

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

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## Background:

The freshwater snail genus *Biomphalaria*, is the intermediate host of the intravascular parasite genus, *Schistosoma*.<sup>1</sup> The leading cause of intestinal schistosomiasis in humans across the globe is caused by *Schistosoma mansoni*, with approximately 83.3 million infected and over 390 million people at risk of infection.<sup>2</sup> When identifying African *Biomphalaria* species using molecular methods, only two species are definitive (*B. camerunensis* and *B. pfeifferi*), with the six remaining species being placed into a poorly defined clade named the "Nilotic Species Complex" (*B. alexandrina*, *B. angulosa*, *B. choanomphala*, *B. smithi*, *B. stanleyi* and *B. sudanica*).<sup>3</sup> Molecular identification methods are seen as more accurate than morphological methods, with the most reliable morphological identification method being genital morphological identification, but requires a lot of training, expertise time and equipment. Alternatively, identifying *Biomphalaria* snails using shell morphology is more rapid and inexpensive, but is not as reliable due to the lack of non-plastic shell characteristics and the indeterminate shell growth during development associated with Planorbidae shells resulting in ecophenotypic variation.<sup>4, 5, 6</sup> A potential solution to the issues of conchological identification methods is to incorporate landmark-based geometric morphometrics, a powerful tool used to quantify and analyse the size and shape variation between organisms. Our study applied landmark-based morphometric techniques to investigation the conchological differences between *Biomphalaria* species collected from the Ugandan shorelines of Lake Albert and Lake Victoria.

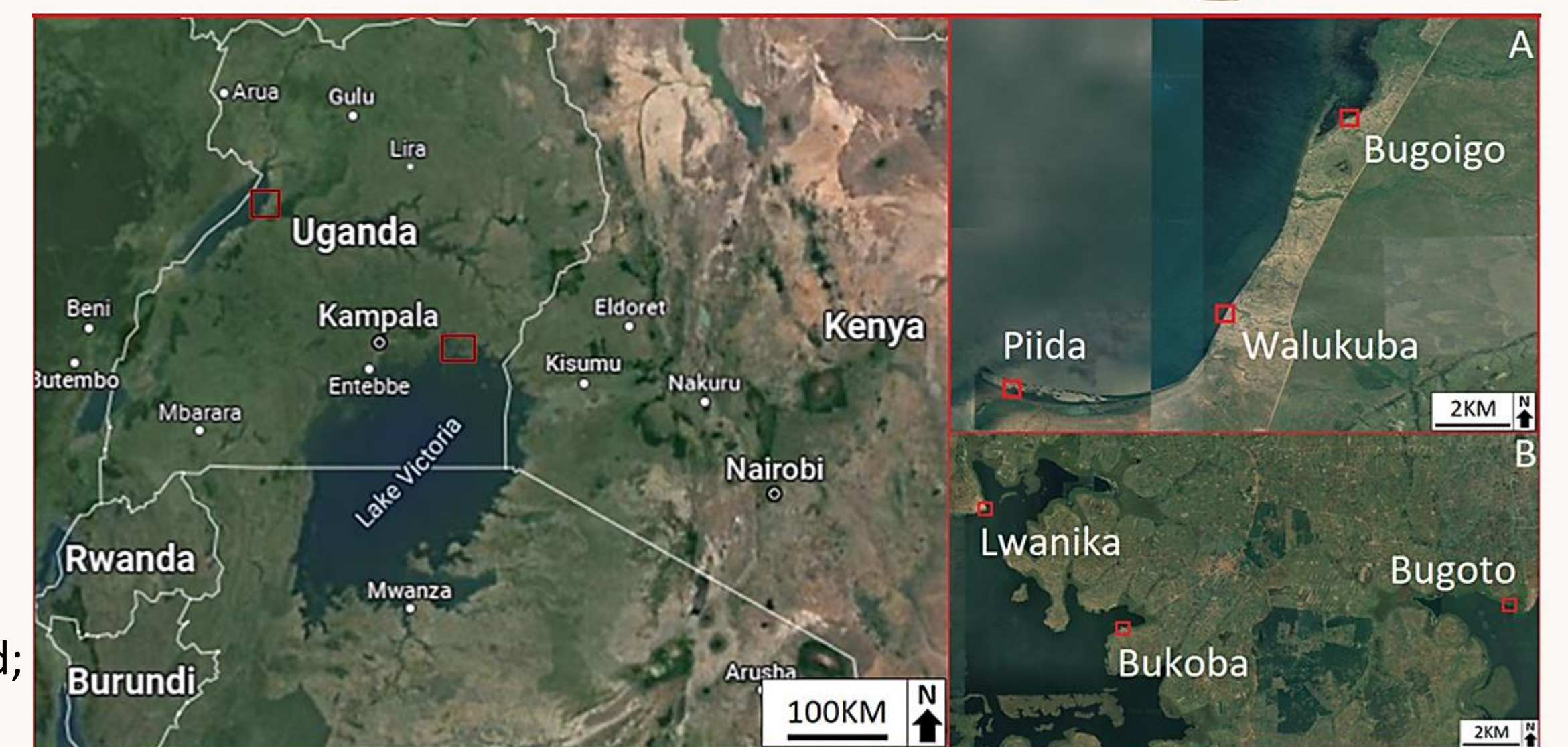
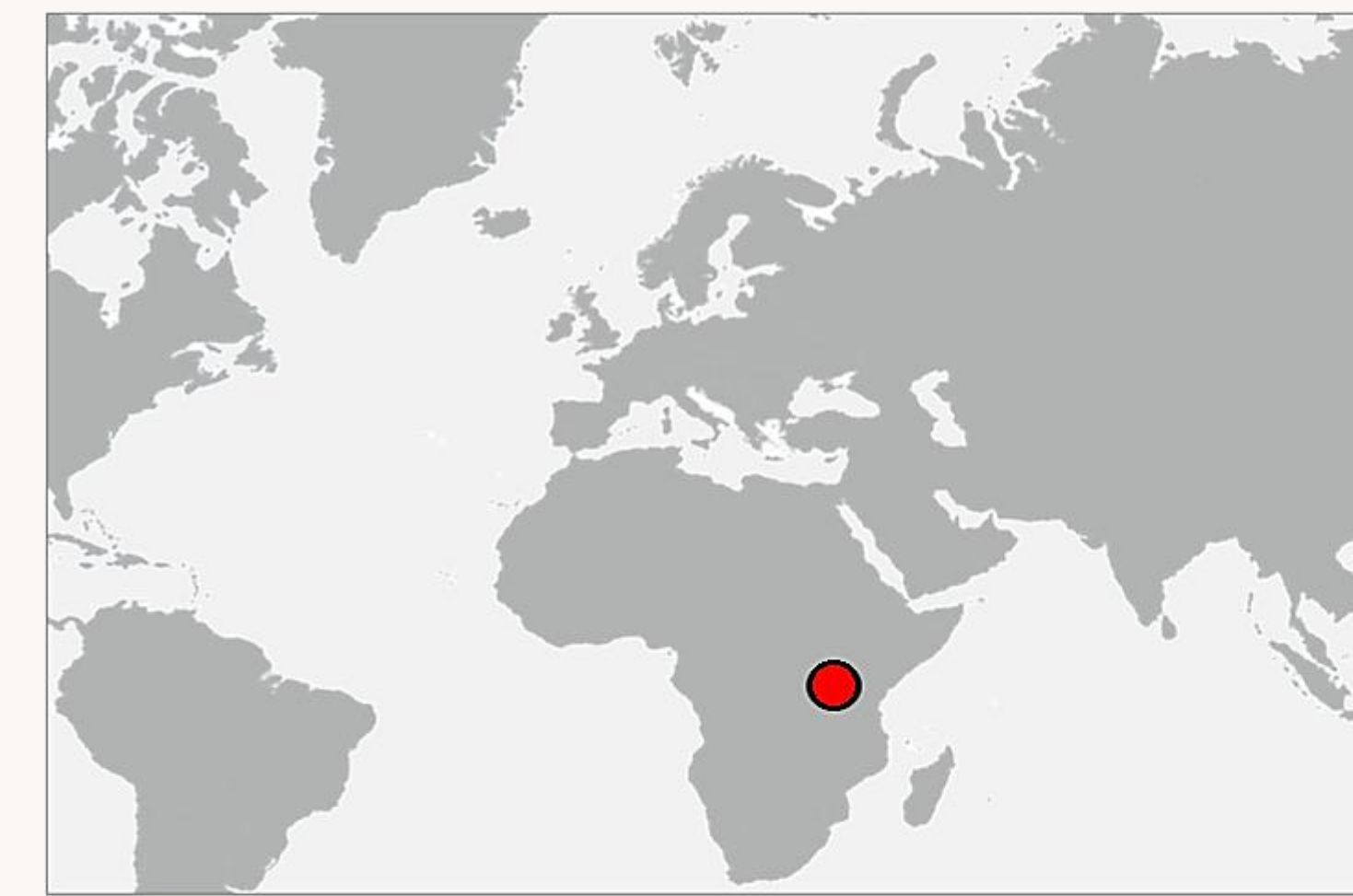
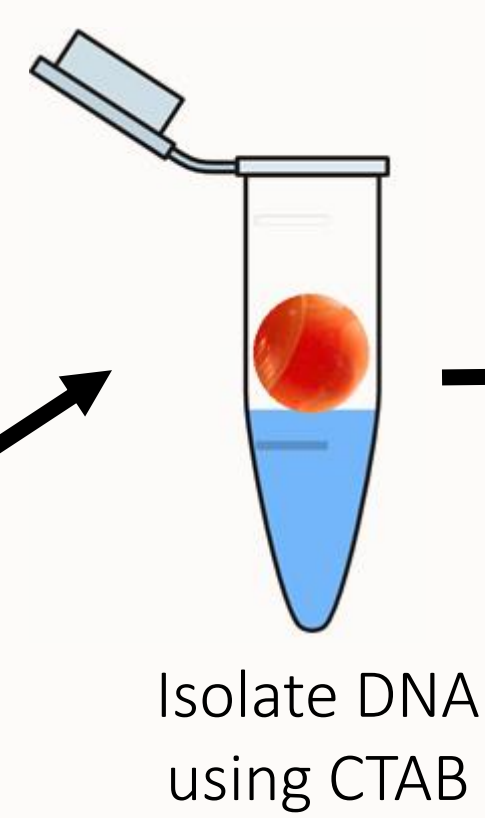


Figure 1. Map of the Great African Lakes of Uganda; Examples of *Biomphalaria* shells recovered; Collection sites of (A) Lake Albert and (B) Lake Victoria

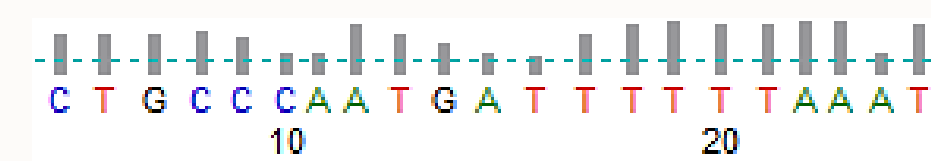
## Materials and Methods:



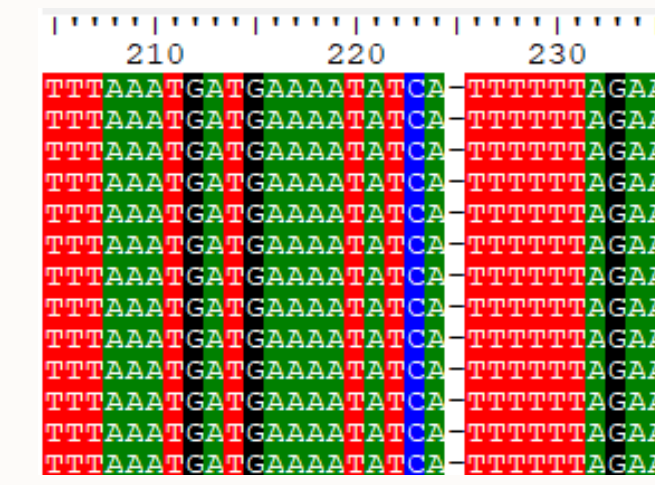
Collect 20 snails from three Lake Albert ( $n=120$ ) and three Lake Victoria ( $n=60$ ) sites.



Isolate DNA using CTAB



DNA was sequenced using rRNA 16S and COI markers



16S and COI sequences were aligned using screened NCBI references from studies with both conchological and molecular identification methods.<sup>3, 4, 5, 6</sup> Species were then identified using a Maximum Likelihood tree (GTR+ $\Gamma$ ) of the combined 16S (330bp) and COI (500bp) gene fragments in PhyML

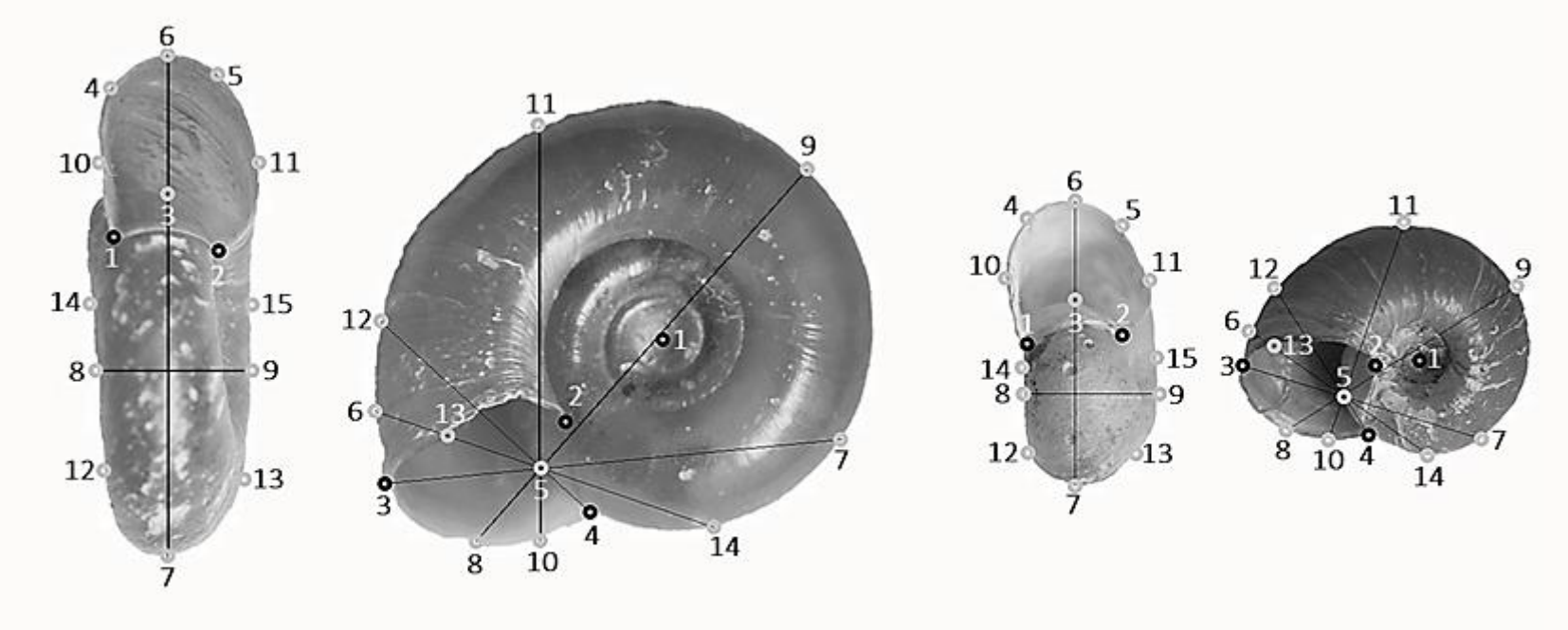
Shells from corresponding isolated samples were preserved



Photographs of the apertural and apical angles were taken for each of the shells using smartphone microscopic photography



Fixed and Semi-fixed landmarks were placed on the apertural and apical angles of each shell using TPS Dig32 and coordinate data was analysed by MorphoJ



## Results:

The ML tree found Lake Albert had three species of *Biomphalaria* (*B. pfeifferi*, *B. stanleyi* and *B. sudanica*) present, while Lake Victoria only had *B. choanomphala* present at all three sites.

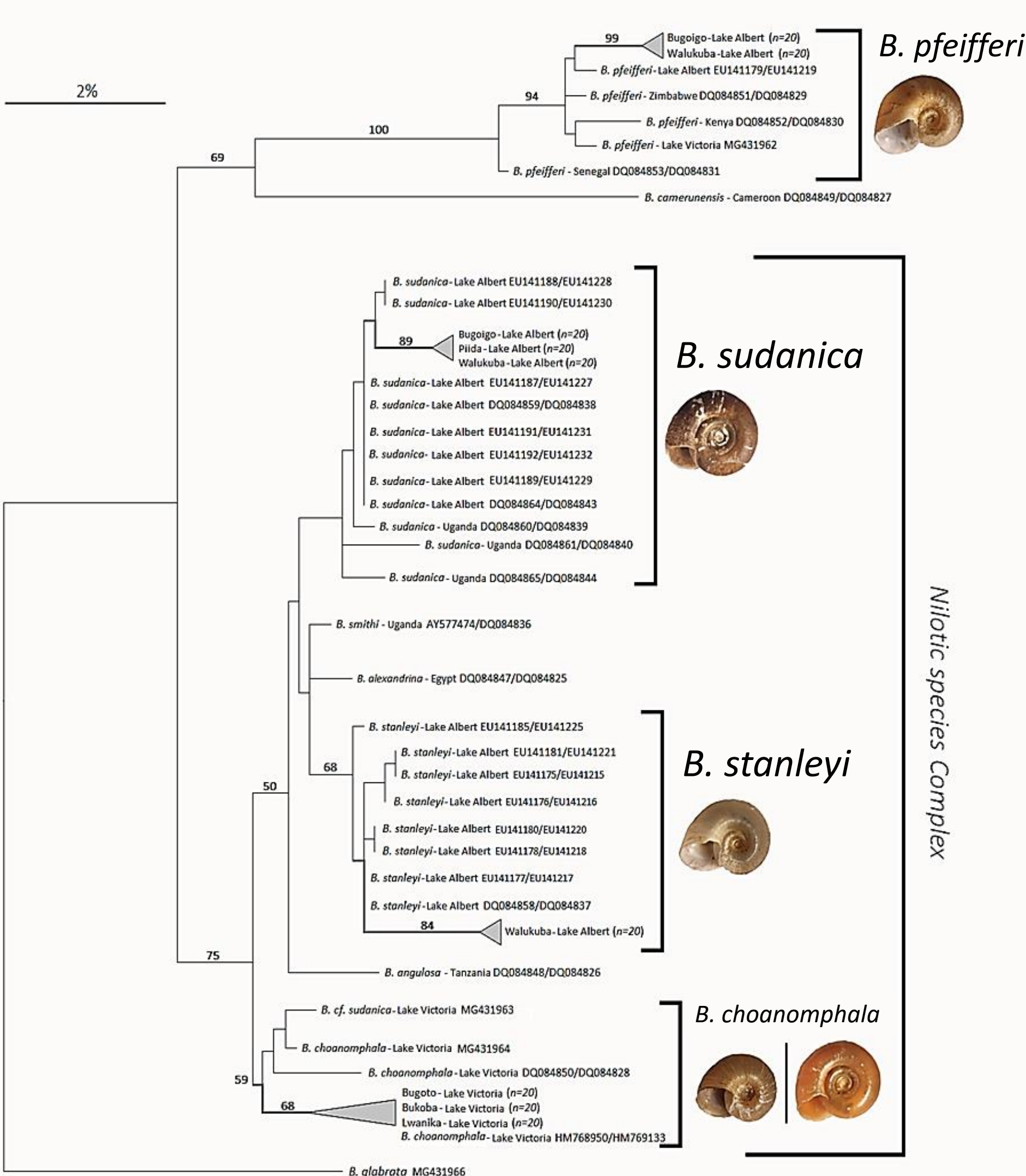


Figure 2. Maximum Likelihood tree (GTR+ $\Gamma$ ) of the combined 16S and COI sequences. Tree is rooted on *B. glabrata*

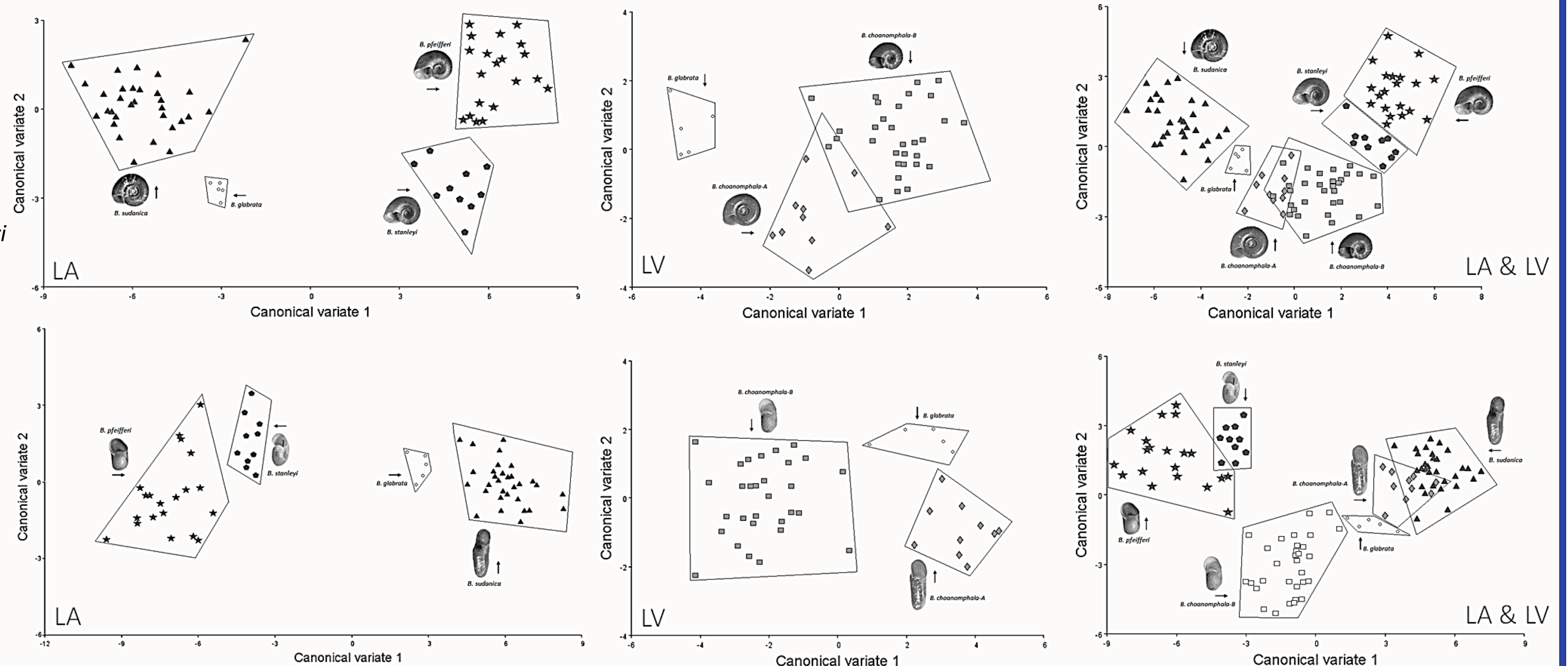


Figure 3. Canonical Variate Analysis (CVA) plots of the apical (Top) and apertural (Bottom) landmark datasets for the *Biomphalaria* species identified at Lake Albert (LA) and Lake Victoria (LV).

## Discussion:

To effectively interrupt the transmission of schistosomiasis, the intermediate snail hosts must be first confidently identified. However, sympatric *Biomphalaria* species cannot be easily identified and distinguished by shell morphology alone. Our study found four *Biomphalaria* species were present on the Ugandan shorelines of Lake Albert and Victoria. Of the species identified, only *B. choanomphala* exhibited two ecological phenotypes. Moreover, we demonstrated that the implementation of landmark-based geometric morphometric techniques can differentiate sympatric *Biomphalaria* species using shell morphology alone, with the apical shell angle being more informative than the apertural shell angle at distinguishing species.

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## References:

<sup>1</sup> Brown, D. S. (2002). *Freshwater snails of Africa and their medical importance*. CRC press; <sup>2</sup> Colley, D. G., Bustinduy, A. L., Secor, W. E., & King, C. H. (2014). Human schistosomiasis. *The Lancet*; <sup>3</sup> Jørgensen, A., Kristensen, T. K., & Stothard, J. R. (2007). Phylogeny and biogeography of African *Biomphalaria* (Gastropoda: Planorbidae), with emphasis on endemic species of the great East African lakes. *Zoological Journal of the Linnean Society*; <sup>4</sup> Plam, M., Jørgensen, A., Kristensen, T. K., & Madsen, H. (2008). Sympatric *Biomphalaria* species (Gastropoda: Planorbidae) in Lake Albert, Uganda, show homoplasies in shell morphology. *African Zoology*; <sup>5</sup> Standley, C. J., Goodacre, S. L., Wade, C. M., & Stothard, J. R. (2014). The population genetic structure of *Biomphalaria choanomphala* in Lake Victoria, East Africa: implications for schistosomiasis transmission. *Parasites & vectors*; <sup>6</sup> Zhang, S. M., Bu, L., Laidemitt, M. R., Lu, L., Mutuku, M. W., Mkoji, G. M., & Loker, E. S. (2018). Complete mitochondrial and rDNA complex sequences of important vector species of *Biomphalaria*, obligatory hosts of the human-infecting blood fluke, *Schistosoma mansoni*. *Scientific reports*.



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