic groups based on a combination of several morphological characters (shell, genital organs and radula).

The four groups of *Biomphalaria* comprise the *B. alexandrina*-group (*B. alexandrina, B. angulosa, B. salinarum* and *B. tchadiensis*), the *B. choanomphala*-group (*B. barthi, B. choanomphala,*

B. smithi and *B. stanleyi*), the *B. pfeifferi*-group (*B. pfeifferi and B. rhodesiensis*) and the *B. sudanica*-group (*B. camerunensis* and *B. sudanica*). Of these morphological identification methods, genital morphology is the most dependable as complementary reproductive organs are essential for intraspecies mating (Gómez, 2001). However, identifying *Biomphalaria* using genital morphology requires both time and expertise, as the genitals need to be cautiously dissected from relaxed snails, then carefully fixed and mounted for viewing. Subsequently, fine detail measurements are collected under a suitable light microscope. This precludes rapid identification of snails at the sight of collection and makes identification of snails by shell morphology more preferrable. Although conchological identification has its drawbacks, its rapid and inexpensive when compared to other morphological, or molecular identification methods.

The introduction of molecular studies has partially clarified the taxonomy and phylogeography of African *Biomphalaria*. Both Dejong et al. (2001) and Jørgensen et al. (2007) found that the only clearly defined African species were *B. camerunensis* and *B. pfeifferi*, while the six other *Biomphalaria* species (*B. alexandrina*, *B. angulosa*, *B. choanomphala*, *B. smithi*, *B. stanleyi* and *B. sudanica*) formed a poorly defined clade named the 'Nilotic species complex'. Dejong et al. (2001) confirmed the topology was consistent with the proposed Neotropical origins of the genus, with the oldest *Biomphalaria* fossils being dated from approximately 60 million years ago (Jarne et al., 2011). However, all of the African *Biomphalaria* species have a low level of genetic diversification (Morgan et al., 2002; Van Damme & Van Bocxlaer, 2009), which is likely the result of their relatively recent evolutionary history. Campbell et al. (2000) places the introduction of proto-*B. glabrata* taxon to the African continent from South America and the evolution of all African *Biomphalaria* at approximately 1.8 to 3.6 Mya, while Morgan et al. (2001) estimated a longer time frame of 2 to 5 Mya based on the current fossil record. Furthermore, the remaining nominal African *Biomphalaria* species (*B. arabica*, *B. barthi, B. rhodesiensis, B. ruppellii, B. salinarum* and *B. tchadiensis*) previously defined exclusively by morphological characteristics are becoming increasingly invalidated by modern molecular methods, with further investigation needed to confirm whether these species are valid taxa (Jørgensen et al., 2007). The large number of invalid taxa within the *Biomphalaria* literature is likely the result of several (if not all) species of *Biomphalaria* being subject to various sources of intraspecific variation such as ecophenotypic variation and indeterminate shell growth (Jarne et al., 2011). Collectively, this can make two individuals within a single nominal species appear taxonomically distinct entities (Jarne et al., 2011). Standley et al. (2011) and Zhang et al. (2018) both found that *B. choanomphala* snails present at Lake Victoria exhibited contrastingly different conchological morphologies, likely due to presence or absence of wave action, but were very genetically similar.

Similarly to *Biomphalaria*, the closely related Planorbidae genus *Helisoma* also exhibits a striking amount of morphological variation (Hoverman et al., 2005). Dillon (2019) discusses the conchological variation found in two genetically identical populations of *H. trivolvis*, which had two contrasting shell morphologies dependent on whether the snails lived in lentic (still) or lotic (flowing) water (Figure 1). Dillon (2019) hypothesised that these two contrasting shell morphologies were ecological phenotypes (or ecophenotypes) that helped the snails adapt to their micro-environments. The lentic morphotype (morphotype-A, Figure 1) is large, narrow, flat and has an arithmetic spiral. This morphology allows for the trapping of air, which the snail uses to regulate its buoyancy in still water to reach and graze on floating vegetation. Conversely, the lotic morphotype (morphotype-B, Figure 1) is small, broad, round and has a logarithmic spiral. This morphology cannot trap air and allows for less drag in flowing water, with their wide aperture/foot being used for better grip while grazing onto rocks in flowing water.

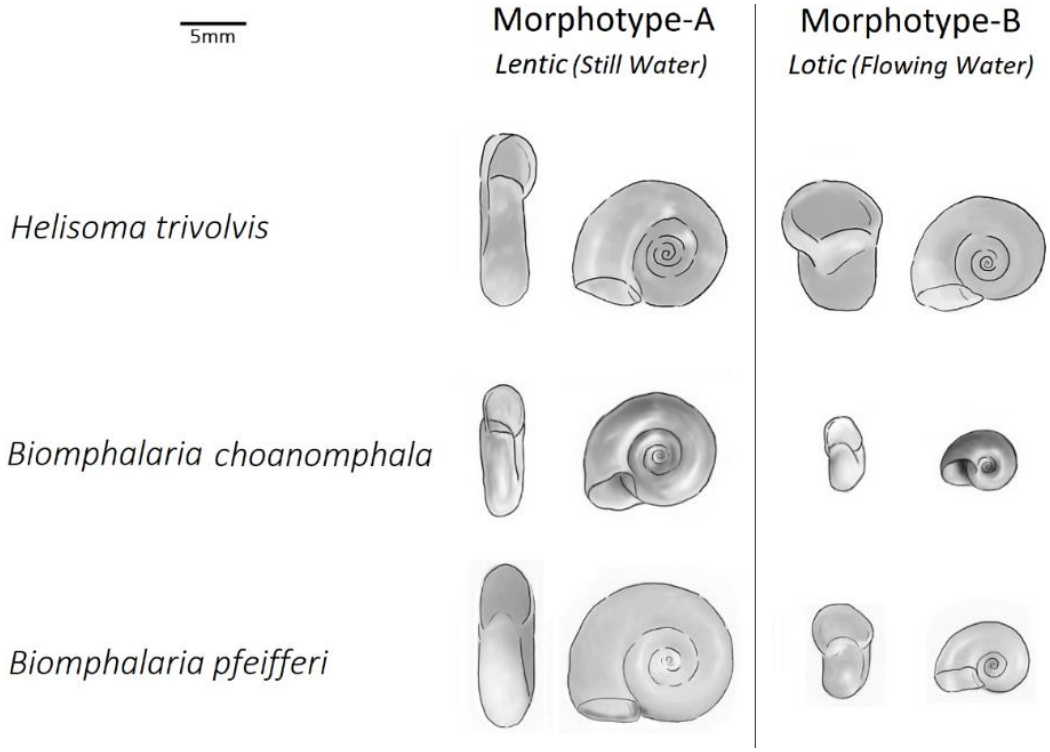

Figure 1. Morphological examples of ecological phenotypic plasticity in Planorbidae snails. Morphotype-A is the form found in lentic (still) water, while morphotype-B is the form found in lotic (flowing) water. Morphotype-A shells are larger, slow-whorling and have narrow apertures compared to morphotype-B shells. The *Biomphalaria pfeifferi* and *Helisoma trivolvis* shells were adapted from Plam et al. (2008) and Dillon (2019), respectively. The shells are viewed from the apertural (left) and apical (right) shell angles.

In light of the morphological comparison within Helisoma described by Dillon (2019), African *Biomphalaria* species frequently resemble shell morphologies that are similar to the lentic (e.g. *B. alexandrina*, *B. angulosa*, *B. camerunensis*, *B. pfeifferi*, and *B. sudanica*) and lotic morphotypes (e.g. *B. choanomphala*, *B. smithi*, and *B. stanleyi*) described by (Brown, 1994; DeJong et al., 2001; Kazibwe et al., 2006; Jørgensen et al., 2007; Plam et al., 2008; Kazibwe et al., 2010; Standley et al., 2011; Zhang et al., 2018). Furthermore, studies that use both conchological and molecular identification methods have shown that *B. choanomphala* and *B. pfeifferi* snails can exhibit these contrasting ecomorphotypes depending on their habitat (Figure 1; Plam et al., 2008; Standley et al., 2011; Standley et al., 2014; Zhang et al., 2018). It is plausible that a parallel adaptation occurs in *Biomphalaria* similar to *Helisoma*.

A potential solution to the issues conchological identification methods have when trying to differentiate *Biomphalaria* species, is to incorporate geometric morphometric techniques. Landmark-based geometric morphometrics is a powerful tool used to quantify and analyse the size and shape variation between organisms (Webster & Sheets, 2010) and has been widely used in differentiating medically important invertebrates, insects in particular (Goncalves et al., 2016; de Souza et al., 2020; Jiménez-Martín et al., 2020). Although landmark-based geometric morphometric techniques have been applied previously to medically important snail genera (Vasallo et al., 2013; Parra & Liria 2017; Hammoud et al., 2022), they are yet to be fully explored and applied for differentiating species within *Biomphalaria*. To this end, we apply landmark-based morphometric techniques to undertake a conchological investigation of *Biomphalaria* snails collected from the Ugandan shorelines of Lake Albert and Lake Victoria.

Materials and Methods:

Sample Sites:

Specimens of *Biomphalaria* used in this study were collected by Rowel et al. (2015), as part of the Wellcome Trust funded Schistosomiasis In Mothers and Infants (SIMI) project. Snails were routinely collected from three disease surveillance sites along the shoreline of Lake Albert and three sites along the shoreline of Lake Victoria between 2009 and 2010 (Figure 2; Table 1). The snails were collected from the lake edge and within the lake (to a depth of ~1m). Approximately half of the snails collected at Lake Albert and Lake Victoria were preserved in 70% ethanol, being held as a reference archival collection at the Liverpool School of Tropical Medicine, UK.

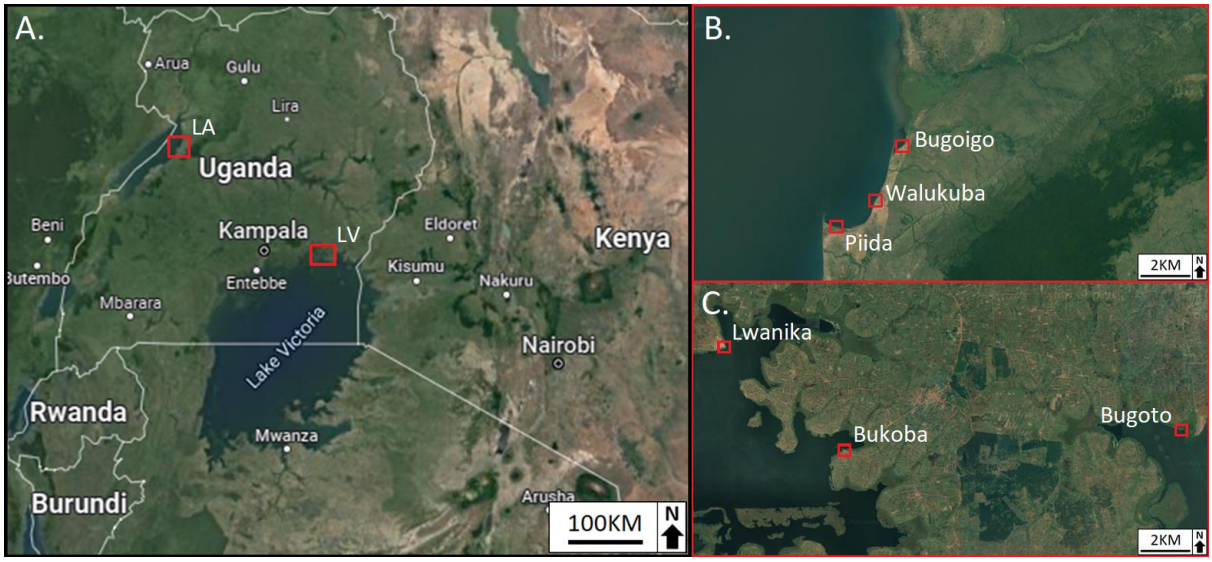

Figure 2. (A) Map showing the location of Lake Albert (LA) and Lake Victoria (LV). (B) Lake Albert collection sites (Bugoigo, Piida and Walukuba). (C) Lake Victoria collection sites (Bugoto, Bukoba and Lwanika). Satellite imaging was provided by Google Maps (Google, 2022).

Table 1. *Biomphalaria* collection information.

	Site	Preserved specimens			Latitude°, Longitude°	Elevation
		No.	A	B		
Lake Albert	Bugoigo	977	84%	16%	1.908, 31.409	615m
	Piida	521	100%	0%	1.819, 31.328	618m
	Walukuba	1147	13%	87%	1.842, 31.378	617m
Lake Victoria	Bugoto	4005	13%	87%	0.319, 33.628	1153m
	Bukoba	1264	44%	56%	0.312, 33.492	1133m
	Lwanika	1113	32%	68%	0.351, 33.446	1128m

Note: A & B indicate what percentage of preserved *Biomphalaria* snails were morphotype-A or -B.

Sample Selection, Shell Categorisation and Species Identification:

All of the preserved *Biomphalaria* snails from Lake Albert and Lake Victoria were first categorised into whether they exhibited a morphotype-A or morphotype-B shell morphology (Figure 1). Once shells were categorised as either morphotype A or morphotype B, they were then placed into species groups based on conchological homogeneity (following the identification guide of Brown, 1994). This was conducted based upon how similar the shells looked to one another using specific shell characteristics such as whorl number, shell diameter, shell height and aperture shape. Once all of the shells were categorised, 20 individuals from each species group were randomly selected from each of the six sites.

For each snail, DNA was extracted using a modified CTAB extraction method as described in Joof et al. (2020) with the extracted samples resuspended in 100-200µl TE, pH 8.0 (10mM Tris-HCl, 0.1mM EDTA) buffer. Species identifications were confirmed by molecular methods using both 16S rRNA and cytochrome c oxidase subunit I genotyping. PCR amplifications were performed using a modified version of the 16S primers designed by Palumbi et al. (1991) (16Sarm: 3'-CTT CTC GAC TGT TTA TCA AAA ACA-'5 and 16Sbrm: 3'-GCC GGT CTG AAC TCA GAT CAT-'5) and the universal COI primers designed by Folmer et al. (1994) (LCO1490: 3'-GGT CAA ATC ATA AAG ATA TTG G-'5 and HCO2198: 3'-TAA ACT TCA GGG TGA CCA AAA AAT CA-'5). All PCR reactions were performed using Promega GoTaq® G2 Master Mix buffer, with 1µl of DNA template added to 24µl of 1X Master Mix buffer (1U TAQ, 0.2µM primers, 200µM dNTP, 3mM $MgCl_2$). The PCR cycling conditions used for both the 16S and COI primer sets were identical, with an initial denaturation at 96°C for 1minute, followed by 34 cycles of 94°C for 1min, 50°C for 1min, 72°C for 1min and a final extension at 72°C for 10mins. PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide and were observed under UV light. All 16S and COI PCR products were purified and sequenced using Macrogen's EZ-Seq service.

Both the 16S and COI sequences had their primer sequences removed and were cut down to match the base pair length of chosen GenBank references. Sequences were aligned using the Muscle algorithm in the program Seaview v5 (Gouy et al., 2021), with misaligned sections of the 16S and COI being fixed manually. Conserved sites were selected using the Gblocks program (Castresana et al., 2000). Samples were identified to the species-level using a concatenated 16S and COI phylogenetic tree incorporating GenBank reference sequences from studies that utilised both conchological and molecular identification methods (Jørgensen et al., 2007; Plam et al., 2008; Standley et al., 2014; Zhang et al., 2018, Supplementary Table 1). Phylogenetic trees were constructed using the Maximum Likelihood method, using a General Time Reversible model incorporating gamma rate correction (GTR+Γ) in the program PhyML v3.1 (Guindon et al., 2010), with bootstrap analysis undertaken using 1000 replicates.

Morphometric analysis:

In order to reduce any error associated with *Biomphalaria* shells due to indeterminate shell growth, several preventive steps were implemented into our morphometric analysis: (I) multiple individuals from different populations were used to average the plastic variation within the dataset; (II) only adult specimens were selected, to minimize the morphological variation between adult and juvenile shells; (III) a Procrustes fit analysis was used to remove the unwanted effects of translation, rotation and scaling of the dataset during landmark placement and (IV) outlier detection was used to excluding individuals that exhibit extreme morphological differences from the final analysis.

After identifying the *Biomphalaria* species found at the great lakes, ten shells from each species with no (or minimal) damage were selected from each site for photography. In addition to the African *Biomphalaria* samples, five laboratory-bred *B. glabrata* were also included as a comparative control. All shells were photographed using a dissection microscope with a 64MegaPixel mobile phone camera attached. All shells were positioned and photographed from the apical and apertural shell angles with a 1mm, 5mm and 10mm scalebar present. Shell diameter and shell height were measured using a dial caliper before each photo. Photographs were imported into the tpsDig2 v2.31 program (Rohlf, 2015), with each image being digitised using 14 landmarks for the apical view (4 fixed and 10 semi-landmark) and 15 landmarks for the apertural view (2 fixed and 13 semi-landmark) of the shell (Figure 3).

The landmark placement of the apical shell photos was guided by the landmark placement of Parra & Liria (2017). The coordinate data for the apical and apertural photos were stored in separate TPS files, and each sample was scaled and had a unique ID (e.g., ID=BS-1). The TPS files were then imported into the MorphoJ v1.07 program (Klingenberg, 2011). The apical shell data was treated as non-symmetrical, while the apertural shell data was treated as symmetrical due to the bilateral symmetry of the 15 landmarks. The data was grouped based on species and a full Procrustes fit was performed to help standardise the data and minimise any differences in object orientation or size.

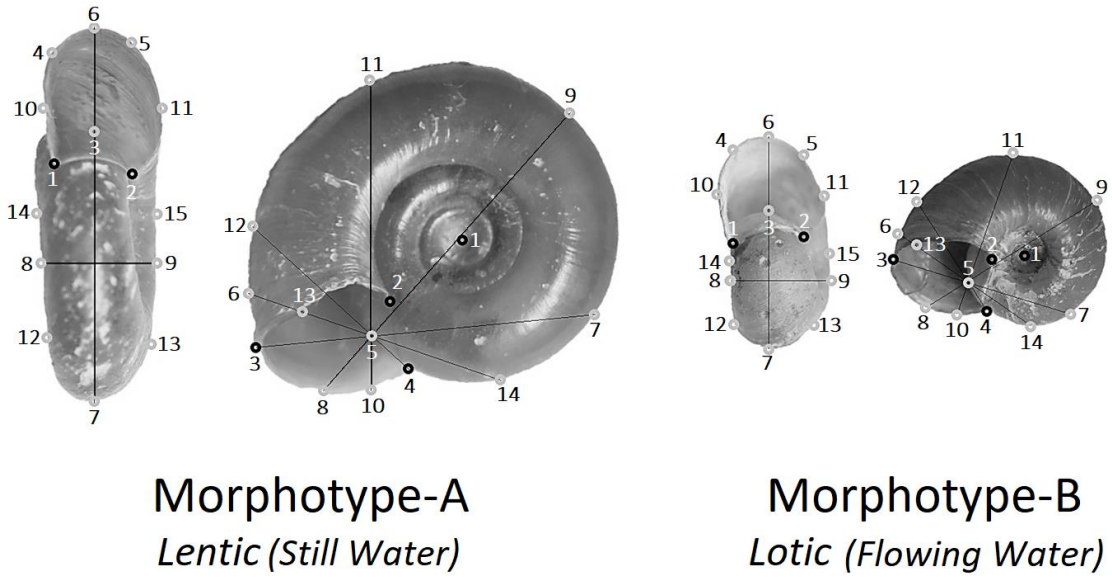

Figure 3. Fixed landmarks (black) and semi-landmarks (grey) on the morphotype-A (lentic) and morphotype-B (lotic) forms of *Biomphalaria* from the apertural (left) and apical (right) shell angles.

A Canonical Variate Analysis (CVA) (also known as Canonical Correlation Analysis or Linear Discrimination Analysis) was used across all landmarks using 10,000 permutations. A CVA is defined as a statistical technique used to analyse the relationship between two sets of variables. In this case,

our landmark coordinate data is the independent variable, while the dependent variables are our species groups as defined by 16S and COI genotyping. CVA is a multivariate analysis that is used to extract the most important information (called canonical variables) from a large and complex dataset. It is particularly useful when the goal is to identify patterns in the data that are not immediately obvious from the raw data itself. These newly created canonical variables are linear combinations of the original variables and are chosen based on how well they explain the variation between the original two datasets, with the first canonical variable (CV1) explaining the most variation, followed by the second (CV2) explaining the second most, and so on. CVA was chosen over other multivariate statistical techniques (like Principal Component Analysis) due to CVA being optimised for the classification and discrimination of groups within large datasets.

GenBank accession numbers

The DNA sequences generated in this study are available in GenBank accession numbers OQ924749-OQ924929 for the 16S gene and OQ849817-OQ849997 for the COI gene (supplementary table 1).

Results:

Species found and shell morphologies:

At Lake Albert, three *Biomphalaria* species (*B. pfeifferi*, *B. stanleyi* and *B. sudanica*) were identified using conchological and molecular methods (Figure 4). Walukuba had all three species present, Bugoigo had two species (*B. pfeifferi* and *B. sudanica*) and Piida had one (*B. sudanica*). At Lake Victoria, only *B. choanomphala* was present and was found at all three sites (Figure 4).

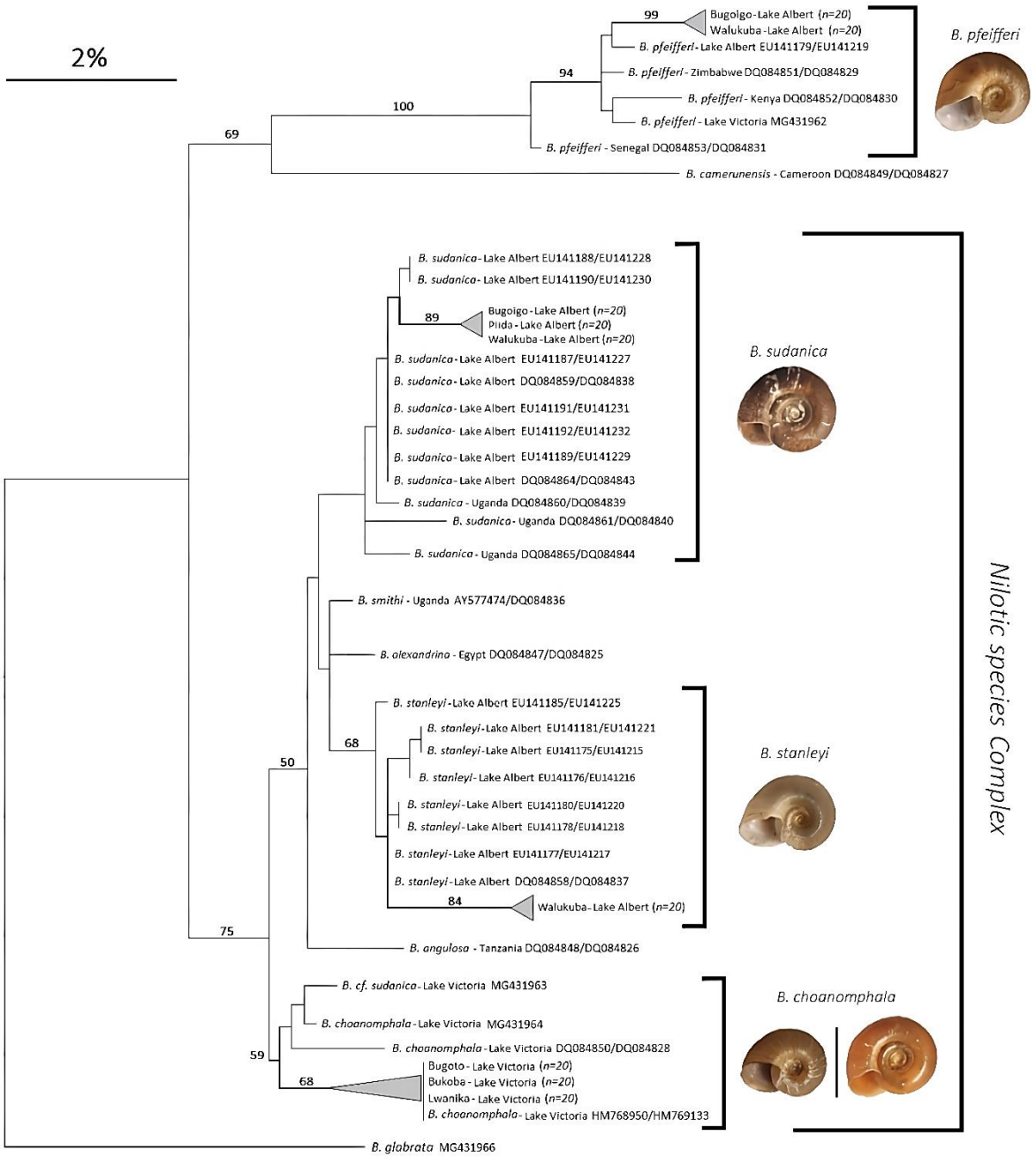

Figure 4. Maximum likelihood tree of the combined 16S rRNA (330bp) and cytochrome c oxidase subunit I (500bp) gene fragments. This tree was generated using PhyML v3.1 using a GTR+Γ model and is rooted on *Biomphalaria glabrata.* Numbers on branches indicate the bootstrap percentages for 1000 replicates (bootstrap values under 50% were not shown). The scale bar represents 2% sequence divergence. Samples labelled '*cf.*' had shell morphologies' that looked like a specific species but were identified as another species using molecular methods (from Jørgensen et al., 2007; Plam et al., 2008; Standley et al., 2014; Zhang et al., 2018).

At Lake Albert, there was no ecophenotypic variation within species. All 60 of the *B. sudanica* identified had morphotype-A shells, all 40 *B. pfeifferi* had morphotype-B shells and all 20 *B. stanleyi*

had morphotype-B shells (Table 2). Of the 2,645 preserved *Biomphalaria* snails from Lake Albert, approximately 54% of the shells were morphotype-A and were morphologically homogenous to *B. sudanica*. The remaining shells were morphotype-B, with approximately 43% of them being morphologically homogenous to *B. pfeifferi* and 3% being morphologically homogenous to *B. stanleyi*. Conversely, at Lake Victoria there was ecophenotypic variation within *B. choanomphala* with 45 of the 60 *B. choanomphala* identified having morphotype-B shells and the remaining 15 having morphotype-A shells. Of the 6,382 preserved *Biomphalaria* snails from Lake Victoria, approximately 75% of the shells were morphologically homogenous to morphotype-B *B. choanomphala*. The remaining 25% were morphologically homogenous to morphotype-A *B. choanomphala*.

The largest species of *Biomphalaria* found at the great lakes was *B. sudanica*, with a mean whorl number of 5.62, a mean shell diameter of 11mm and a mean shell height of 3.3mm (Table 2). The second largest species was the morphotype-A form of *B. choanomphala* with a mean whorl number of 5.86, a mean shell diameter of 9.9mm and a mean shell height of 3.6mm (Table 2). The third largest species was *B. pfeifferi* with a mean whorl number of 3.18, a mean shell diameter of 7.7mm and a mean shell height of 3.8mm (Table 2). The fourth largest species was the Morphotype-B form of *B. choanomphala* with a mean whorl number of 4.22, a mean shell diameter of 6.7mm and a mean shell height of 3.1mm. The smallest species found was *B. stanleyi* with a mean whorl number of 3.35, a mean shell diameter of 5.3mm and a mean shell height of 2.4mm (Table 2). In addition to the four *Biomphalaria* species found, an invasive *Gyraulus* species was identified at both Lake Albert and Lake Victoria (Supplementary figure 1). It had an appearance similar to juvenile *B. sudanica* but was significantly thinner with a mean shell height of 0.9mm, a mean whorl number of 4.55 and a mean shell diameter of 3.7mm.

Table 2. Mean shell diameter and height of photographed *Biomphalaria* shells.

	Sites	Species	Morphotype	Mean Shell Dimensions (mm)	
				Diameter (±SD)	Height (±SD)
-	Control	*B. glabrata (n=10)*	A	15.5 (±4.1)	5.2 (±0.9)
Lake Albert	Bugoigo	*B. sudanica* (*n=10*)	A	11.7 (±1.6)	3.3 (±0.4)
		B. pfeifferi (*n=10*)	B	7.6 (±1.8)	3.7 (±0.8)
	Piida	*B. sudanica* (*n=10*)	A	11.6 (±2.9)	3.4 (±0.3)
	Walukuba	*B. sudanica* (*n=10*)	A	9.5 (±2.1)	3.1 (±0.4)
		B. pfeifferi (*n=10*)	B	7.8 (±1.6)	3.9 (±0.8)
		B. stanleyi (*n=10*)	B	5.3 (±0.5)	2.4 (±0.1)
Lake Victoria	Bugoto	*B. choanomphala* (*n=10*)	B	6.6 (±0.5)	3.1 (±0.2)
	Bukoba	*B. choanomphala* (*n=10*)	B	6.2 (±0.6)	3.3 (±0.1)
	Lwanika	*B. choanomphala* (*n=10*)	B	7.2 (±0.5)	3 (±0.4)
	All Sites	*B. choanomphala* (*n=10*)	A	9.9 (±1.3)	3.6 (±0.3)

Note: 'S.D' stands for standard deviation.

Morphometrics:

When a canonical variate analysis (CVA) was performed on the *Biomphalaria* samples found at Lake Albert, we found that the three species of *Biomphalaria* present at Lake Albert were clearly separated from one other when using both the apical (CV1: 95.6% and CV2: 4.4%) and apertural (CV1: 98.1% and CV2: 1.9%) datasets (Figure 5a). For Lake Victoria, the two morphotypes of *B. choanomphala* overlapped with one another in CVA analysis using the apical dataset (CV1: 85.6% and CV2: 14.4%) (Figure 5b) but were separated when using the apertural dataset (CV1: 95% and CV2: 5%) (Figure 5b).

When a CVA was performed on all *Biomphalaria* samples obtained from both lakes combined, we found that all four species were clearly separated from one another using the apical dataset (CV1: 79.3% and CV2: 17.3%) (Figure 5c). For the apertural dataset, *B. pfeifferi* showed overlap (albeit minimal) with B. stanleyi, and B. sudanica showed large amounts of overlap with the morphotype-A form of *B. choanomphala* (CV1: 76.9% and CV2: 16.9%) (Figure 5c). The two morphotypes of *B. choanomphala* (morphotypes A and B) overlapped for the apical dataset but when using the apertural dataset the morphotype A and B forms of *B. choanomphala* were separate (Figure 5c).

Our CVA plots found the apical dataset more informative at differentiating the Lake Albert species from one another than the apertural dataset (Figure 5a). The apical dataset was also capable of differentiating the Lake Albert species from *B. choanomphala* (Figure 5c). While the apertural

dataset was able to differentiate the Lake Albert species and both of the *B. choanomphala* morphotypes when tested separately, it was only able to differentiate the morphotype-B form of *B. choanomphala* when testing all samples together. Additional information relating to the morphological differences between species that had overlapping morphological characteristics can be found in supplementary figure 2.

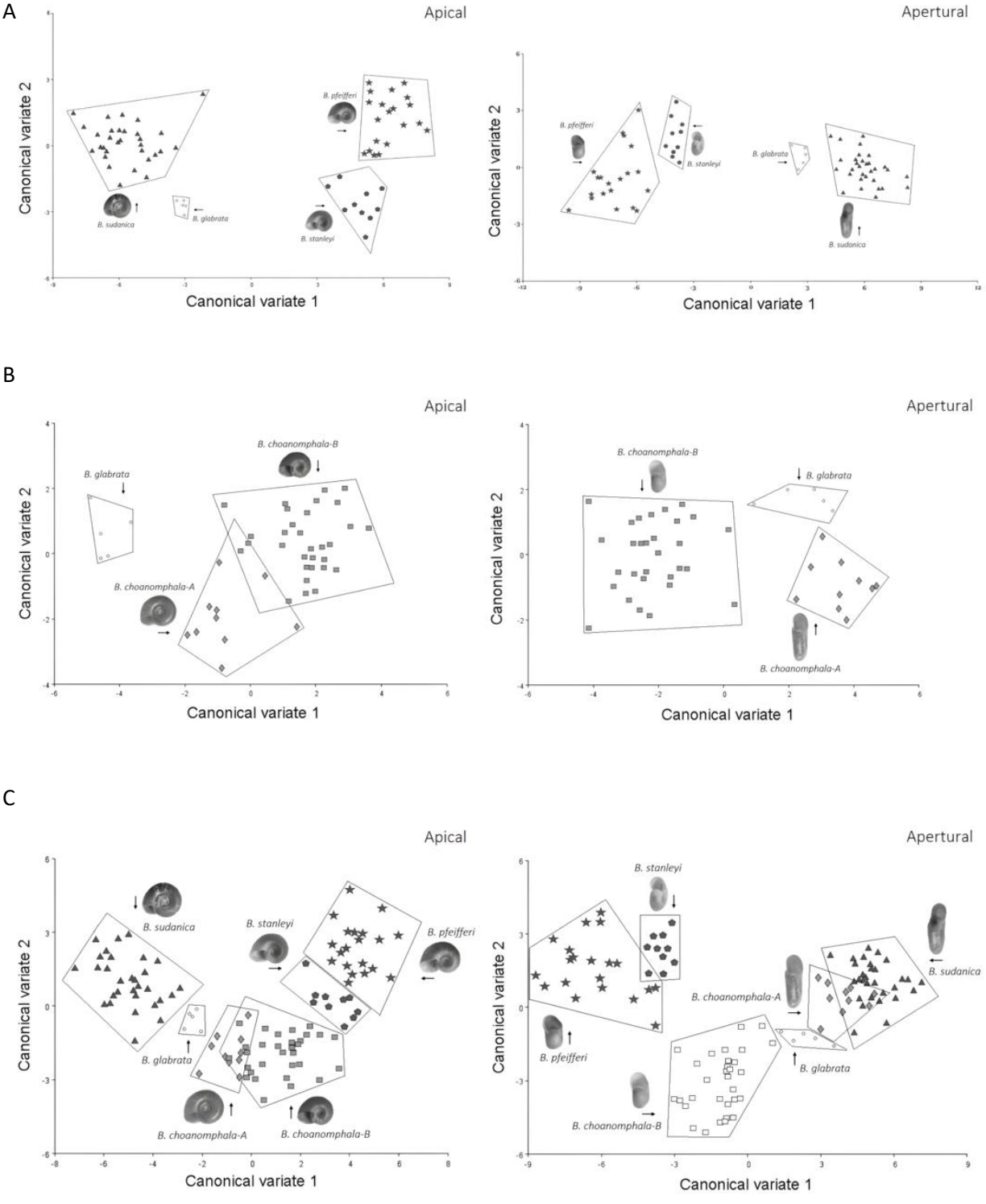

Figure 5. Canonical variate analysis plots of the apical and apertural shell landmark datasets. (A) CVA plot of the *Biomphalaria* species present at Lake Albert (*B. pfeifferi* (*n=20*), *B. stanleyi* (*n=10*) and *B. sudanica* (*n=30*)). (B) CVA plot of *Biomphalaria* species present at Lake Victoria (*B. choanomphala* morphotype-A (*n=10*) and *B. choanomphala* morphotype-B (*n=30*)). (C) CVA plot of *Biomphalaria* species present at both lakes (*B. choanomphala* morphotype-A (*n=10*), *B. choanomphala* morphotype-B (*n=30*), *B. pfeifferi* (*n=20*), *B. stanleyi* (*n=10*) and *B. sudanica* (*n=30*)). All CVA plots contain *B. glabrata* (*n=5*) as control. All samples from Lake Albert are coloured black, samples from Lake Victoria are coloured grey and the *B. glabrata* samples are coloured white. *B. choanomphala-A* = ◊, *B. choanomphala-B* = □, *B. glabrata* = ○, *B. stanleyi* = ⬠, *B. sudanica* = △ and *B. pfeifferi* = ☆.

Discussion:

The morphological identification of intermediate snail hosts is the first step in efficiently interrupting schistosomiasis transmission (Abe et al., 2018). Whilst identification conducted in the field using morphology is useful due to its simplicity and low cost, certain species (such as sympatric *Biomphalaria*) cannot be easily distinguished by morphology alone. Alternatively, more precise methods are required when trying to identify similar species that co-inhabit the same environment (Webster, & Sheets, 2010; Palasio et al., 2017; Vaux et al., 2018). Our study is the first contribution that utilises landmark-based geometric morphometric techniques to differentiate sympatric *Biomphalaria* species. Previous conchological morphology studies of *Biomphalaria* have categorised species based on whether they exhibited a “lacustrine” morphology (found within a lake) or a “non-lacustrine” morphology (found elsewhere) (DeJong et al., 2001; Kazibwe et al., 2006; Plam et al., 2008; Kazibwe et al., 2010). However, these categories are contradictory to the terminology proposed by Dillon (2019) as the lacustrine morphology is equivalent to the lotic (morphotype-B) morphotype, but lakes are described as lentic ecosystems. Moreover, we found both “lacustrine” (morphotype-B) and “non-lacustrine” (morphotype-A) shells at both Lake Albert and Lake Victoria, making the differentiation arbitrary.

The ratio of morphotype-A and morphotype-B shells present at each site varied (Table 1). This could indicate that some of the sites were more preferable for one of the morphotype than another. For example, at Lake Albert, morphotype-A shells were predominantly found at Bugoigo and Piida, while morphotype-B shells were predominantly found at Walukuba. It is worth noting that the shoreline of Walukuba is much more open to wave action than that at Bugoigo and Piida, which are each nested behind large spits that protrude into the lake. Bugoigo and Piida both have lentic ecosystems in the form of sheltered marshlands, while Walukuba is a more lotic ecosystem (Figure 2; Supplementary figure 3). Likewise, Bugoto had the lowest number of morphotype-A shells and was an

unprotected shoreline, while Bukoba (and Lwanika) had the highest number of morphotype-A shells and had a lentic ecosystem in the form of sheltered vegetation protected by coves (Figure 2; Supplementary figure 3). Alternatively, shell morphology could also be influenced by other factors besides the flowrate of the ecosystem such as parasitism, predation and temperature (Haas, 2003; Hoverman et al. 2005; Holomuzki & Biggs, 2006; Hoverman & Relyea, 2007; Lagrue et al., 2007; Vasallo et al., 2013; Parra & Liria, 2017; Tamburi et al., 2018). Of the four *Biomphalaria* species we identified at the great lakes, only *B. choanomphala* was found to exhibit more than one ecophenotype. Our findings are consistent with both Standley et al. (2011) and Zhang et al. (2018) who found that *B. sudanica*-like snails at Lake Victoria were more genetically similar to *B. choanomphala* than to *B. sudanica* from other African countries and should in fact be classified as *B. choanomphala* and not *B. sudanica*. Similarly, Standley et al. (2012) found the morphotype-A and morphotype-B forms of *B. choanomphala* were present across the entire shoreline of Lake Victoria. Moreover, their Bayesian analysis found each ecophenotype inhabited separate ecological niches from one another, with specific abiotic variables (e.g. chloride, nitrate, sulphate, pH and water depth) being significant predictors of which morphotype would be present in a given ecosystem.

When measuring each of the *Biomphalaria* species found at the great lakes, *B. sudanica* had the largest mean shell diameter (11mm) followed by the morphotype-A form of *B. choanomphala* (9.9mm), then *B. pfeifferi* (7.7mm), then the morphotype-B form of *B. choanomphala* (6.7mm) and finally *B. stanleyi* (5.3mm). Conversely, the *Biomphalaria* species with the largest mean shell height was the morphotype-A form of *B. choanomphala* (3.6mm) followed by *B. pfeifferi* (3.8mm), then *B. sudanica* (3.3mm), then the morphotype-B form of *B. choanomphala* (3.1mm) and finally *B. stanleyi* (2.4mm). However, these shell characteristics alone were not dependable enough to distinguish *Biomphalaria* species. Further examination using a CVA found the apical shell angle was more informative at distinguishing *Biomphalaria* species from one another than using the apertural shell angle (Figure 5). The *Biomphalaria* species found at Lake Albert were morphologically distinct from each other when using both the apical and (to a lesser extent) the apertural dataset (Figure 5a). When a CVA was performed on the two *B. choanomphala* morphotypes found at Lake Victoria, the apical dataset showed an overlap between the two, while the apertural dataset did not (Figure 5b). This showed despite the apparent difference in shell diameter and height, the apical dataset was able to find homogenous characteristics between the two *B. choanomphala* morphotypes.

Previous studies of the great lakes consistently report *B. pfeifferi*, *B. sudanica* and *B. stanleyi* at Lake Albert and *B. choanomphala* at Lake Victoria (Brown, 1994; Jørgensen et al., 2007; Plam et al., 2008; Adriko et al., 2013; Zhang et al., 2018; Rowel et al., 2015; Mutuku et al., 2019). However, despite the long-established history of *B. choanomphala* being endemic to Lake Victoria, Plam et al. (2008)

found *B. choanomphala* at Lake Albert, though this might have been an ephemeral presence. Similarly, Zhang et al. (2018) found *B. pfeifferi* in streams leading into Lake Victoria. These cases of atypical *Biomphalaria* species being found at each of the great lakes is likely due to the invasive nature of *Biomphalaria* (Pointer et al., 2005). Therefore, the possibility of *Biomphalaria* species from one lake being introduced to another is very likely. Newly introduced *Biomphalaria* species could affect the transmission rates of intestinal schistosomiasis at the great lakes as some *Biomphalaria* species are less resistant to *S. mansoni* infection than others (Brown, 1994; Morgan et al., 2001; Campbell et al., 2010; Stensgaard et al., 2013; Lu et al., 2016).

When CVA was used to compare the *Biomphalaria* species at both Lake Albert and Lake Victoria using the apical dataset, the Lake Albert species (*B. pfeifferi*, *B. stanleyi* and *B. sudanica*) were distinguishable from each other and from *B. choanomphala*. However, the two *B. choanomphala* morphotypes overlapped with each other (Figure 5c). The apertural dataset was not as effective as the apical dataset at distinguishing the species, with only the morphotype-B form of *B. choanomphala* being distinct. The morphotype-A form of *B. choanomphala* overlaps with *B. sudanica*. Likewise, *B. pfeifferi* overlaps with *B. stanleyi* (Figure 5c).

Our novel use of CVA has been proven able to differentiate the shell morphologies of four *Biomphalaria* species. CVA in future has the potential for improving conchological identification methods used in the field. Further research is needed encompassing a larger variety of specimens, populations, species, and locations to confirm the true effectiveness and integrity of this technique.

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Conflicts of interest:

None

Ethical statement:

None

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Supplementary:

Supplementary Table 1. GenBank accession numbers and corresponding references for the 16S/COI phylogenetic tree.

GenBank				
Code	Accession no.		Species	Reference
	16S rRNA	COI		
BsmRwe1	AY577474	DQ084836	*smithi*	Jørgensen et al. (2007)
BalDBL1	DQ084847	DQ084825	*alexandrina*	Jørgensen et al. (2007)
BanRua1	DQ084848	DQ084826	*angulosa*	Jørgensen et al. (2007)
BcaBak1	DQ084849	DQ084827	*camerunensis*	Jørgensen et al. (2007)
BchVic1	DQ084850	DQ084828	*choanomphala*	Jørgensen et al. (2007)
BpfChi1	DQ084851	DQ084829	*pfeifferi*	Jørgensen et al. (2007)
BpfKib1	DQ084852	DQ084830	*pfeifferi*	Jørgensen et al. (2007)
BpfDeG1	DQ084853	DQ084831	*pfeifferi*	Jørgensen et al. (2007)
BstBut1	DQ084858	DQ084837	*stanleyi*	Jørgensen et al. (2007)
BsuBut1	DQ084859	DQ084838	*sudanica*	Jørgensen et al. (2007)
BsuKin1	DQ084860	DQ084839	*sudanica*	Jørgensen et al. (2007)
BsuMah1	DQ084861	DQ084840	*sudanica*	Jørgensen et al. (2007)
BsuNto1	DQ084864	DQ084843	*sudanica*	Jørgensen et al. (2007)
BsuRut1	DQ084865	DQ084844	*sudanica*	Jørgensen et al. (2007)
FL1	EU141175	EU141215	*stanleyi*	Plam et al. (2008)
FL2	EU141176	EU141216	*stanleyi*	Plam et al. (2008)
FL3	EU141177	EU141217	*stanleyi*	Plam et al. (2008)
FL4	EU141178	EU141218	*stanleyi*	Plam et al. (2008)
FL5	EU141179	EU141219	*pfeifferi*	Plam et al. (2008)
FL6	EU141180	EU141220	*stanleyi*	Plam et al. (2008)
FN1	EU141181	EU141221	*stanleyi*	Plam et al. (2008)
FN5	EU141185	EU141225	*stanleyi*	Plam et al. (2008)
SN1	EU141187	EU141227	*sudanica*	Plam et al. (2008)
SN2	EU141188	EU141228	*sudanica*	Plam et al. (2008)
SN3	EU141189	EU141229	*sudanica*	Plam et al. (2008)
SN4	EU141190	EU141230	*sudanica*	Plam et al. (2008)
SN5	EU141191	EU141231	*sudanica*	Plam et al. (2008)
SN6	EU141192	EU141232	*sudanica*	Plam et al. (2008)
-	MG431962	MG431962	*pfeifferi*	Zhang et al. (2018)
-	MG431963	MG431963	*cf. sudanica*	Zhang et al. (2018)
-	MG431964	MG431964	*choanomphala*	Zhang et al. (2018)
-	MG431966	MG431966	*glabrata*	Zhang et al. (2018)
CJS-2010	HM768950	HM769133	*choanomphala*	Standley et al. (2014)

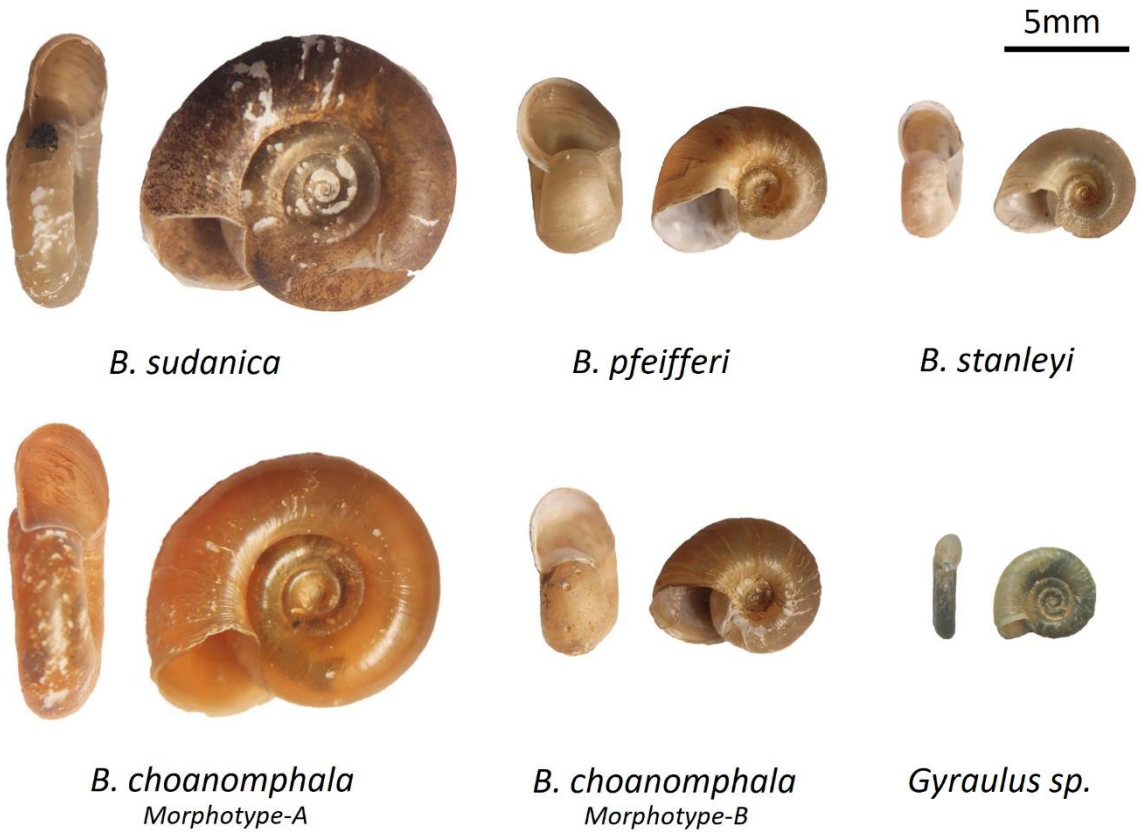
Our sequences				
Code	Accession no.		Species	Site/Lake
	16S	COI		
BA1R1	OQ924829	OQ849897	*sudanica*	Bugoigo/LA
BA102	OQ924830	OQ849898	*sudanica*	Bugoigo/LA
BA103	OQ924831	OQ849899	*sudanica*	Bugoigo/LA
BA104	OQ924832	OQ849900	*sudanica*	Bugoigo/LA
BA105	OQ924833	OQ849901	*sudanica*	Bugoigo/LA
BA106	OQ924834	OQ849902	*sudanica*	Bugoigo/LA
BA107	OQ924835	OQ849903	*sudanica*	Bugoigo/LA
BA108	OQ924836	OQ849904	*sudanica*	Bugoigo/LA
BA109	OQ924837	OQ849905	*sudanica*	Bugoigo/LA
BA111	OQ924838	OQ849906	*sudanica*	Bugoigo/LA
BA112	OQ924839	OQ849907	*sudanica*	Bugoigo/LA
BA113	OQ924840	OQ849908	*sudanica*	Bugoigo/LA
BA114	OQ924841	OQ849909	*sudanica*	Bugoigo/LA
BA115	OQ924842	OQ849910	*sudanica*	Bugoigo/LA
BA116	OQ924843	OQ849911	*sudanica*	Bugoigo/LA
BB103	OQ924844	OQ849912	*sudanica*	Bugoigo/LA
BB110	OQ924845	OQ849913	*sudanica*	Bugoigo/LA
BB111	OQ924846	OQ849914	*sudanica*	Bugoigo/LA
BB113	OQ924847	OQ849915	*sudanica*	Bugoigo/LA
B2G04	OQ924848	OQ849916	*sudanica*	Bugoigo/LA
B1B15	OQ924789	OQ849857	*pfeifferi*	Bugoigo/LA
B1B17	OQ924790	OQ849858	*pfeifferi*	Bugoigo/LA
B1B19	OQ924791	OQ849859	*pfeifferi*	Bugoigo/LA
B1B20	OQ924792	OQ849860	*pfeifferi*	Bugoigo/LA
B2B01	OQ924793	OQ849861	*pfeifferi*	Bugoigo/LA
B2B03	OQ924794	OQ849862	*pfeifferi*	Bugoigo/LA
B2B04	OQ924795	OQ849863	*pfeifferi*	Bugoigo/LA
B2B07	OQ924796	OQ849864	*pfeifferi*	Bugoigo/LA
B2B08	OQ924797	OQ849865	*pfeifferi*	Bugoigo/LA
B7A10	OQ924798	OQ849866	*pfeifferi*	Bugoigo/LA
B7B10	OQ924799	OQ849867	*pfeifferi*	Bugoigo/LA
B7C10	OQ924800	OQ849868	*pfeifferi*	Bugoigo/LA
B7D08	OQ924801	OQ849869	*pfeifferi*	Bugoigo/LA
B7D09	OQ924802	OQ849870	*pfeifferi*	Bugoigo/LA
B7E09	OQ924803	OQ849871	*pfeifferi*	Bugoigo/LA
B7E10	OQ924804	OQ849872	*pfeifferi*	Bugoigo/LA
B7F07	OQ924805	OQ849873	*pfeifferi*	Bugoigo/LA
B7F09	OQ924806	OQ849874	*pfeifferi*	Bugoigo/LA
B7H07	OQ924807	OQ849875	*pfeifferi*	Bugoigo/LA
B7H09	OQ924808	OQ849876	*pfeifferi*	Bugoigo/LA
PA101	OQ924849	OQ849917	*sudanica*	Piida/LA
PA102	OQ924850	OQ849918	*sudanica*	Piida/LA
PA103	OQ924851	OQ849919	*sudanica*	Piida/LA
PA107	OQ924852	OQ849920	*sudanica*	Piida/LA
PA108	OQ924853	OQ849921	*sudanica*	Piida/LA
PA109	OQ924854	OQ849922	*sudanica*	Piida/LA

PA110	OQ924855	OQ849923	*sudanica*	Piida/LA
PA112	OQ924856	OQ849924	*sudanica*	Piida/LA
PA117	OQ924857	OQ849925	*sudanica*	Piida/LA
PA118	OQ924858	OQ849926	*sudanica*	Piida/LA
PA119	OQ924859	OQ849927	*sudanica*	Piida/LA
PA201	OQ924860	OQ849928	*sudanica*	Piida/LA
PA203	OQ924861	OQ849929	*sudanica*	Piida/LA
PA207	OQ924862	OQ849930	*sudanica*	Piida/LA
PA211	OQ924863	OQ849931	*sudanica*	Piida/LA
PA212	OQ924864	OQ849932	*sudanica*	Piida/LA
PA214	OQ924865	OQ849933	*sudanica*	Piida/LA
PA215	OQ924866	OQ849934	*sudanica*	Piida/LA
PA217	OQ924867	OQ849935	*sudanica*	Piida/LA
PA219	OQ924868	OQ849936	*sudanica*	Piida/LA
WA109	OQ924809	OQ849877	*sudanica*	Walukuba/LA
WA111	OQ924810	OQ849878	*sudanica*	Walukuba/LA
W2B09	OQ924811	OQ849879	*sudanica*	Walukuba/LA
W2B10	OQ924812	OQ849880	*sudanica*	Walukuba/LA
W2E9	OQ924813	OQ849881	*sudanica*	Walukuba/LA
W2E10	OQ924814	OQ849882	*sudanica*	Walukuba/LA
W2F01	OQ924815	OQ849883	*sudanica*	Walukuba/LA
W2F02	OQ924816	OQ849884	*sudanica*	Walukuba/LA
W2F03	OQ924817	OQ849885	*sudanica*	Walukuba/LA
W2F04	OQ924818	OQ849886	*sudanica*	Walukuba/LA
W2F05	OQ924819	OQ849887	*sudanica*	Walukuba/LA
W2F06	OQ924820	OQ849888	*sudanica*	Walukuba/LA
W2F07	OQ924821	OQ849889	*sudanica*	Walukuba/LA
W2F08	OQ924822	OQ849890	*sudanica*	Walukuba/LA
W2F09	OQ924823	OQ849891	*sudanica*	Walukuba/LA
W2G01	OQ924824	OQ849892	*sudanica*	Walukuba/LA
W2G03	OQ924825	OQ849893	*sudanica*	Walukuba/LA
W2G06	OQ924826	OQ849894	*sudanica*	Walukuba/LA
W2G07	OQ924827	OQ849895	*sudanica*	Walukuba/LA
W2G08	OQ924828	OQ849896	*sudanica*	Walukuba/LA
WA103	OQ924769	OQ849837	*pfeifferi*	Walukuba/LA
WA104	OQ924770	OQ849838	*pfeifferi*	Walukuba/LA
WA107	OQ924771	OQ849839	*pfeifferi*	Walukuba/LA
WA108	OQ924772	OQ849840	*pfeifferi*	Walukuba/LA
WA112	OQ924773	OQ849841	*pfeifferi*	Walukuba/LA
WB101	OQ924774	OQ849842	*pfeifferi*	Walukuba/LA
WB102	OQ924775	OQ849843	*pfeifferi*	Walukuba/LA
WB103	OQ924776	OQ849844	*pfeifferi*	Walukuba/LA
WB104	OQ924777	OQ849845	*pfeifferi*	Walukuba/LA
WB105	OQ924778	OQ849846	*pfeifferi*	Walukuba/LA
WB106	OQ924779	OQ849847	*pfeifferi*	Walukuba/LA
WB107	OQ924780	OQ849848	*pfeifferi*	Walukuba/LA
WB108	OQ924781	OQ849849	*pfeifferi*	Walukuba/LA
WB109	OQ924782	OQ849850	*pfeifferi*	Walukuba/LA
WB110	OQ924783	OQ849851	*pfeifferi*	Walukuba/LA

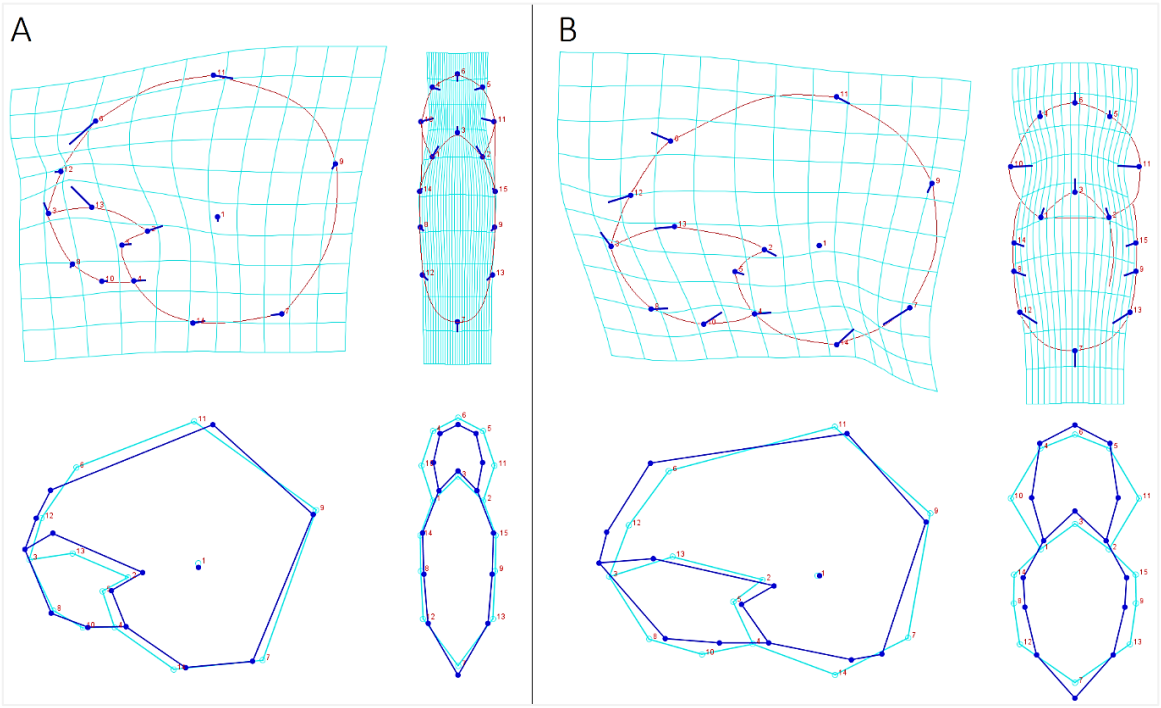
WB111	OQ924784	OQ849852	*pfeifferi*	Walukuba/LA
WB112	OQ924785	OQ849853	*pfeifferi*	Walukuba/LA
WB114	OQ924786	OQ849854	*pfeifferi*	Walukuba/LA
WB116	OQ924787	OQ849855	*pfeifferi*	Walukuba/LA
WB118	OQ924788	OQ849856	*pfeifferi*	Walukuba/LA
WA101	OQ924749	OQ849817	*stanleyi*	Walukuba/LA
WA102	OQ924750	OQ849818	*stanleyi*	Walukuba/LA
WA105	OQ924751	OQ849819	*stanleyi*	Walukuba/LA
W6A03	OQ924752	OQ849820	*stanleyi*	Walukuba/LA
W6A04	OQ924753	OQ849821	*stanleyi*	Walukuba/LA
W6A05	OQ924754	OQ849822	*stanleyi*	Walukuba/LA
W7A07	OQ924755	OQ849823	*stanleyi*	Walukuba/LA
W7C09	OQ924756	OQ849824	*stanleyi*	Walukuba/LA
W7D10	OQ924757	OQ849825	*stanleyi*	Walukuba/LA
W7F10	OQ924758	OQ849826	*stanleyi*	Walukuba/LA
W9A01	OQ924759	OQ849827	*stanleyi*	Walukuba/LA
W9B01	OQ924760	OQ849828	*stanleyi*	Walukuba/LA
W9C01	OQ924761	OQ849829	*stanleyi*	Walukuba/LA
W9D01	OQ924762	OQ849830	*stanleyi*	Walukuba/LA
W9D02	OQ924763	OQ849831	*stanleyi*	Walukuba/LA
W9E01	OQ924764	OQ849832	*stanleyi*	Walukuba/LA
W9F01	OQ924765	OQ849833	*stanleyi*	Walukuba/LA
W9F03	OQ924766	OQ849834	*stanleyi*	Walukuba/LA
W9G01	OQ924767	OQ849835	*stanleyi*	Walukuba/LA
W9H01	OQ924768	OQ849836	*stanleyi*	Walukuba/LA
BG2A08	OQ924869	OQ849937	*choanomphala*	Bugoto/LV
BG2A10	OQ924870	OQ849938	*choanomphala*	Bugoto/LV
BG2E04	OQ924871	OQ849939	*choanomphala*	Bugoto/LV
BG2G09	OQ924872	OQ849940	*choanomphala*	Bugoto/LV
BG6B05	OQ924873	OQ849941	*choanomphala*	Bugoto/LV
BG6C05	OQ924874	OQ849942	*choanomphala*	Bugoto/LV
BG6C06	OQ924875	OQ849943	*choanomphala*	Bugoto/LV
BG6C07	OQ924876	OQ849944	*choanomphala*	Bugoto/LV
BG6D05	OQ924877	OQ849945	*choanomphala*	Bugoto/LV
BG6D08	OQ924878	OQ849946	*choanomphala*	Bugoto/LV
BG6D09	OQ924879	OQ849947	*choanomphala*	Bugoto/LV
BG6E05	OQ924880	OQ849948	*choanomphala*	Bugoto/LV
BG6E06	OQ924881	OQ849949	*choanomphala*	Bugoto/LV
BG6E07	OQ924882	OQ849950	*choanomphala*	Bugoto/LV
BG6E09	OQ924883	OQ849951	*choanomphala*	Bugoto/LV
BG6F08	OQ924884	OQ849952	*choanomphala*	Bugoto/LV
BG6F09	OQ924885	OQ849953	*choanomphala*	Bugoto/LV
BG6G06	OQ924886	OQ849954	*choanomphala*	Bugoto/LV
BG6G07	OQ924887	OQ849955	*choanomphala*	Bugoto/LV
BG6G09	OQ924888	OQ849956	*choanomphala*	Bugoto/LV
BKLV01	OQ924889	OQ849957	*choanomphala*	Bukoba/LV
BKLV08	OQ924890	OQ849958	*choanomphala*	Bukoba/LV
BKLV10	OQ924891	OQ849959	*choanomphala*	Bukoba/LV
BK2A04	OQ924892	OQ849960	*choanomphala*	Bukoba/LV

BK2A07	OQ924893	OQ849961	*choanomphala*	Bukoba/LV
BK2C06	OQ924894	OQ849962	*choanomphala*	Bukoba/LV
BK2E01	OQ924895	OQ849963	*choanomphala*	Bukoba/LV
BK2E03	OQ924896	OQ849964	*choanomphala*	Bukoba/LV
BK2E05	OQ924897	OQ849965	*choanomphala*	Bukoba/LV
BK2E06	OQ924898	OQ849966	*choanomphala*	Bukoba/LV
BK2E07	OQ924899	OQ849967	*choanomphala*	Bukoba/LV
BK2E08	OQ924900	OQ849968	*choanomphala*	Bukoba/LV
BK6G08	OQ924901	OQ849969	*choanomphala*	Bukoba/LV
BK7D11	OQ924902	OQ849970	*choanomphala*	Bukoba/LV
BK7E11	OQ924903	OQ849971	*choanomphala*	Bukoba/LV
BK7E12	OQ924904	OQ849972	*choanomphala*	Bukoba/LV
BK7H12	OQ924905	OQ849973	*choanomphala*	Bukoba/LV
BK9B03	OQ924906	OQ849974	*choanomphala*	Bukoba/LV
BK9C03	OQ924907	OQ849975	*choanomphala*	Bukoba/LV
BK9F04	OQ924908	OQ849976	*choanomphala*	Bukoba/LV
LW2D02	OQ924909	OQ849977	*choanomphala*	Lwanika/LV
LW2D03	OQ924910	OQ849978	*choanomphala*	Lwanika/LV
LW2D08	OQ924911	OQ849979	*choanomphala*	Lwanika/LV
LW2H07	OQ924912	OQ849980	*choanomphala*	Lwanika/LV
LW2H09	OQ924913	OQ849981	*choanomphala*	Lwanika/LV
LW6B02	OQ924914	OQ849982	*choanomphala*	Lwanika/LV
LW6C02	OQ924915	OQ849983	*choanomphala*	Lwanika/LV
LW6D03	OQ924916	OQ849984	*choanomphala*	Lwanika/LV
LW6D10	OQ924917	OQ849985	*choanomphala*	Lwanika/LV
LW6E10	OQ924918	OQ849986	*choanomphala*	Lwanika/LV
LW6F02	OQ924919	OQ849987	*choanomphala*	Lwanika/LV
LW6F04	OQ924920	OQ849988	*choanomphala*	Lwanika/LV
LW6F10	OQ924921	OQ849989	*choanomphala*	Lwanika/LV
LW6G01	OQ924922	OQ849990	*choanomphala*	Lwanika/LV
LW6G02	OQ924923	OQ849991	*choanomphala*	Lwanika/LV
LW7B11	OQ924924	OQ849992	*choanomphala*	Lwanika/LV
LW7C11	OQ924925	OQ849993	*choanomphala*	Lwanika/LV
LW7D12	OQ924926	OQ849994	*choanomphala*	Lwanika/LV
LW7F12	OQ924927	OQ849995	*choanomphala*	Lwanika/LV
LW9E04	OQ924928	OQ849996	*choanomphala*	Lwanika/LV
Gsp	OQ924929	OQ849997	*Gyraulus sp.*	-

Note: '*cf.*' indicates the shell morphology looked like a specific species but was identified as a different species using molecular methods.



Supplementary Figure 1. Shell morphologies of Planorbidae snails found at the Ugandan shorelines of Lake Albert and Lake Victoria. *Biomphalaria pfeifferi, B. stanleyi, B. sudanica* and the unknown *Gyraulus sp.* were present at Lake Albert. *Biomphalaria choanomphala* and the unknown *Gyraulus sp.* were present at Lake Victoria. The shells are viewed from the apertural (left) and apical (right) shell angles.



Supplementary Figure 2. Morphometric comparison between (A) *B. choanomphala*-A and *B. sudanica* and (B) *B. pfeifferi* and *B. stanleyi* using a lollipop graph (top) and wireframe graph (bottom) of the apical and apertural shell angles.

Supplementary Figure 3. Satellite views of the Lake Albert (A: Bugoigo; B: Piida; C: Walukuba) and Lake Victoria (D: Bugoto; E: Bukoba; F: Lwanika) collection sites. Satellite imaging was provided by Google Maps (Google, 2022).