



Diversity and systematics of three freshwater bivalve families from South Africa

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ABSTRACT

Bivalves play an important role in the environment as they are filter feeders and contribute to the biogenic transfer of particles in the water column. Additionally, they can increase the oxygen level in sediment as they are burrowers. Freshwater bivalves, specifically in the Sphaeriidae, Iridinidae, and Unionidae families are understudied in South Africa. This study aimed to revise the species that occur in South Africa and update the distribution to ultimately contribute to the molecular, morphological, and distribution data. Sampling sites were determined based on previous distribution records and consisted of 78 sites in the northern provinces of South Africa. The bivalves that were found were morphologically identified based on existing keys considering key morphological characteristics as *Euglesa* sp., *Euepra ferruginea*, *Chambardia wahlbergi*, and *Unio caffer*. Molecular methods were used to generate sequences for each species, where possible, whereafter GenBank and BOLD sequences were aligned with the novel sequences to determine their phylogenetic relationships. In total, 35 COI and 47 16S sequences were generated and identified as the following species: *Euglesa costulosa*, *Euglesa viridaria*, *Euepra ferruginea*, *Chambardia wahlbergi*, and *Unio caffer*. The study found that intraspecific variations are more prevalent than interspecific variations, with morphological differences observed within species that are phylogenetically identical. This suggests that there may be fewer species than previously proposed in the literature. The current distribution of these species, as observed in this study, was compared to data from GBIF and the NWU Snail Collection, leading to the conclusion that species richness and diversity have declined for sphaeriids, iridinids, and unionids. Further research is needed to address conservation concerns for these bivalves, particularly in relation to identified research gaps, such as the lack of distribution records and molecular data.

Key terms: Sphaeriidae, Iridinidae, Unionidae, taxonomy, morphology, distribution, conservation.

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CHAPTER 1 GENERAL INTRODUCTION

1.1 Mollusca

Molluscs are bilaterally symmetrical eumetazoans. This phylum encompasses a diverse range of body forms and sizes, spanning from giant squids to organisms measuring down to 500 μm (Lindberg et al., 2004). Mollusca belongs to the second largest phylum after Arthropoda (Lindberg et al., 2004; Ponder et al., 2020). According to Ponder et al. (2020), it is difficult to provide a straightforward definition of Mollusca because of its diverse morphological and molecular traits. Although there are enough overlapping morphological characteristics to characterise Mollusca as monophyletic, such as having a body that typically consists of a head, visceral mass, and foot encased in a mantle that is covered by a shell. These features vary from genus to genus where some animals may only possess two or three of the features (Lindberg et al., 2004).

Tripathy et al. (2018) highlights the importance of Mollusca by explaining that they contribute significantly to the biomass of both terrestrial and freshwater habitats, serve an important function in ecosystem services, and act as bio-indicators. Furthermore, molluscs are important in maintaining the aquatic food chain by playing a vital role in predator-prey interactions (Tripathy and Mukhopadhyay, 2015). Their importance also lies in their comprehensive fossil record extending back about 560 million years ago, which makes them useful in understanding evolutionary history and extinction patterns (Lindberg et al., 2004).

Many molluscs have beneficial economic value as food or cultural items (Lindberg et al., 2004; Vaughn and Hoellein, 2018). They create a product that can be used for jewellery as pearls can be harvested from certain mussels. Snails have also been known to secrete a substance called, mucin, which is highly sought after for their use in skin products. Furthermore, marine molluscs are of commercial importance as mussels, clams, snails and oysters are a popular food source for humans (Gosling, 2008).

Molluscs can also have negative economic impacts as some can be hosts for parasites, or function as pests (Lindberg et al., 2004; Vaughn and Hoellein, 2018). An example of molluscs that are seen as pests, could be the invasiveness of some species, for example, the alien species of *Tarebia* snails that overpopulate ecosystems and outcompete some native species (Pearson et al., 2024). An example is where gastropods are of both economic and public health significance because of their role as intermediate hosts for parasites that cause diseases like bilharzia and liver-fluke disease, leading to health care costs and productivity losses (Figure 1.1; Appleton, 2002). Bilharzia, also known as schistosomiasis, is a human

parasitic disease caused by blood flukes (flatworms). Certain freshwater South African snails serve as intermediate hosts for these blood flukes. When infected, the snails shed the infective stage of the parasites (cercariae) into the water where they can infect humans (Appleton and Miranda, 2015).

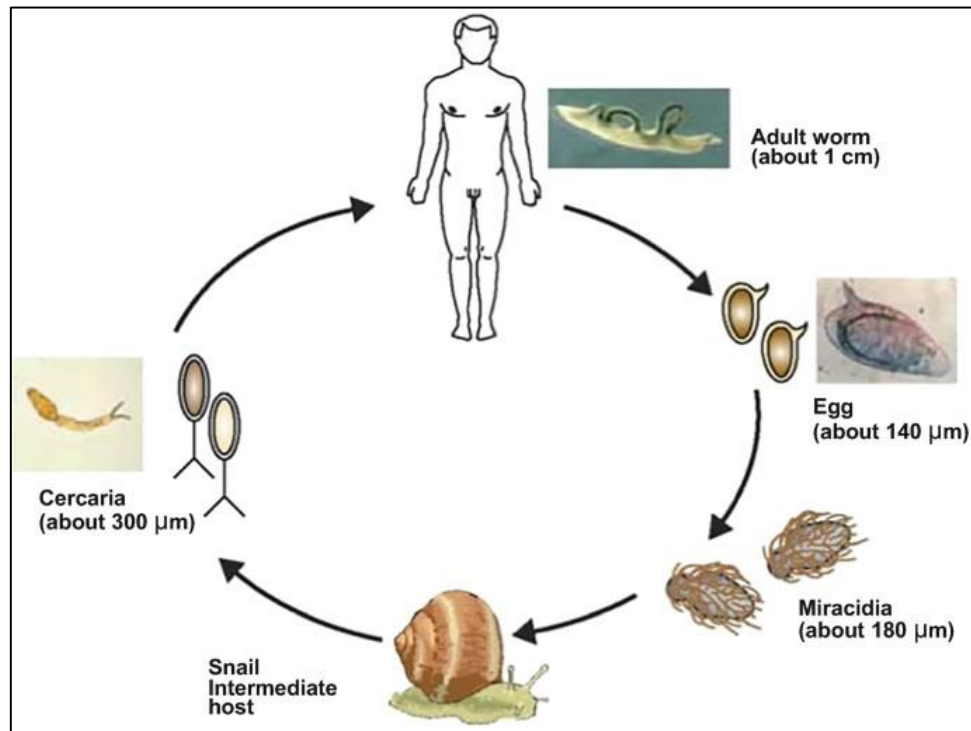


Figure 1.1 The life cycle of the parasites responsible for bilharzia in humans, with intermediate hosts being snails as illustrated in Danso-Appiah et al. (2024).

Even though Mollusca form an important part of the ecosystem, there is still much to learn about them. For example, unionid mussels have a complex life cycle that depends on migratory fish to carry their parasitic larvae (Figure 1.2; Tripathy and Mukhopadhyay, 2015). However, it is unclear what species of fish in South Africa are more suited to carry the larvae of iridinids and unionids respectively (De Kock and Wolmarans, 2010). As the absence of these specific fish species could directly influence the presence of these bivalves (Haag and Stoeckel, 2015), more research is necessary to preserve these important mollusc species in South Africa.

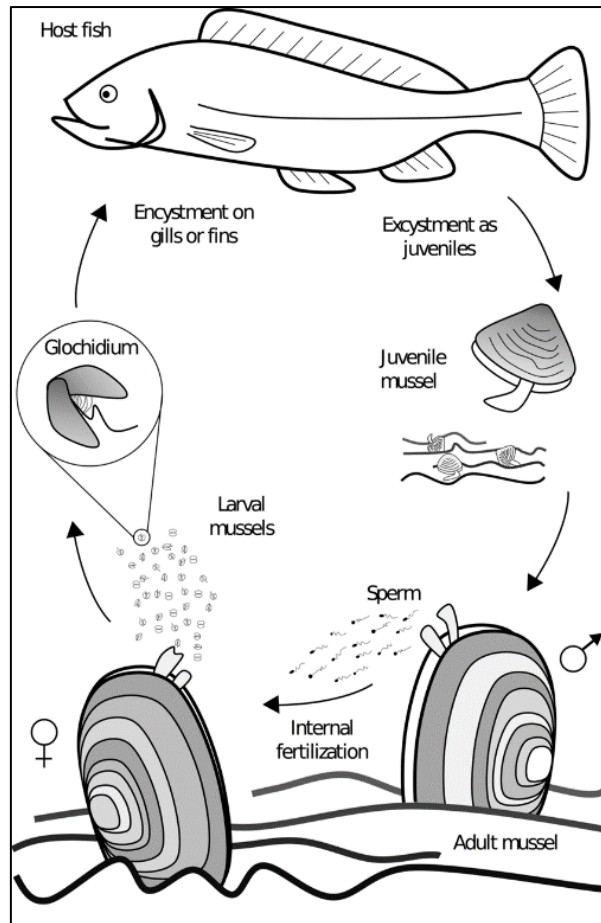


Figure 1.2 Lifecycle of unionids bivalves as illustrated by Rock et al (2022).

Appleton (2002) highlights how little we know about molluscs as their exact numbers of abundance in Africa are still up for debate. State authorities discontinued systematic surveys of freshwater Mollusca in South Africa in the 1980s, and most collection localities have not been sampled since. As a result, information on their present conservation status and species diversity is limited (De Kock and Wolmarans, 2010).

There are eight classes belonging to Mollusca: Bivalvia, Caudofoveata, Gastropoda, Cephalopoda, Monoplacophora, Polyplacophora, Scaphopoda, and Soleonogastres (Ponder et al., 2020). Molluscs are most abundant and diverse in the marine environment, but two classes live in freshwater or brackish water, namely Bivalvia and Gastropoda. In the case of this study, the focus will only be on Bivalvia.

1.2 Bivalvia

The class Bivalvia is an ancient taxon, that was first described in 1758 (Linnaeus, 1758) with 25,000 surviving species (Ghiselli et al., 2021). Taylor et al. (1973) emphasises the rich history

of this class, indicating that bivalve lineages have extensive and continuous records stretching deep into the Palaeozoic era. Throughout their extended evolutionary history, bivalves have adapted to a variety of physicochemical conditions, habitats, biological interactions, and feeding patterns.

Bivalves have been extensively studied in zoology and palaeontology because they fossilise well (Bieler and Mikkelsen, 2006). However, this has resulted in different species names and phylogenetic hypotheses which may have contributed to the lack of taxonomic structure. According to Bieler et al. (2014), their extensive fossil record has emerged as one of the most effective tools for describing global ecological and biogeographical patterns.

Bivalves vary in size, and despite their seemingly basic body plan, they evolved highly varied shell forms and intricate anatomical systems (Ghiselli et al., 2021). Hypotheses about Bivalvia's higher-level internal structure have evolved throughout time, influenced by corroborating and contradictory signals from character systems derived from shell morphology, as well as organisational patterns of the gills or stomach (Bieler et al., 2014). Different techniques like shell morphology and anatomy can be used to distinguish species (Taylor et al., 1973). Another technique that can be used is phenotype analyses that analyse an organism's physical and biological features. This technique to distinguish between species has become more reliable over time (Adamkewicz, et al., 1997).

Bivalves are of economic and ecological importance (Bieler and Mikkelsen, 2006), but regardless of their rich potential, this group remains understudied (Bieler et al., 2013). Bieler et al. (2014) mention a few instances where bivalves have assisted in breakthroughs in research. Examples include new transcriptomic resources, ageing research, the discovery of new antibiotics, and understanding diversification in the deep sea. They have also been used in cancer studies concerning contagious tumours and gene expression (Lemer et al., 2019). Their economic and ecological importance is further highlighted as they have been known to accumulate metals in their shells from industrial or mining activities (Craeymeersch and Jansen, 2019), as well as toxic substances like mercury (Tripathy and Mukhopadhyay, 2015) allowing them to be used in environmental monitoring studies (Vaughn and Hoellein, 2018).

Bivalves have a significant impact on ecosystem processes. They play a key role in energy and nutrient cycling through nutrient deposition and the breakdown of organic material in the water column and sediment as they are filter feeders (Lemer et al., 2019). They are also responsible for the biogenic transfer of sediment particles by moving around in the sediment. This allows for an increase in oxygen in the sediment and the release of organic material that was compacted in the sediment.

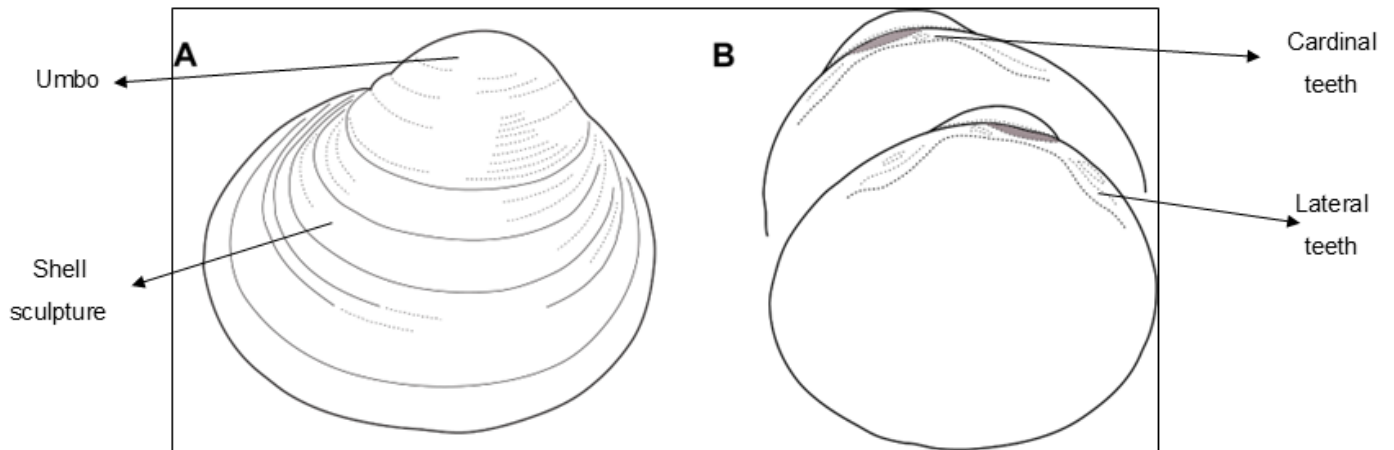
The eight families of freshwater bivalves that occur in South Africa fall in the subclass Autobranchia, namely Cyrenidae, Etheriidae, Iridinidae, Mytilidae, Psammobiidae, Sphaeriidae, and Unionidae (Appleton, 2002). In this study, the focus will be on Sphaeriidae, Iridinidae, and Unionidae. While conducting research on the South African pea clam, *Euglesa langleyana*, belonging to the family Sphaeriidae, De Kock and Wolmarans (2008) found that the abundance and diversity of freshwater bivalves have decreased over time. These same authors expressed a similar concern over iridinids too, as their sampling efforts showed a decrease in *Chambardia petersi* and *Chambardia wahlbergi* from previously known locations (see De Kock and Wolmarans, 2012). Likewise, for the family Unionidae, De Kock and Wolmarans (2010) conducted sampling at sites where *Unio caffer* had previously been found but were unable to locate this species, suggesting a potential threat to its survival. While these references are over 10 years old, there is a trend indicating that molluscs, specifically freshwater bivalves in Sphaeriidae, Iridinidae, and Unionidae, are declining in South Africa. Böhm et al. (2021) illustrated with the help of IUCN Red List statuses that there is a worldwide decrease in freshwater molluscs, including bivalves, where 34% of freshwater bivalve species are already extinct or threatened.

1.3 Morphology of South African bivalve families

MolluscaBase and MUSSELP database are two separate databases that outline the taxonomic structure and evolutionary history of molluscs. Both databases have been used as reliable sources in the past, but for the sake of this project, MolluscaBase was used to outline the taxonomy and provide information on the selected bivalves (MolluscaBase, 2024).

The morphological characteristics of bivalves are uniform across all genera, but they are limited. Some examples of the key characteristics are the umbo orientation, the presence of cardinal and lateral teeth, and shell sculpture (Figure 1.3 – Figure 1.5; Kuiper, 1964). The umbo of a mussel is where the two valves connect on the dorsal side and depending on the species, can be placed more anteriorly or posteriorly. The difference between cardinal and lateral teeth is the placing. Cardinal teeth can be found on the inside of the umbo, i.e. on the dorsal side, and can vary in size, shape, and abundance. Whereas the lateral teeth almost always come in pairs with at least one on each posterior and anterior side. The shell sculpture can be seen on the outside of the shells and can vary in symmetry, concentricity, and texture. Though there are limited characteristics, they can still be useful in identifying species (Kuiper, 1964). The focus of this project will be on the following three families known to occur in South Africa i.e. Sphaeriidae, Iridinidae and Unionidae.

The family Sphaeriidae was first described by Deshayes (1853). They are referred to as pea or fingernail clams because they are the smallest freshwater bivalves. There are four genera in this family that occur in South Africa: *Euglesa* Jenyns, 1832 (10 species in SA), *Eupera* Bourguignat, 1854 (1 species in SA), *Pisidium* Pfeiffer, 1821 (1 species in SA), and *Sphaerium* Scopoli, 1777 (2 species in SA). Originally, there were seven species from South Africa in the



genus *Pisidium* according to Kuiper (1964), however, six of these were recently moved to *Euglesa*. All the bivalves in *Euglesa* have different morphological characteristics but a distinguishing feature of this genus is that they are all ovate (oval-shaped) (see Figure 1.3). *Eupera* species are noticeably larger and more elongate, and they only have one cardinal tooth (Appleton, 2002). Species in the genus *Sphaerium* look very similar to *Euglesa* species with slight differences in colour, texture, and teeth.

Figure 1.3 General morphological characteristics of sphaeriid bivalves redrawn from Kuiper (1964).

The family Iridinidae was originally described by Servain (1891). It contains the largest freshwater bivalve in southern Africa: *Chambardia wahlbergi* (see Appleton, 2002). Morphological characteristics of this family are lacking, but Mandahl-Barth (1988) discussed some features of Mutelidae (a junior synonym for Iridinidae). There are three genera from this family that occur in South Africa, namely: *Aspatharia* Bourguignat, 1885 (1 species in SA), *Chambardia* Bourguignat, 1891 (in Servain, 1891) (2 species in SA), and *Mutela* Scopoli, 1777 (1 species in SA). There are no external features that distinguish Iridinidae mussels from Unionidae mussels, but in Africa, *Unio* is usually smaller (Mandahl-Barth, 1988) (see Figure 1.4). Appleton (2002) differs in opinion, by pointing out that Iridinidae mussels are different because they don't have hinge teeth or sculpture like *Unio*. Identification based on

morphological characteristics has proven to be unreliable for iridiniids (Fol et al., 2021). A more reliable way of classifying these species should use a combination of morphometrics and molecular analysis.

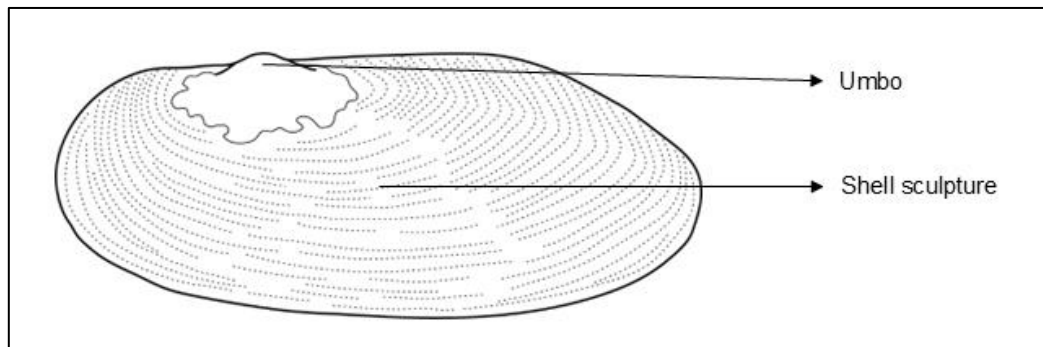


Figure 1.4 A redrawn image of the general morphology of iridiniid bivalves as illustrated by Mandahl-Barth (1988).

The family Unionidae was originally described by Rafinesque (1820). In South Africa, there are two genera known, namely *Coelatura* Conrad, 1853 (2 species in SA) and *Unio* Philipsson, 1788 (In Retzius, 1788) (1 species in SA). Swainson (1840) provided key characteristics for bivalves in the family Unionidae, indicating a distinct characteristic that distinguished *Unio* from the other genera was its cardinal teeth. The umbo of these bivalves is prominent and distended, with their cardinal teeth situated more towards the back and the posterior hinge in line with the margin (Swainson, 1840). According to Appleton (2002), *Unio* may have an angular hinge with a smooth cardinal tooth, but the author doesn't specify whether it is receded or not. According to Conrad (1853), *Coelatura* can be identified by its cardinal teeth that are quite elongate and parallel to the anterior hinge margin. This genus also has a shell which is thin and not elongated, with corrugated or sub-nodular umbones.

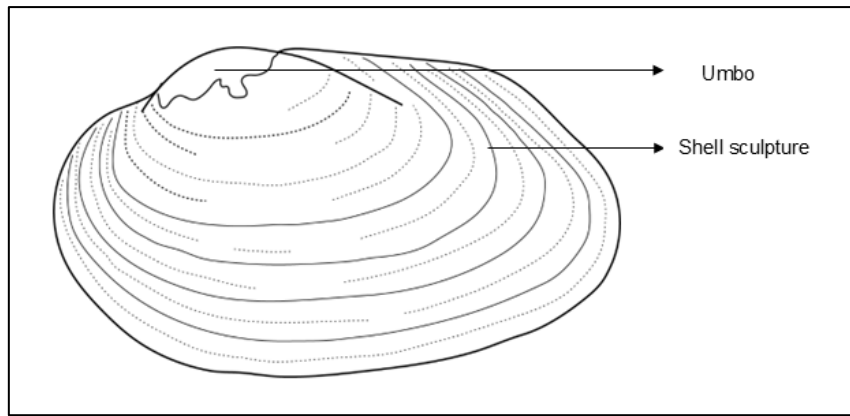


Figure 1.5 A redrawn image of the general morphology of unionids as illustrated by Mandahl-Barth (1988).

1.4 General geographical distribution of the South African bivalve families

Sphaeriids occur right across the African continent but based on the Global Biodiversity Information Facility (GBIF) they seem to congregate more towards the south, with most occurrences being reported from the Uganda and South African region. Three sphaeriids are endemic to South Africa, namely *Euglesa costulosa* (Connolly, 1931), *Euglesa harrisoni* (Kuiper, 1964), and *Euglesa langleyana* (Melvill & Ponsoby, 1891) (see Kuiper, 1964; De Kock 2008). *Euglesa casertana* (Connolly, 1931) is the most widespread, not only in Africa but worldwide (Bespalaya et al., 2019).

Iridinids seem to be more evenly spread throughout Africa but occur less frequently in the northern parts and most southern parts (GBIF). Only one species is endemic to southern Africa: *Mutela zambesiensis* Mandahl-Barth, 1988. Appleton (2002) and Mandahl-Barth (1988) agree that species in the family have limited distribution, with only *Chambardia wahlbergi* (Krauss, 1848) being well represented in southern Africa.

Unionids have also been reported from the whole of the African continent, but according to GBIF, most occurrences were localised in the southern region of the continent. According to Appleton (2002), the most widespread species of this family, *Unio caffer* Krauss, 1848, is also endemic to southern Africa.

1.5 Molecular studies

Molecular studies of freshwater molluscs are still developing, and techniques and protocols are yet to be perfected (David and Savini, 2011). Few taxa have been used to test theories on higher-level freshwater mollusc relationships, and the data have often been reanalysed. Limited sampling and false replication have contributed to bias in the understanding of

freshwater mollusc evolution (Graf, 2013). These issues have been related to the inability of mitochondrial DNA to retrieve Mesozoic divergences. The use of molecular phylogenetics has changed taxonomic viewpoints by providing information that alters freshwater mollusc systematics (Pfeiffer et al., 2019). Furthermore, misidentifications of mollusc species have been corrected with genetic analyses (Fol et al., 2021).

While the morphology is relatively well-known among sphaeriid species, phylogenetic relationships are severely understudied (Clewing et al. 2013). Research on sphaeriids has increased in recent years (Clewing et al., 2020; Bepalaya et al., 2024) although this is still lacking in South Africa. Recently, Clewing et al. (2022) conducted research on an array of sphaeriids, including species from Africa, and even South Africa, focusing on their phylogeny. This was the first of its kind, as the existing studies done on South African sphaeriids focused on identification based on morphology (Kuiper, 1964; De Kock and Wolmarans 2008a; De Kock and Wolmarans, 2008b). Sphaeriids have also been noted to be an ideal model for phylogeographic investigations because of their ability to spread across continents (Bepalaya et al., 2020; Clewing et al., 2013). However, there are still several unanswered questions concerning phylogenetic relationships and taxonomic stance of sphaeriids (Clewing et al., 2013; Clewing et al., 2020). According to Graf (2013), this has led to many misidentifications.

Iridinidae is polyphyletic according to Combosch et al. (2017). According to Fol et al. (2021), this family will need to rely on molecular analyses to determine taxonomy as their morphological features vary too much and are influenced by environmental changes and distribution. This has led to significant confusion in their taxonomy in the past. Bolotov et al. (2024) believe that molecular, morphological, anatomical, and biogeographical investigations need to be combined to identify taxa to species level. Currently, there is not a lot of information available concerning this family's phylogeny, and more research is required on this.

Phylogenetic consensus has not been developed regarding the early evolution of Unionidae (Pfeiffer et al., 2019). The monophyly of unionoids is supported by recent palaeoheterodont findings, indicating that all unionids share a common ancestor. However, extinctions have created a phylogenetic gap, leaving the timing of the separation of the clades' stem groups uncertain. Although the Unionidae phylogeny has gained much attention, its applicability is limited due to biased sampling and inconsistent conclusions (Graf, 2013). Inter- and intraspecific morphological adaptability and evolutionary convergence (Inoue et al., 2014) have held taxonomists back from their conservation efforts in relation to taxonomy (Lopes-Lima et al., 2017). Lopes-Lima et al. (2017) conducted thorough research on the phylogeny of Unionidae outlining and adding novel taxonomic structures and nomenclature, but unfortunately, this is still lacking in South Africa. In comparison to the preceding two families,

Unionidae has gained much attention and thus has a more detailed taxonomic background (Huang et al., 2019; Pfeiffer et al., 2019; Lopes-Lima et al., 2020). The existing methods could be extrapolated to gain the same insight on South African unionid taxa, and potentially for iridiniids and sphaeriids too.

1.6 National Freshwater Snail Collection (NFSC)

The NFSC was initiated in 1956 by Professor JA van Eeden, a Zoology researcher from Potchefstroom, South Africa. He was awarded the prestigious Nuffield scholarship in 1954 for malacological research and a Council for Scientific and Industrial Research (CSIR) grant in 1960 to conduct research on the taxonomy of South African water snails (Hadfield, 2020), which also resulted in a comprehensive overview of bilharzia in South Africa. Following this, the researchers for the NFSC set out to map the distribution of snails throughout the whole of South Africa. By 2015, they managed to collect 33,000 records, and this came to be known as the National Freshwater Snail Collection (referred to as the NWU Snail Collection from here on out). While this was important research for bilharzia, they also collected other species in the Molluscan group, such as bivalves, from each site they visited (Van Eeden, 2017). The NWU Zoology department now holds this snail collection, with thousands of documented snails in over 60,000 specimen jars (Hadfield, 2020). These historical records can provide vital information on previous distributions and biodiversity and can be compared to more recent data for updated information on these molluscs.

1.7 Conservation

The greatest threat towards all molluscan fauna in South Africa, as in the rest of the world, seems to be caused by human impact. When considering freshwater habitats, the main concern is habitat destruction and fragmentation (Herbert, 1998). The latest threats have been pollution, both chemical and physical, and the modification of freshwater systems, e.g., dam building and water abstraction (Böhm et al., 2021). Comprehensive surveys of freshwater Mollusca were discontinued in the early 1980s (De Kock and Wolmarans, 2010). Herbert (1998) believes this contributes to the lack of conservation of sphaeriid bivalves in South Africa. Knowledge of the current state of the distribution and diversity of South African freshwater Mollusca is deficient, and information on their conservation status is therefore speculative and unsatisfactory (De Kock and Wolmarans, 2010). Given South Africa's diverse climates and rich biological heritage, it is crucial to ensure their proper conservation (Herbert, 1998).

De Kock and Wolmarans (2008a) noted that there is still much to learn about the conservation status of South African sphaeriids. There have been differing opinions on how many native South African sphaeriid species are present. Connolly (1939) reported three species from South Africa: *Euglesa langleyana*, *Euglesa ovampica*, and *Euglesa costulosa*, whereafter Kuiper (1964) added four additional species: *Euglesa pirothi*, *Euglesa viridaria*, *Euglesa harrisoni*, and *Euglesa casertana*. However, based on the survey done as part of the National Freshwater Snail Collection, only three species were represented: *Euglesa langleyana*, *Euglesa viridaria*, and *Euglesa costulosa*. Following this timeline, there is reason to believe that the species in this family are decreasing in abundance and diversity. De Kock and Wolmarans (2008b) mention that sites where these bivalves were previously found before the 1980s have not been visited again. If these sites are visited again, it would be a good indication of the status of the sphaeriid abundance and diversity in South Africa.

De Kock and Wolmarans (2010) expressed their concern over *Unio caffer* as they believed this species should be categorised as vulnerable or endangered on the IUCN based on their findings, whereas it is currently listed as 'of least concern' (Seddon et al., 2011). The decrease in *Unio* numbers could be attributed to their unique life cycle where they depend on fish to complete their life cycle (Figure 1.2). There is little information on the host fish for the parasitic larvae of unionid freshwater mussels, making it difficult to assess the specific effects of dam construction, overfishing, or aquaculture (Graf, 2011). Other possible reasons for the decline of these bivalves include the development of dams, the introduction of invasive species, and pollution (De Kock and Wolmarans, 2010). Though this is still hypothetical in South Africa, it has been proven around the world as the cause of the global freshwater bivalve decline (Böhm et al., 2021; De Kock and Wolmarans, 2010).

The research efforts of De Kock and Wolmarans (2012) in South Africa between 1980 to 2007 as part of the National Freshwater Snail Collection, brought to light that Iridinidae distributions, specifically, *Chambardia petersi* and *Chambardia wahlbergi*, stretch more westwards than originally thought. These species were previously only known from Mpumalanga and Limpopo, but new records were found in North West, Free-State, and Gauteng by De Kock and Wolmarans (2012). Their research done in the Kruger National Park, located in the Mpumalanga and Limpopo provinces, indicated a decrease in the abundance and diversity of this species compared to previous data. The specimens found in the North West were also larger than previously reported. The capability of these bivalves to disperse is supported by Seddon et al. (2011), where they mentioned that *C. wahlbergi* has been reported from the whole of Africa. These changes call for a re-evaluation of this family to update the distribution and monitor the possible invasion of alien species (De Kock and Wolmarans, 2012).

1.8 Aims and objectives

Based on the above information, the selected three bivalve families located in South Africa (Sphaeriidae, Iridinidae, and Unionidae) need revision. With a better understanding of current distribution patterns, and accurate taxonomy based on both morphology and molecular identification, potentially threatened mollusc species could be conserved. Therefore, the following aims and objectives were put forth for this project.

Aims:

- Revise the taxonomy of three freshwater bivalve families (Sphaeriidae, Iridinidae, Unionidae) in the northern regions of South Africa.
- Update the distribution of three freshwater bivalve families (Sphaeriidae, Iridinidae, Unionidae) in the northern regions of South Africa.

Objectives:

- Sample in previously known locations in the northern provinces of South Africa for selected bivalves to determine current distribution.
- Use existing keys and high-resolution images to morphologically identify the collected bivalves to species level.
- Perform molecular analyses on the bivalves to determine the genetic diversity in each of the selected families: Sphaeriidae, Iridinidae, and Unionidae.

1.9 Chapter layout

Chapter 1: Provides background information on Mollusca, Bivalvia and South African bivalves. This chapter also includes a brief overview of all elements to be used in this study.

Chapter 2: Indicates the study area and site selection for this project (which applies to all chapters).

Chapter 3: Provides a detailed revision of the South African bivalves in the family Sphaeriidae.

Chapter 4: Gives a detailed revision of the South African bivalves in the family Iridinidae.

Chapter 5: Provides a detailed revision of the South African bivalves in the family Unionidae.

Chapter 6: Gives concluding remarks on each respective family by referring to key findings. Recommendations are also provided for future studies.

Chapter 7: References used in all chapters.

1.10 Project conference contributions

There were three outputs of this dissertation in the form of national conference contributions:

- Janse van Rensburg, S., Malherbe, W., de Necker, L. (2023) Updated distribution of South African freshwater bivalves in the Sphaeriidae family from lowveld to highveld. 60th Southern African Society of Aquatic Scientists (SASAqS), 25-29 June 2023, Lord Charles Hotel, South Africa.
- Janse van Rensburg, S., Malherbe, W. (2023) The distribution and taxonomy of Sphaeriidae bivalves in South African wetland systems. National Wetlands Indaba (NWI), 23-26 October 2023, ATKV Buffelspoort, South Africa.
- Janse van Rensburg, S., Hadfield, K.A., de Necker, L., Malherbe, W. (2024) Unveiling the identity of South African mollusc species from the family Sphaeriidae. Joint Southern African Society of Aquatic Scientists (SASAqS) and Society of Environmental Toxicology and Chemistry (SETAC Southern Africa) regional conference, 23–26 June 2024, Gold Reef City, Johannesburg, South Africa.

CHAPTER 2 SITE SELECTION AND STUDY AREA

2.1 Natural Science Collections Facility and North-West University Snail Collection

The Natural Science Collections Facility (from here on out referred to as the Collections Facility) operates as a virtual hub, connecting a network of institutions that maintain natural science collections. These collections are accessible to external researchers, with the facility created as part of the Department of Science and Innovation (DSI)'s Research Infrastructure Roadmap and coordinated by the South African National Biodiversity Institute (SANBI).

This collection facility makes use of a Quantum Geographical Information System (QGIS) tool named the Collections Facility Georefencer to plot coordinates of historical data of South African molluscs to make them usable in distribution studies. Each georeference contains a locality name, coordinates, the uncertainty parameter, and the identity and abundance of organisms found. An uncertainty parameter is a measure of distance around a coordinate point that accounts for any uncertainty concerning the coordinates, e.g., if the coordinates are in a river, an uncertainty parameter will be added as the exact known location of the collected data point is unknown.

The Potchefstroom campus of the North-West University (NWU) also houses data used for georeferencing. This includes original handwritten cards with relevant historical information and the associated preserved organisms, forming part of the NWU Snail Collection (see Chapter 1, section 1.6). This collection served as a valuable resource for identifying potential sampling sites, for comparing species and morphologically characterising specimens found during this project.

2.2 Site selection

Most sites were selected based on the historic distribution records as reported in the NWU Snail Collection. Some sites were selected based on the type locality of selected bivalves as stated in Kuiper (1964), and some others were selected based on the accessibility and type of habitat present. The preferred habitat for smaller bivalves belonging to Sphaeriidae was waterbodies with shallow, still-standing to slow-flowing water on the margins (De Kock and Wolmarans, 2008). As some sphaeriids attach to vegetation with byssus threads, sites with vegetation were also favoured (Appleton, 2002). For the larger bivalves belonging to Iridinidae and Unionidae, the deeper areas along the margin of the waterbodies were targeted. These waterbodies included rivers, streams, wetlands, and dams for all three bivalve families.

Elevation, ecoregion type, and rainfall were also considered factors when selecting sites as sphaeriids prefer certain temperatures, habitats, and higher elevation.

A total of 78 sites were sampled for the present study from 2022–2024 in the Northern provinces of South Africa (Figure 2.1). The provinces included: North West, Gauteng, Mpumalanga, Limpopo, and KwaZulu-Natal.

The coordinates obtained from the NWU snail collection database were used to deliver an updated distribution of the three bivalve families and sites were then selected. Maps were constructed with the help of QGIS version 3.22 (long-term release).

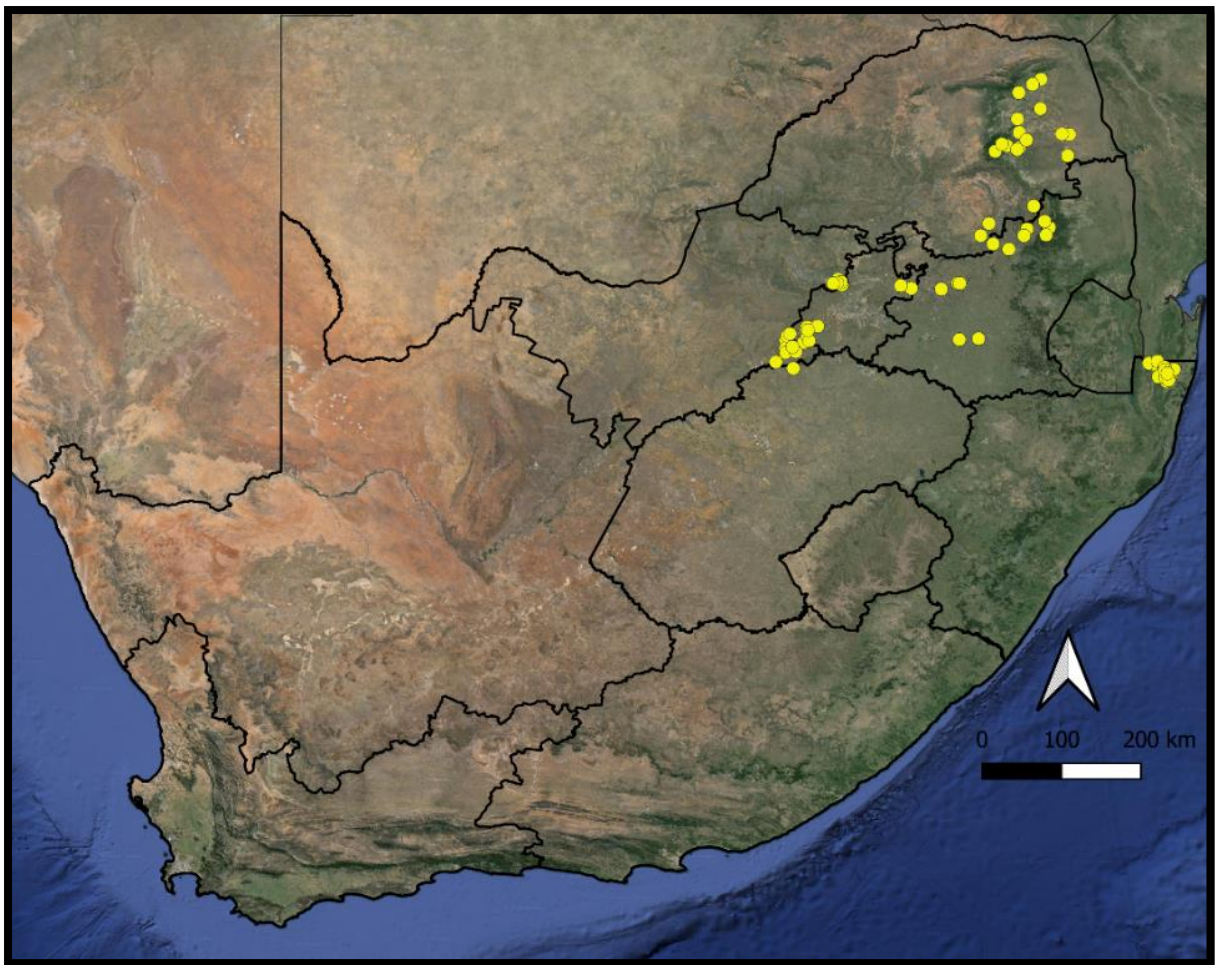


Figure 2.1 A map of South Africa depicting the 78 sites sampled during this project.

A world elevation layer from QGIS was used to compile a map (Figure 2.2) to illustrate South Africa's elevation. Dark orange indicates the highest elevation, while green indicates the lowest elevation. Most of the selected sites were located at higher elevations, with only a few

at moderate or low elevations. The higher elevation sites were prioritised based on literature, indicating that the target bivalves predominantly occur in these areas.

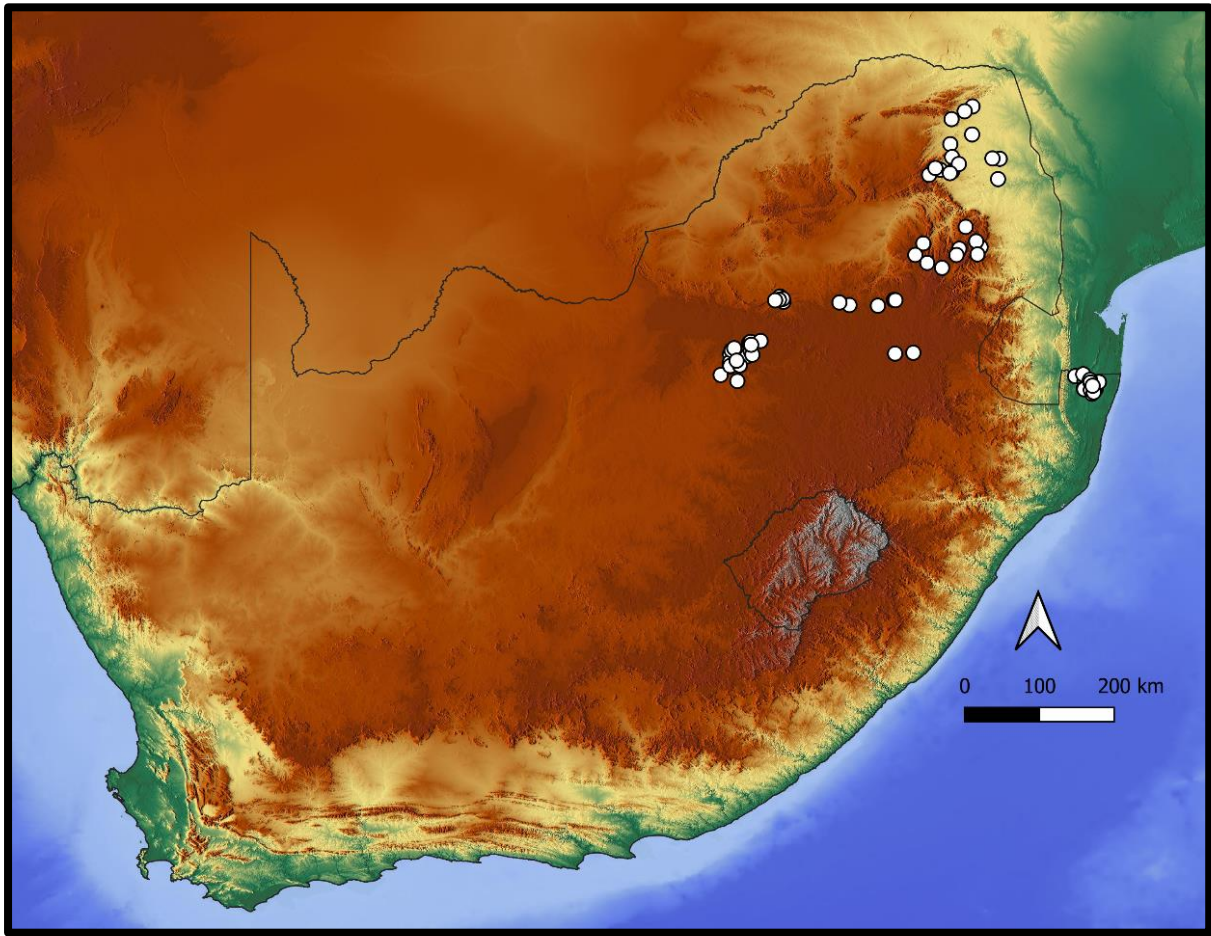


Figure 2.2 A map of South Africa illustrating elevation with all sample sites (white dot).

An ecoregion level 1 layer from SANBI was imported into QGIS to illustrate the different ecoregions of South Africa. The regions were defined based on the classification system by Kleynhans et al., (2005). The sites were selected based on previous data, for example, from the NWU Snail Collection which fell in these seven different ecoregions: Highveld, Lowveld, Natal Coastal Plain, Eastern Bankenveld, Northern Escarpment Mountains, Western Bankenveld, and North Eastern Highlands (Figure 2.3).

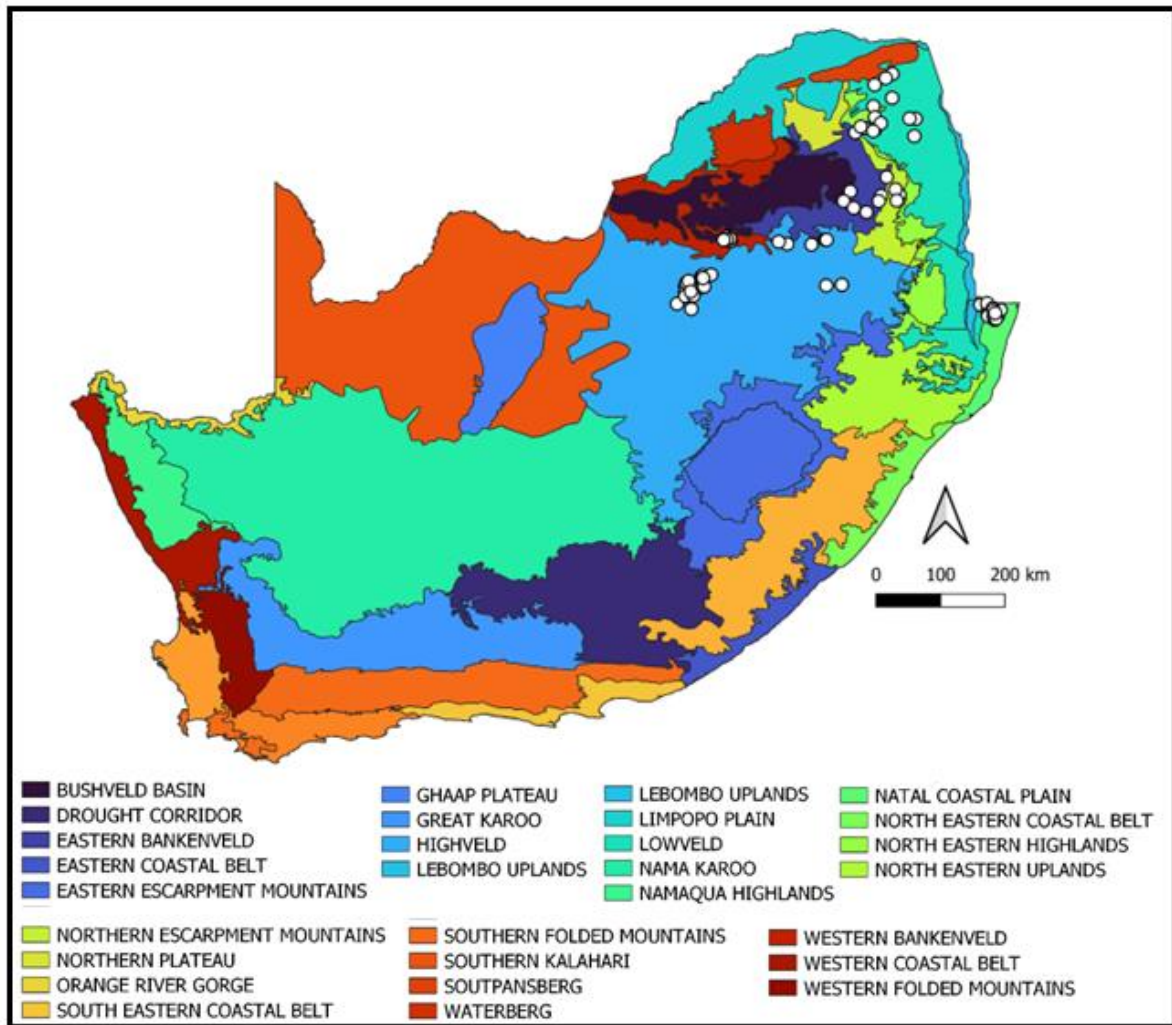


Figure 2.3 A map of South Africa illustrating different ecoregions with all sample sites (white dot).

A rainfall layer imported from SANBI (Department of Agriculture, Forestry and Fisheries portal) was used to create a map in QGIS illustrating the mean annual rainfall in South Africa. As these bivalves do not favour dry regions, higher rainfall areas were targeted as there would generally be more stable water bodies in these areas. Most sites were in the 601–800 mm region, with only a few sites in the 401–600 mm region.

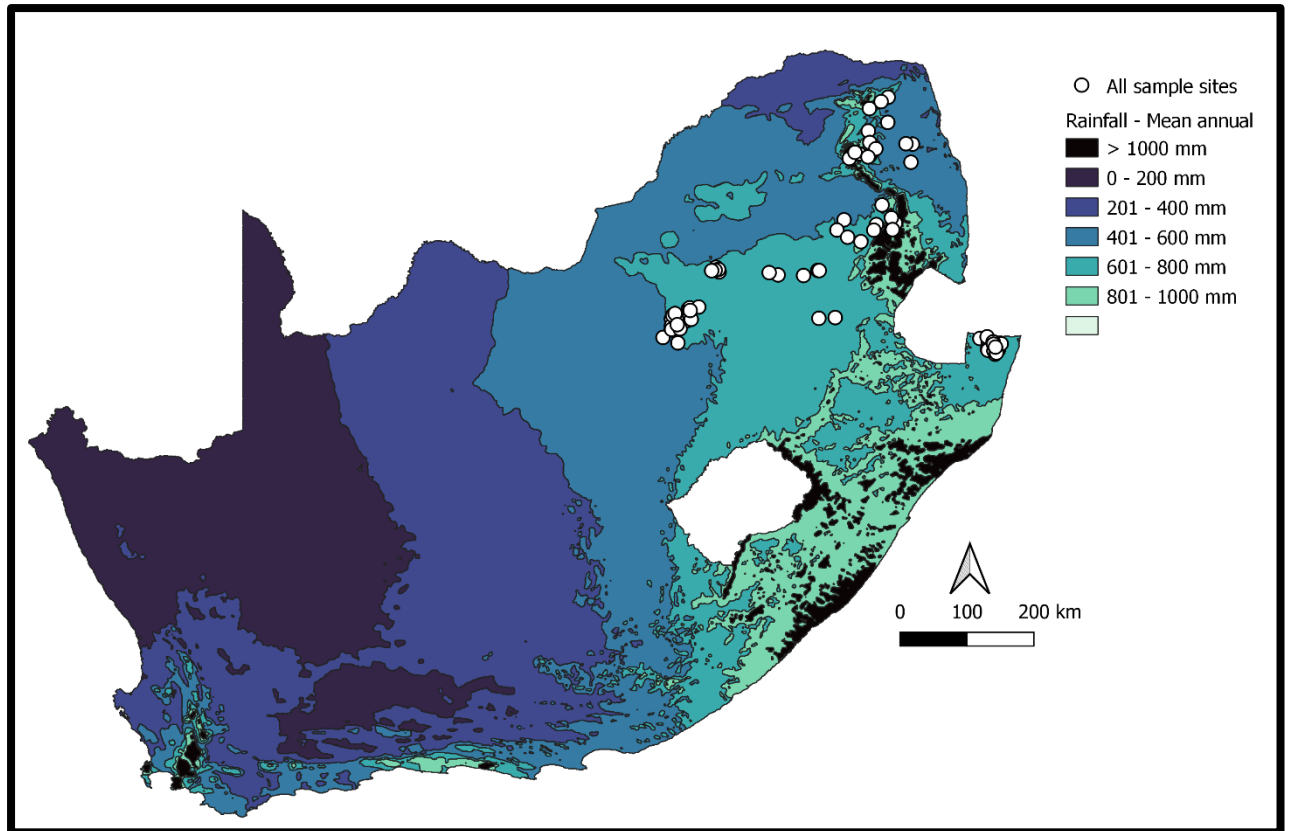


Figure 2.4 A map of South Africa illustrating the mean annual rainfall with all sample sites (white dot).

The elevation of each site chosen can be seen in Tables 2.1–2.20, as well as the temperature of the water which can be associated with the elevation.

2.3 Study area

Out of the 78 sites assessed, the bivalves from the three selected families were present at only 20 sites (Figure 2.5). Bivalves were found in each province that was sampled, with the highest occurrences being in North West and Mpumalanga (Figure 2.5).

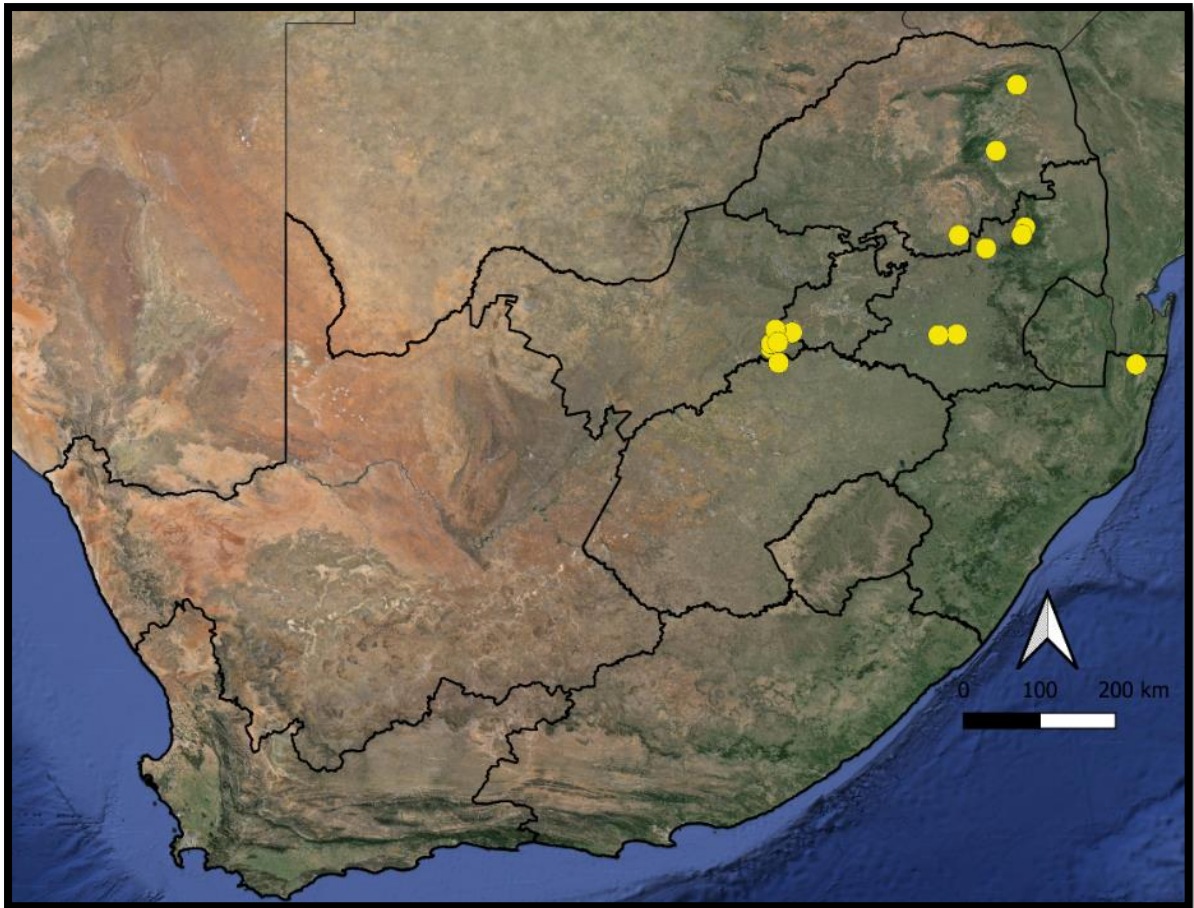


Figure 2.5 A map of Southern Africa depicting the 20 sites where selected bivalves were found.

The following tables (Tables 2.1–2.20) provide a detailed summary of the sites where selected bivalves were found in each province. Each table includes an image of the site, along with the habitat and site, as well as descriptions of flow, depth, vegetation, and substrate type. The sampling for this project started mid-2022 and ended mid-2024, primarily during summer, autumn, and spring. Each site was visited one time, and they consisted of rivers, dams, springs, and wetlands.

Table 2.1 Habitat and site information for Olifants River.

Site name	Olifants River
Province	Mpumalanga
Date	2023-03-28
Coordinates	-25.853858; 29.2934
Elevation (m)	1273
Dissolved oxygen (%)	86.5
Dissolved oxygen (mg/l)	7.44
Water temperature (°C)	23
Electrical conductivity (µS/cm)	504
pH	8.07
Habitat description: The habitat was well-vegetated along the margins. The water was shallow and slow-flowing, with still-standing pools on the margins.	

Table 2.2 Habitat and site information for Wilge River.

Site name	Wilge River
Province	Mpumalanga
Date	2023-03-28
Coordinates	-25.844472; 28.871664
Elevation (m)	1207
Dissolved oxygen (%)	79.1
Dissolved oxygen (mg/l)	7.28
Water temperature (°C)	19.3
Electrical conductivity (µS)	606
pH	7.66
Habitat description: There was a lot of overhanging vegetation at this site. It was shallow on the margins but deeper towards the middle. The water was slow-flowing to still-standing.	

Table 2.3 Habitat and site information for Steelpoort River (upstream). **Table 2.4** Habitat and site information for Spekboom River.

Site name	Steelpoort River (upstream)
Province	Limpopo
Date	2023-03-29
Coordinates	-25.100833; 29.847061
Elevation (m)	867
Dissolved oxygen (%)	93.4
Dissolved oxygen (mg/l)	8.47
Water temperature (°C)	20.1
Electrical conductivity (µS)	221
pH	7.88
Habitat description: There was mainly still-standing to slow-flowing water which was deep towards the middle but shallow on the margins. There was no aquatic vegetation, but there was marginal vegetation.	



Site name	Spekboom River
Province	Mpumalanga
Date	2023-03-29
Coordinates	-25.009453; 30.500106
Elevation (m)	1218
Dissolved oxygen (%)	79.1
Dissolved oxygen (mg/l)	7.01
Water temperature (°C)	20.5
Electrical conductivity (µS)	46
pH	7.55
Habitat description: The substrate consisted mainly of rocks and boulders with limited gravel areas. The water was fast-flowing with a few still-standing pools in the marginal areas. There was overhanging and marginal vegetation.	

Table 2.5 Habitat and site information for Sabie River.

Site name	Sabie River
Province	Mpumalanga
Date	2023-03-30
Coordinates	-25.093278; 30.768975
Elevation (m)	882
Dissolved oxygen (%)	102.7
Dissolved oxygen (mg/l)	9.96
Water temperature (°C)	16.9
Electrical conductivity (µS)	62.5
pH	7.01
Habitat description: The substrate was mostly sandy. The water was flowing at a moderate speed and had still-standing pools along the margins. There was marginal vegetation.	

Table 2.6 Habitat and site information for Luvuvhu River.

Site name	Luvuvhu River
Province	Limpopo
Date	2023-03-31
Coordinates	-22.900447; 30.695956
Elevation (m)	361
Dissolved oxygen (%)	90.8
Dissolved oxygen (mg/l)	7.4
Water temperature (°C)	25.8
Electrical conductivity (µS)	142.6
pH	7.30
Habitat description: The water was deep and slow-flowing to fast-flowing, with shallow areas at the entry points and along the margins. There was a lot of marginal and aquatic vegetation.	

Table 2.7 Habitat and site information for Groot Letaba River.

Site name	Groot Letaba River
Province	Limpopo
Date	2023-03-31
Coordinates	-23.86655; 30.391981
Elevation (m)	368
Dissolved oxygen (%)	60.7
Dissolved oxygen (mg/l)	5.27
Water temperature (°C)	22.2
Electrical conductivity (µS)	92.5
pH	7.41
Habitat description: The substrate was sandy, and the water was still-standing to slow-flowing. It was shallow on the margin but got deeper towards the middle. There was no vegetation in the area that was sampled.	

Table 2.8 Habitat and site information for Mac Mac River.

Site name	Mac Mac River
Province	Mpumalanga
Date	2023-03-30
Coordinates	-24.986261; 30.815606
Elevation (m)	1088
Dissolved oxygen (%)	95.7
Dissolved oxygen (mg/l)	9.22
Water temperature (°C)	17
Electrical conductivity (µS)	104.7
pH	7.42
Habitat description: The substrate was sandy and rocky. The water was shallow and slow-flowing, accompanied by a lot of overhanging vegetation.	

Table 2.9 Habitat and site information for Vaal River.

Site name	Vaal River
Province	Free State
Date	2023-03-01
Coordinates	-26.970549; 27.210896
Elevation (m)	1400
Dissolved oxygen (%)	-
Dissolved oxygen (mg/l)	-
Water temperature (°C)	-
Electrical conductivity (µS)	-
pH	-

Habitat description:

The substrate varied across the site (with sandy, muddy and rocky areas). There were shallow pools along the margin with quite a lot of aquatic, marginal and overhanging vegetation. It was fast-flowing in the headwaters, and slow-flowing towards the margins, with a few rapids nearby.

Table 2.10 Habitat and site information for Wonderfonteinspruit (site 3).

Site name	Wonderfonteinspruit (site 3)
Province	North West
Date	2023-09-27
Coordinates	-26.5233333; 27.4075
Elevation (m)	1400
Dissolved oxygen (%)	42.9
Dissolved oxygen (mg/l)	
Water temperature (°C)	
Electrical conductivity (µS)	9.66
pH	8.71

Habitat description:

The substrate was very muddy, with a few sandy spots. The water was slow flowing to still standing in the area sampled, but there were a few rapids nearby. Marginal, aquatic, and overhanging vegetation was present.

Table 2.11 Habitat and site information for Wonderfonteinspruit (site 10).

Site name	Wonderfonteinspruit (site 10)
Province	North West
Date	2023-09-27
Coordinates	-26.4841667; 27.164167
Elevation (m)	1400
Dissolved oxygen (%)	58.3
Dissolved oxygen (mg/l)	-
Water temperature (°C)	-
Electrical conductivity (µS)	8.58
pH	8.26
Habitat description: The substrate was sandy to rocky. The water was shallow and still-standing with a lot of aquatic and marginal vegetation.	

Table 2.12 Habitat and site information for Mooi River (Muiskraal).

Site name	Mooi River (Muiskraal)
Province	North West
Date	2022
Coordinates	-26.572158; 27.103367
Elevation (m)	1400
Dissolved oxygen (%)	-
Dissolved oxygen (mg/l)	-
Water temperature (°C)	-
Electrical conductivity (µS)	-
pH	-
Habitat description: The water was fast-flowing with still-standing to slow-flowing pools on the margins. The substrate was mostly rocky in the headwaters, but sandy in pools. There was overhanging vegetation but not aquatic vegetation.	

Table 2.13 Habitat and site information for Mooi River (Oewersig).

Site name	Mooi River (Oewersig)
Province	North West
Date	2023
Coordinates	-26.688826; 27.102764
Elevation (m)	1400
Dissolved oxygen (%)	-
Dissolved oxygen (mg/l)	-
Water temperature (°C)	-
Electrical conductivity (µS)	-
pH	-
Habitat description:	
The substrate was mostly sandy, with a few muddy/silty spots. It is mostly shallow, with fast-flowing areas in the headwaters, and slow-flowing to still-standing areas along the margin. There was mostly overhanging vegetation.	

Table 2.14 Habitat and site information for Mooi River (upstream).

Site name	Mooi River (upstream)
Province	North West
Date	2023-09-27
Coordinates	-26.6686111; 27.2
Elevation (m)	1400
Dissolved oxygen (%)	55.5
Dissolved oxygen (mg/l)	-
Water temperature (°C)	-
Electrical conductivity (µS)	7.13
pH	8.11
Habitat description:	
The substrate was mostly sandy. The water was slow-flowing to still-standing, and deep towards the middle but shallow along the margins. There was aquatic and overhanging vegetation.	

Table 2.15 Habitat and site information for X-spruit (site 1).

Site name	Site 1
Province	Mpumalanga
Date	2024-04-19
Coordinates	-26.4484722; 29.79733333
Elevation (m)	1700
Dissolved oxygen (%)	65.6
Dissolved oxygen (mg/l)	6.16
Water temperature (°C)	17.6
Electrical conductivity (µS)	512
pH	8.51
Habitat description:	
The substrate was sandy to muddy. The water was slow-flowing to still-standing and quite deep towards the middle, but shallower on the margins. There was mostly marginal vegetation.	

Table 2.16 Habitat and site information for X-spruit (site 2).

Site name	Site 2
Province	Mpumalanga
Date	2024-04-19
Coordinates	-26.5510833; 29.81811111
Elevation (m)	1700
Dissolved oxygen (%)	65.4
Dissolved oxygen (mg/l)	5.78
Water temperature (°C)	20.1
Electrical conductivity (µS)	550
pH	8.35
Habitat description:	
The substrate was muddy/silty. There was a lot of aquatic vegetation. The water was slow-flowing to still-standing with shallower areas along the margin but got deep very fast towards the middle.	

Table 2.17 Habitat and site information for X-spruit (site 3).

Site name	X-spruit (site 3)
Province	Mpumalanga
Date	2024-04-19
Coordinates	26.7808333; 29.80652778
Elevation (m)	1700
Dissolved oxygen (%)	56
Dissolved oxygen (mg/l)	5.1
Water temperature (°C)	17.6
Electrical conductivity (µS)	278
pH	7.92

Habitat description:

The water was fast-flowing with a few pools along the margin that had still-standing to slow-flowing water. There was a lot of overhanging and aquatic vegetation. The substrate consisted of boulders along the headwaters but had sandy areas along the margins.

Table 2.18 Habitat and site information for X-spruit (site 4).

Site name	X-spruit (site 4)
Province	Mpumalanga
Date	2024-04-20
Coordinates	-26.563490; 29.547855
Elevation (m)	1700
Dissolved oxygen (%)	-
Dissolved oxygen (mg/l)	-
Water temperature (°C)	-
Electrical conductivity (µS)	-
pH	-

Habitat description:

The substrate was sandy to muddy. The water was slow-flowing to still-standing, and had a maximum depth of about 1 m. There was a lot of marginal vegetation, with some overhanging vegetation.

Table 2.19 Habitat and site information for Phongolo River (bridge).

Site name	Phongolo River (bridge)
Province	KwaZulu-Natal
Date	2022-09-19
Coordinates	-26.9916667; 32.4427778
Elevation (m)	62
Dissolved oxygen (%)	35.3
Dissolved oxygen (mg/l)	2.97
Water temperature (°C)	22.91
Electrical conductivity (µS)	322
pH	7.7
Habitat description: The substrate was sandy to muddy with a few rocks present. The headwaters were fast-flowing, but there was still-standing to slow-flowing water on the margins. There was some marginal vegetation, but very little aquatic vegetation.	

Table 2.20 Habitat and site information for Klein Letaba River.

Site name	Klein Letaba River
Province	Limpopo
Date	2023-03-31
Coordinates	-23.316128; 30.689567
Elevation (m)	322
Dissolved oxygen (%)	61.3
Dissolved oxygen (mg/l)	4.93
Water temperature (°C)	25.5
Electrical conductivity (µS)	520
pH	7.19
Habitat description: The substrate was mostly sandy. There were a few still-standing isolated pools, with a fast-flowing stream nearby. In some isolated pools, there was aquatic vegetation, but no marginal vegetation. All pools were relatively shallow. There was a sand mining operation close to this site.	

CHAPTER 3 A REVIEW OF FRESHWATER BIVALVES IN THE FAMILY SPHAERIIDAE IN SOUTH AFRICA

3.1 Introduction

Bivalves play a critical role in freshwater environments but have received little attention concerning studies of their taxonomy, phylogeny, distribution, and their role in aquatic environments. These molluscs are burrowers and filter feeders, and their primary food sources are phytoplankton, bacteria, and particulate organic matter. They also contribute to nutrient cycling through bio-deposition and excretion (Vaughn and Hakenkamp, 2001). Bivalves increase oxygen concentrations in water and sediment through their burrowing activity in the sediment. These movements allow nutrients found in sediments to be discharged back into the water column. Bivalve shells play an important function in epiphytic and epizoid populations because they provide habitat for other freshwater organisms. When contemplating the benthic region, creatures that live there can also use the shells as a refuge, which stabilises the sediment (Vaughn and Hakenkamp, 2001).

Sphaeriidae Deshayes, 1855 (1820) consists of the smallest freshwater bivalves with a size of 2 – 3 mm (Korniushin, 2000), and are commonly referred to as pill-, fingernail-, and pea clams (Graf, 2013). They are the second most diverse family of freshwater bivalves, following Unionidae Rafinesque, 1820 (see Clewing et al., 2013). These bivalves can survive in habitats that no other bivalves can, such as peat bogs, springs, and small creeks, which makes them a valuable target for research (Korniushin, 2000). They are also able to survive in extreme environments with high altitudes and have a high passive dispersal capacity (Clewing et al., 2013). This could allow them to assist in biomonitoring, but unfortunately, they remain understudied because of their small size, mode of life (in sediment), and the lack of distinguishing morphological characteristics leads to misidentifications (Clewing, et al., 2013).

Sphaeriids often dominate the benthic environment and form an essential part of the food chain by acting as an important food source for fish and birds (Kubikova et al., 2011; Bessalaya et al., 2015). They also serve as a transitory connection between primary producers and consumers since bivalves consume phytoplankton and crustaceans consume bivalves. For this reason, they can be used in biomonitoring studies because the health and conditions of multiple levels of the food chain can be targeted and monitored (Bessalaya et al., 2015). This was shown by Raikow and Hamilton (2001) who used sphaeriids, specifically *Sphaerium*, as part of a stable isotope study.

Most *Euglesa* bivalves can be found in cold water streams and rivers, within fine mud and leaf litter (Nesemann and Sharma, 2005). Other bivalves can attach to aquatic vegetation and sometimes to the surface film of water by slime threads. Some bivalves can also be found in shallow and deep lakes (both oligotrophic and eutrophic), as well as roadside ditches, and temporary ponds and swamps (Herrington, 1962; Mackie, 2007). According to Hamill (1975), they are most abundant in shallow (2–3 m) littoral areas where sediment is composed of fine particles and detritus, and current flow is low. While sphaeriids may differ in where they occur in freshwater habitats, all are bottom dwellers and filter-feeders (De Kock, 2008).

Members of Sphaeriidae provide parental care through ovovivipary (or euvivipary) and skip the planktonic larval stage that other bivalves go through, resulting in crawl-away juveniles. These species are hermaphroditic and may self-fertilise (Cummings and Graf, 2010). This is an adaptation that enables survival in variable freshwater environments (Bespalaya et al., 2018). Sphaeriids, including juveniles and small adults, may disperse and colonise isolated habitats and ephemeral bodies of water via waterbirds (Graf, 2013). The diversity of life-history features, including age at initial reproduction, egg-laying time, embryo release time, brood size, and number of generations each season, contributes to their vast dispersion throughout continents. These features vary based on geographical location, habitat type, and ecological considerations (Holopainen and Jonasson, 1983). Juveniles are released when conditions are favourable (regarding oxygen and temperature), and the number of juveniles depends on the size of the parent. Their lifespan is usually 4–5 years (Holopainen and Jonasson, 1983).

Kuiper (1946) described seven sphaeriid species from South Africa, including their taxonomy, morphology, distribution, and type locality. He also introduced the idea that three species might be endemic to this region, and that *Pisidium* Pfeiffer, 1821 (now moved to *Euglesa* Jenyns, 1832) might be more diverse than originally thought (Kuiper, 1964). He was one of the first authors to express the difficulty of distinguishing between different species, attributing it to the discontinuous distribution and limited information on relationships. Understanding the diversity within *Euglesa* and accurately identifying species is challenging due to substantial intraspecific conchological variation and consistent shell shape shared across different species (Groh et al., 2020). There are currently three genera in South Africa that form part of Sphaeriidae: *Euglesa*, *Eupera* Bourguignat, 1854, and *Sphaerium* Scopoli, 1777 (see Appleton, 2002; Kuiper, 1964).

Before the end of 2023, all the South African *Euglesa* species were synonymised with the genus *Pisidium* (see Bespalaya et al., 2024) but are now part of the genus *Euglesa*. Currently, there are seven South African *Euglesa* species known to science (Table 3.1). The NWU Snail Collection has catalogued three out of the seven species (De Kock and Wolmarans, 2008), identified solely on shell characteristics (Kuiper, 1964; Appleton, 2002), highlighting the need for molecular work

on this family. Identification of *Euglesa* still relies heavily on their morphology, with differing opinions in literature, where Korniushev (2000) believes that the presence and density of pores are important, while Kuiper (1983) and De Kock and Wolmarans (2008) strongly believe that the shell characteristics are the most important. Another example of contradicting information in the literature is where Mandahl-Barth (1988) disagrees with Kuiper (1966) that there are very similar characteristics for *Euglesa viridaria* and *Euglesa ovampica*; and Mandahl-Barth (1988) questions the validity of *Euglesa viridaria*. Thus, the genus *Euglesa* is in dire need of revision (Appleton, 2002). These differences in opinions, though originating from a knowledgeable background, could be attributed to a lack of molecular studies that were not available in past decades. Thus, this study aims to address some of these challenges.

Two *Eupera* species were originally noted to occur in South Africa (Appleton, 2002): *Eupera ferruginea* (Krauss, 1848) and *Eupera parasitica* (Deshayes, 1854). However, in August 2024 *E. parasitica* was synonymised with *Eupera ferruginea* (Table 3.1). *Eupera ferruginea* has been reported from South Africa several times, as part of a survey done in 1964 (by the NWU Snail Collection) and another survey done in 1995. Although the authors mention that *E. ferruginea* was not found in a 2001 survey, they were able to collect it again in 2006 (Wolmarans and De Kock, 2006). This trend suggests that this species is well established in South Africa, or at least in Kruger National Park, where the surveys were conducted.

There is a scarcity of literature on *Sphaerium* in South Africa. Only two species in this genus are reported from South Africa: *Sphaerium capense* (Krauss, 1848) and *Sphaerium incomitatum* (Kuiper, 1966) (Table 3.1). Other than Appleton (2002), Mandahl-Barth (1988), and Rapalai (2024) providing basic information about the species such as type locality, distribution and habitat, there is not much information on these species. Wolmarans and De Kock discussed most molluscs in South Africa, however, they did not discuss these two species. This highlights the limited knowledge available about these species in the region.

Species in the Sphaeriidae family have been previously studied, with their distribution and morphology documented as part of the NWU Snail Collection (De Kock and Wolmarans, 2008; 2010; 2012). However, they did not include molecular methods as part of their work. In other countries, this has become a popular way of identifying sphaeriid species (Clewings et al., 2013; Bieler et al., 2014; Bessalaya et al., 2024). But unfortunately, South African sphaeriids remain understudied. Korniushev (2000) and De Kock and Wolmarans (2008) expressed the need for further research as it could lead to a better understanding of their history and structure as well as provide insight into their conservation status and threat level.

The IUCN (International Union for Conservation of Nature) Red List is the most extensive resource available on the protection status of animal, fungal, and plant species worldwide. It provides an understanding of the current conservation status of species and whether more research is required. Species are assigned a threat status, which serves to indicate species are in immediate need of conservation (Rodrigues et al., 2006). The threat status of each of the South African species in Sphaeriidae can be found in Table 3.1.

This chapter therefore aimed to revise the family Sphaeriidae in South Africa by updating distribution records in the relevant provinces, as well as adding to the current molecular and morphological knowledge of these bivalves.

The hypothesis for this chapter was that there is a less diverse array of species present than originally assumed, with differences likely reflecting variations among morphospecies rather than distinct species, the implication being that there are infraspecific variations instead of interspecific variations.

Table 3.1 All documented South African sphaeriids (Mollusca: Bivalvia) (n = 10) as well as their synonyms, presence and abundance of sequences, and their category as per the IUCN red list.

Species name	Synonyms	Sequences	IUCN red list category
<i>Euglesa casertana</i> (Poli, 1791)	• <i>Cardium casertanum</i> Poli, 1791 (original name) • <i>Euglesa (Euglesa) casertana</i> Poli, 1791	• 94 on GenBank (16S, 28S, COI) • 354 on BOLD (COI)	Least concern
<i>Euglesa costulosa</i> (Connolly, 1931)	• <i>Pisidium costulosum</i> Connolly, 1931 (original name) • <i>Euglesa (Pseudeupera) costulosa</i> Connolly, 1931	• 2 on GenBank (16S, COI)	Least concern
<i>Euglesa harrisoni</i> (Kuiper, 1964)	• <i>Pisidium harrisoni</i> Kuiper, 1964 (original name)	None	Data deficient
<i>Euglesa langleyana</i> (Melvill & Ponsoby, 1891)	• <i>Pisidium langleyanum</i> Melvill & Ponsonby, 1891 (original name)	None	Least concern
<i>Euglesa ovampica</i> (Ancey, 1890)	• <i>Pisidium ovampicum</i> Ancey, 1890 (original name) • <i>Euglesa (Pseudeupera) ovampica</i> Ancey, 1890	None	Least concern
<i>Euglesa pirothi</i> (Jickeli, 1881)	• <i>Pisidium pirothi</i> Jickeli, 1881 (original name)	• 1 on GenBank (COI)	Least concern

Species name	Synonyms	Sequences	IUCN red list category
	• <i>Euglesa (Afropisidium) pirothi</i> Jickeli, 1881 • <i>Pisidium pirothi</i> Jickeli, 1881 and <i>Afropisidium pirothi</i> Jickeli, 1881		
<i>Euglesa viridaria</i> (Kuiper, 1956)	• <i>Pisidium viridarium</i> Kuiper, 1956 (original name) • <i>Euglesa (Euglesa) viridaria</i> Kuiper, 1956	• 1 on GenBank (COI)	Least concern
<i>Eupera ferruginea</i> (Krauss, 1848)	• <i>Cyclas ferruginea</i> Krauss, 1848 (original name) • <i>Byssanodonta parasitica</i> Deshayes, 1854 • <i>Eupera parasitica</i> Deshayes, 1854	• 2 on GenBank (COI)	Least concern
<i>Sphaerium capense</i> (Krauss, 1848)	• <i>Cyclas capensis</i> Krauss, 1848 (original name) • <i>Sphaerium capense capense</i> Krauss, 1848	None	Least concern
<i>Sphaerium incomitatum</i> (Kuiper, 1966)	• <i>Pisidium incomitatum</i> Kuiper, 1966 (original name) • <i>Sphaerium (Musculium) incomitatum</i> Kuiper, 1966	None	Least concern

3.2 Materials and methods

3.2.1 Collection

Bivalves were collected at all 78 sites mentioned in Chapter 2. Collection was done by sieving through gravel with a metal-frame snail sampler, with a mesh size of 1 mm x 1 mm to ‘filter’ through the sand and trap the small bivalves (Figure 3.1). Shallow, slow-flowing parts of the waterbody were targeted as the bivalves prefer this kind of habitat. The type of sediment they usually occur in is sandy, although they have also been known to attach to rhizomes of aquatic plants (Appleton, 2002). When a scoop of sediment was retrieved, it was thoroughly scanned for the bivalves by carefully scanning through the sediment from one corner of the scoop to the other, periodically discarding of the sediment that has already been searched. The scoop could also be dipped in water while dragging it backwards multiple times between scanning to ensure increase visibility. Once a bivalve was observed, it was placed in a tube with 96% ethanol to properly preserve the specimens for molecular analyses and marked accordingly based on where and when it was found. Ethical clearance for this project was granted by the North-West University Animal Care, Health and Safety Research Ethics Committee (NWU ethics number: NWU-01264-23-A9).



Figure 3.1 (A) Bivalves being collected using a snail sampler, (B) a metal frame snail sampler.

3.2.2 Morphological methods

All bivalves collected were taken back to the North-West University, Potchefstroom to be further analysed in the laboratory. With the aid of a AZ100 dissection microscope (Nikon, Japan), Z-stacking was used to capture images at different levels of the bivalve and compile them into a single image, allowing for the observation of various focal points simultaneously. As the shell is rounded, this step is important to ensure that all morphological characteristics can be observed. Following this, the bivalves were dissected to remove tissue for molecular analysis. Thereafter, the shells were placed into separate tubes and air-dried for morphological studies.

Scanning Electron Microscopy (SEM) was performed on at least one bivalve per site when available. The bivalve shells were left in a desiccator to dry completely before they were mounted on individual stubs using carbon glue. A Spi-module Sputter Coater (Spi, Netherlands) was used to coat the bivalves with 60% gold and 40% palladium for two minutes. Images were captured with the Phenom Pro Desktop SEM (ThermoFischer Scientific).

Collected bivalves were identified to species level using available keys (Kuiper, 1964; Appleton, 2002). The characteristics that were observed included umbo orientation, shell sculpture, shell

shape, presence of teeth, and size (Figure 3.2). The bivalves were measured using ImageJ, by adjusting the scale relevant to the one provided.

Drawings of all newly collected and previously published specimens were made using Adobe Illustrator (Creative Cloud, 2024) and a Wacom Intuos 4 pro graphic tablet (PTK-640).

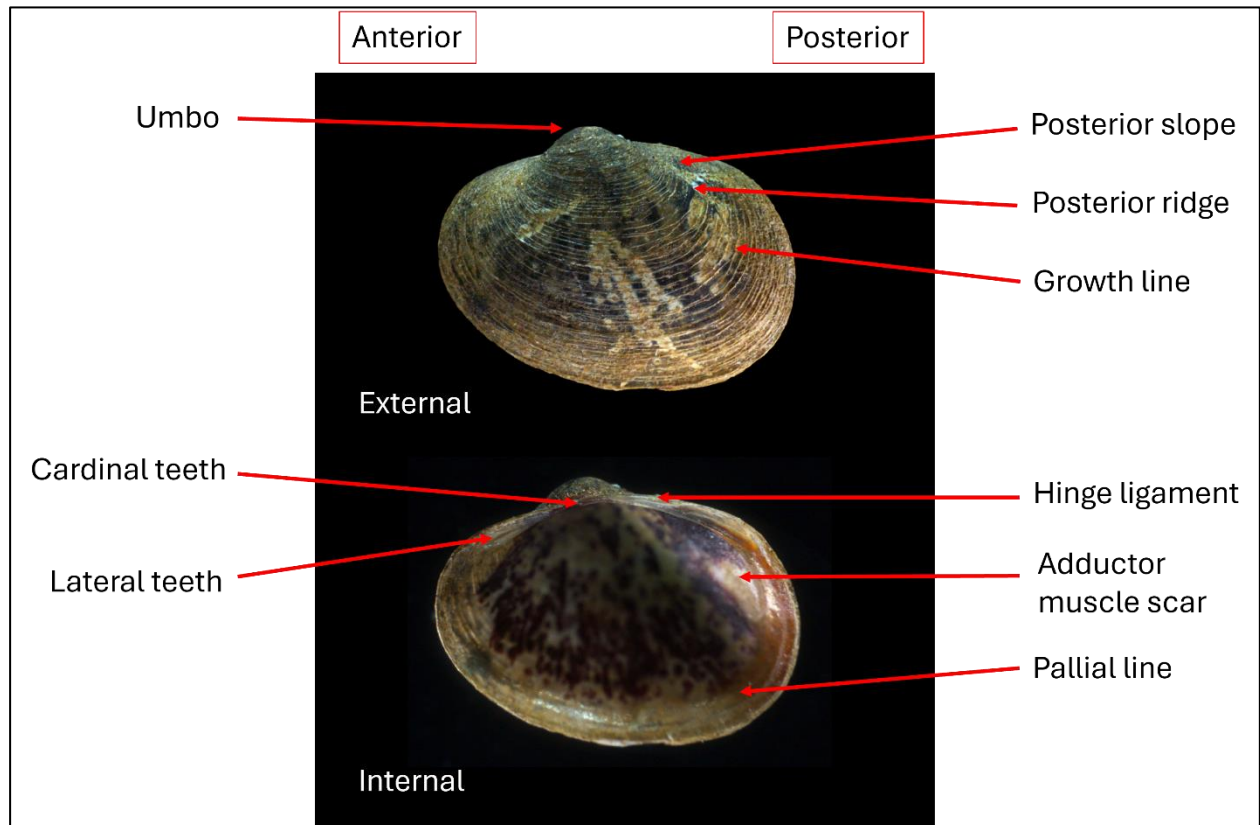


Figure 3.2 A redrawn image based on Kuiper (1964) illustration indicating the key morphological features of sphaeriids.

3.2.3 Molecular methods

After dissections of the bivalves, tissue was carefully removed, because of the fragility of the bivalve shells. Sterilised, soft tweezers and surgical blades were used to cut off a small piece of the tissue (or the entire body if it was very small) and placed in marked tubes in the freezer.

Two different DNA extraction methods were used for these bivalves. Firstly, the PCR Biosystems Rapid extractions kit (Analytical Solutions, Randburg, South Africa), following the manufacturer's guidelines, was followed. The following was added to each sample: 20 μ L lysis buffer, 10 μ L proteinase K buffer, and 70 μ L molecular grade water. This mixture was incubated at 75 °C for 5 minutes, mixed twice by vortex during this time, with the final incubation taking place at 95 °C for

10 minutes. After incubation, the samples were diluted using 150 μ L molecular grade water. According to manufacturer guidelines, 900 μ L ought to be used in this step, but less was used to yield a higher DNA concentration. After dilution, the samples were centrifuged at 15,596 RCF (Relative Centrifugal Force) for one minute, whereafter 150 μ L of the supernatant was transferred for each sample to sterile tubes and frozen at -20 °C until further use.

Secondly, 10% Chelex (Bio-Rad, South Africa) was used to extract DNA. The 10% Chelex is made up of Chelex beads and Milli-Q water at a 1:10 ratio. First, the tissue of each sample had to be completely dried. This was done by placing the open tubes in an incubator at 56 °C until dry. Approximately 200 μ L of 10% Chelex was then added to the tissue and incubated for 20 min at 95 °C, mixing by vortex every 5 min. The final step was centrifugation at 3000 rpm for 5 min. The extractions were stored in the freezer until further use.

After extraction, Polymerase chain reaction (PCR) techniques were used to amplify the DNA (different conditions are referred to by A and B in Table 3.2). For the cytochrome oxidase I (COI) gene, two different primer combinations were used for the two different genera; the other reagents and conditions stayed the same throughout. The primer combination for *Euglesa* species was COI PisInt-F and HCO2198 (Table 3.2). For *Eupera* species the universal invertebrate primers, LCO1490 and HCO2198 (Table 3.2) were used. Additionally, two ribosomal RNA gene regions were targeted: 16S and 28S. The generic primer combinations were used for both *Euglesa* and *Eupera* species, namely: 16Sar and 16Sbr as well as 28SF and 28SR (Table 3.2). In each respective small PCR tube, the following was added for a total volume of 25 μ L: 1.4 μ L forward primer, 1.4 μ L reverse primer, 6.8 μ L Kapa Hifi HotStart ReadyMix, 4 μ L DNA, and 10.4 μ L double distilled water.

The PCR results were analysed using 1% agarose gel electrophoresis at 90V for 30 minutes, then visualised under ultraviolet light using a gel documentation imaging system. Successful samples were transported to Inqaba Biotechnical Industries, a commercial sequencing business in Pretoria, South Africa, for purification and sequencing. Geneious Prime bioinformatics software (v2.1) was used to align, trim, and assemble newly produced sequences. The jModelTest 2.1.4 (Posada, 2008; Darriba et al., 2012) made use of the Alkaline Information Criterion (AIC) to calculate the best nucleotide substitution model for each dataset, which was the GTR + G model. MrBayes 3.2.7a was utilised with modified parameters on the computational resource CIPRES Science Gateway v3.3 (Miller et al., 2010) to generate the Bayesian Inference (BI) values. For Maximum Likelihood (ML) values, RA x ML HPC v.8 was used on CIPRES with modified parameters. Both the ML and BI trees were visualised in the FigTree software before the Adobe Illustrator program was used for the final editing of the combined tree.

For phylogenetic analysis, comparable sequences were identified using the Basic Local Alignment Tool (BLAST) (NCBI, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Sequences of the same genus were extracted from GenBank and BOLD to add to the alignment (Table 3.2). All sequences were acquired before 31 October 2024, thus any relevant sequences added after this date were not included. As there are not many sequences of this group on GenBank, representative sequences of all those present were added to the alignment (Table 3.3). *Corbicula africana* (Bivalvia: Cyrenidae) was used as the outgroup for both COI and 16S.

Table 3.2 Information of primers used for molecular analyses of *Euglesa* and *Eupera*.

Genetic marker	Primer	Sequence	Reference	PCR conditions*	Genus
COI	LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	Folmer et al. (1994)	Figure 3.3	<i>Eupera</i>
	HCO2198	5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'			
COI	COI PisInt-F	5' -GGNTCTGCN TAYGTNGARAG-3'	Bößneck et al. (2016)	Figure 3.3	<i>Euglesa</i>
	HCO2198	5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'	Folmer et al. (1994)		
16S	16Sar	5'-CGCCTGTTTAACAAAAACAT-3'	Hata et al. (2017)	Figure 3.4	<i>Euglesa</i> and <i>Eupera</i>
	16Sbr	5'-CCGGTCTGAACTCAGATCATGT-3'			
28S	28SF	5' – ACA ACT GTG ATG CCC TTA G – 3'	Song et al. (2008)	Figure 3.3	<i>Euglesa</i> and <i>Eupera</i>
	28SR	5' – TGG TCC GTG TTT CAA GAC G – 3'			

* PCR conditions can be found below (after Table 3.3).

Table 3.3 Sequences obtained from GenBank and BOLD (*) along with their name, accession number, reference, and country of origin. COI sequences are provided first, followed by 16S sequences. The outgroups are shaded in grey.

Sequence name	Updated name and authority [#]	Accession number	Reference for sequence	Country of origin
COI gene sequences				
<i>Corbicula africana</i> (Outgroup)	<i>Corbicula africana</i> (Krauss, 1848)	KC211266	Pigneur et al. (2014)	South Africa
<i>Euglesa casertana</i>	<i>Euglesa casertana</i> (Poli, 1791)	KF483418	Clewing et al. (2013)	Turkey

Sequence name	Updated name and authority [#]	Accession number	Reference for sequence	Country of origin
<i>Euglesa casertana</i>	<i>Euglesa casertana</i> (Poli, 1791)	KF483394	Clewing et al. (2013)	Iran
<i>Euglesa casertana</i>	<i>Euglesa casertana</i> (Poli, 1791)	*MOSK159.23		Slovakia
<i>Euglesa casertana</i>	<i>Euglesa casertana</i> (Poli, 1791)	KF483356	Clewing et al. (2013)	Albania
<i>Euglesa casertana</i>	<i>Euglesa casertana</i> (Poli, 1791)	KF483392	Clewing et al. (2013)	Greece
<i>Euglesa casertana</i>	<i>Euglesa casertana</i> (Poli, 1791)	*EINVA177.19		USA
<i>Euglesa casertana</i>	<i>Euglesa casertana</i> (Poli, 1791)	*ANNMO192.19		USA
<i>Euglesa compressa</i>	<i>Euglesa compressa</i> (Prime, 1852)	*EINVA424.19		USA
<i>Euglesa globularis</i>	<i>Euglesa globularis</i> (Clessin, 1873)	*MOSK093.22		Slovakia
<i>Euglesa globularis</i>	<i>Euglesa globularis</i> (Clessin, 1873)	KF483423	Clewing et al. (2013)	Germany
<i>Euglesa henslowana</i>	<i>Euglesa henslowana</i> (Sheppard, 1825)	KF483398.1	Clewing et al. (2013)	Macedonia
<i>Euglesa japonica</i>	<i>Odhneripisidium japonicum</i> (Pilsbry & Y. Hirase, 1908)	LC703749	Saito et al. (2022)	Japan
<i>Euglesa japonica</i>	<i>Odhneripisidium japonicum</i> (Pilsbry & Y. Hirase, 1908)	LC703756	Saito et al. (2022)	Japan
<i>Euglesa lilljeborgii</i>	<i>Euglesa lilljeborgii</i> (Clessin, 1886)	KF483406.1	Clewing et al. (2013)	Norway
<i>Euglesa nitida</i>	<i>Euglesa nitida</i> (Jenyns, 1832)	KF483364	Clewing et al. (2013)	China
<i>Euglesa obtusalis</i>	<i>Euglesa obtusalis</i> (Lamarck, 1818)	KF483387	Clewing et al. (2013)	Germany
<i>Euglesa obtusalis</i>	<i>Euglesa obtusalis</i> (Lamarck, 1818)	*MOLCA273.22		Spain
<i>Euglesa personata</i>	<i>Euglesa personata</i> (Malm, 1855)	*MN514187.1		Germany
<i>Euglesa sp.</i>	<i>Euglesa sp.</i> Jenyns, 1832	*BEISA175.19		South Africa
<i>Euglesa subtruncata</i>	<i>Euglesa subtruncata</i> (Malm, 1855)	*ANNMO233.19		USA
<i>Euglesa supina</i>	<i>Euglesa supina</i> (A. Schmidt, 1851)	KF483388.1	Clewing et al. (2013)	Germany
<i>Eupera ferruginea</i>	<i>Eupera ferruginea</i> (Krauss, 1848)	KF483421	Clewing et al. (2013)	Uganda
<i>Hindupisidium nevilleianum</i>	<i>Hindupisidium nevilleianum</i> (Theobald, 1876)	KU76207.1	Bößneck et al. (2016)	Nepal

Sequence name	Updated name and authority [#]	Accession number	Reference for sequence	Country of origin
<i>Pisidium amnicum</i>	<i>Pisidium amnicum</i> (O. F. Müller, 1774)	MK584324	Gauthier et al. (2020)	France
<i>Pisidium annandalei</i>	<i>Odhneripisidium annandalei</i> (Prashad, 1925)	LC703739	Saito et al. (2022)	Japan
<i>Pisidium cf. casertanum</i>	<i>Euglesa casertana</i> (Poli, 1791)	KF483396	Clewing et al. (2013)	Lesotho
<i>Pisidium costulosum</i>	<i>Euglesa costulosa</i> (Connolly, 1931)	KF483422	Clewing et al. (2013)	South Africa
<i>Pisidium ferrugineum</i>	<i>Euglesa ferruginea</i> (Prime, 1852)	*ANNMO210.19		USA
<i>Pisidium henslowanum</i>	<i>Euglesa henslowana</i> (Sheppard, 1825)	*ANNMO228.19		USA
<i>Pisidium kuiperi</i>	<i>Odhneripisidium kuiperi</i> (Dance, 1967)	KF483410	Clewing et al. (2013)	Nepal
<i>Pisidium milium</i>	<i>Euglesa milium</i> (Held, 1836)	*ANNMO264.19		Canada
<i>Pisidium milium</i>	<i>Euglesa milium</i> (Held, 1836)	MK584326	Gauthier et al. (2020)	France
<i>Pisidium nitidum</i>	<i>Euglesa nitida</i> (Jenyns, 1832)	*MOLCA275.22		Spain
<i>Pisidium subtruncatum</i>	<i>Euglesa subtruncata</i> (Malm, 1855)	KF483395	Clewing et al. (2013)	Italy
<i>Pisidium subtruncatum</i>	<i>Euglesa subtruncata</i> (Malm, 1855)	KF483402	Clewing et al. (2013)	Macedonia
<i>Pisidium subtruncatum</i>	<i>Euglesa subtruncata</i> (Malm, 1855)	KF483372	Clewing et al. (2013)	China
<i>Pisidium supinum</i>	<i>Euglesa supina</i> (A. Schmidt, 1851)	*ANNMO240.19		USA
<i>Pisidium variabile</i>	<i>Euglesa variabilis</i> (Prime, 1852)	*EINVA353.19		USA
<i>Pisidium viridarium</i>	<i>Euglesa viridaria</i> (Kuiper, 1956)	KF483397	Clewing et al. (2013)	Lesotho
16S gene sequences				
<i>Afropisidium nevillianum</i>	<i>Hindupisidium nevillianum</i> (Theobald, 1876)	LC703871	Saito et al. (2022)	Bangladesh
<i>Corbicula madagascariensis</i> (outgroup)	<i>Corbicula madagascariensis</i> Smith, 1882	AF152022	(Cooley and O Foighil, 2000)	Madagascar
<i>Euglesa casertana</i>	<i>Euglesa casertana</i> (Poli, 1791)	KY202903	Mouthon and Forcellini (2017)	France
<i>Euglesa casertana</i>	<i>Euglesa casertana</i> (Poli, 1791)	KY202860	Mouthon and Forcellini (2017)	France
<i>Euglesa casertana</i>	<i>Euglesa casertana</i> (Poli, 1791)	LC703828	Saito et al. (2022)	Russia

Sequence name	Updated name and authority [#]	Accession number	Reference for sequence	Country of origin
<i>Euglesa compressa</i>	<i>Euglesa compressa</i> (Prime, 1852)	KY202891	Mouthon and Forcellini (2017)	France
<i>Euglesa compressa</i>	<i>Euglesa compressa</i> (Prime, 1852)	KY202893	Mouthon and Forcellini (2017)	France
<i>Euglesa globularis</i>	<i>Euglesa globularis</i> (Clessin, 1873)	KY126450	Bespalaya et al. (2017)	Russia
<i>Euglesa globularis</i>	<i>Euglesa globularis</i> (Clessin, 1873)	MK492506	Bespalaya et al. (2017)	Russia
<i>Euglesa globularis</i>	<i>Euglesa globularis</i> (Clessin, 1873)	LC703827	Saito et al. (2022)	Russia
<i>Euglesa globularis</i>	<i>Euglesa globularis</i> (Clessin, 1873)	LC703849	Saito et al. (2022)	Russia
<i>Euglesa japonica</i>	<i>Odhneripisidium japonicum</i> (Pilsbry & Y. Hirase, 1908)	LC703806	Saito et al. (2022)	Japan
<i>Euglesa supina</i>	<i>Euglesa supina</i> (A. Schmidt, 1851)	KY126443	Bespalaya et al. (2017)	Russia
<i>Euglesa supina</i>	<i>Euglesa supina</i> (A. Schmidt, 1851)	KY202897	Mouthon and Forcellini (2017)	France
<i>Euglesa waldeni</i>	<i>Euglesa waldeni</i> (Kuiper, 1975)	KY126441	Bespalaya et al. (2017)	Russia
<i>Eupera ferruginea</i>	<i>Eupera ferruginea</i> (Krauss, 1848)	KF483320	Clewing et al. (2013)	Uganda
<i>Pisidium casertanum</i>	<i>Euglesa casertana</i> (Poli, 1791)	AY957795	(Guralnick, 2005)	
<i>Pisidium casertanum</i>	<i>Euglesa casertana</i> (Poli, 1791)	KF483316	Clewing et al. (2013)	Turkey
<i>Pisidium costulosum</i>	<i>Euglesa costulosa</i> (Connolly, 1931)	KF483323	Clewing et al. (2013)	South Africa
<i>Pisidium dubium</i>	<i>Pisidium dubium</i> (Say, 1817)	AF152027	Cooley and O Foighil (2000)	USA
<i>Pisidium edlaueri</i>	<i>Euglesa edlaueri</i> (Kuiper, 1960)	KF483302	Clewing et al. (2013)	Macedonia
<i>Pisidium javanum</i>	<i>Hindupisidium javanum</i> (van Benthem Jutting, 1931)	KU376190	Böðneck et al. (2016)	Indonesia
<i>Pisidium lilljeborgii</i>	<i>Euglesa lilljeborgii</i> (Clessin, 1886)	KY126447	Bespalaya et al. (2017)	Russia
<i>Pisidium maasseni</i>	<i>Euglesa maasseni</i> (Kuiper, 1987)	KF483298	Clewing et al. (2013)	Macedonia
<i>Pisidium personatum</i>	<i>Euglesa personata</i> (Malm, 1855)	KF483318	Clewing et al. (2013)	Turkey

Sequence name	Updated name and authority [#]	Accession number	Reference for sequence	Country of origin
<i>Pisidium pulchellum</i>	<i>Euglesa pulchella</i> (Jenyns, 1832)	KY202894	Mouthon and Forcellini (2017)	France
<i>Pisidium viridarium</i>	<i>Euglesa viridaria</i> (Kuiper, 1956)	KF483296	Clewing et al. (2013)	Lesotho
<i>Sphaerium nucleus</i>	<i>Sphaerium nucleus</i> (Studer, 1820)	HM208273	Petkevičiūtė et al. (2018)	Czech Republic

*BOLD sequences do not have references.

[#]Names last updated on 31 October 2024.

Two different PCR protocols were followed. The first protocol (Figure 3.3) made use of a modified touchdown PCR from Bößneck et al. (2016) (where the annealing temperature decreases in each cycle). The initial denaturation started at 95°C for 1 minute, followed by 7 cycles with a denaturation of 95°C for 30 seconds (s) and an annealing temperature lasting for 30s starting at 52°C and decreasing with each cycle by 1°C, to end at 46°C. An extension of 72°C for 30s formed part of these 7 cycles. This was followed by 33 cycles of 95°C for 30s, 45°C for 30s, and 72°C for 30s. The final extension was at 72°C for 3 min and it was held at 4°C till further analysis.

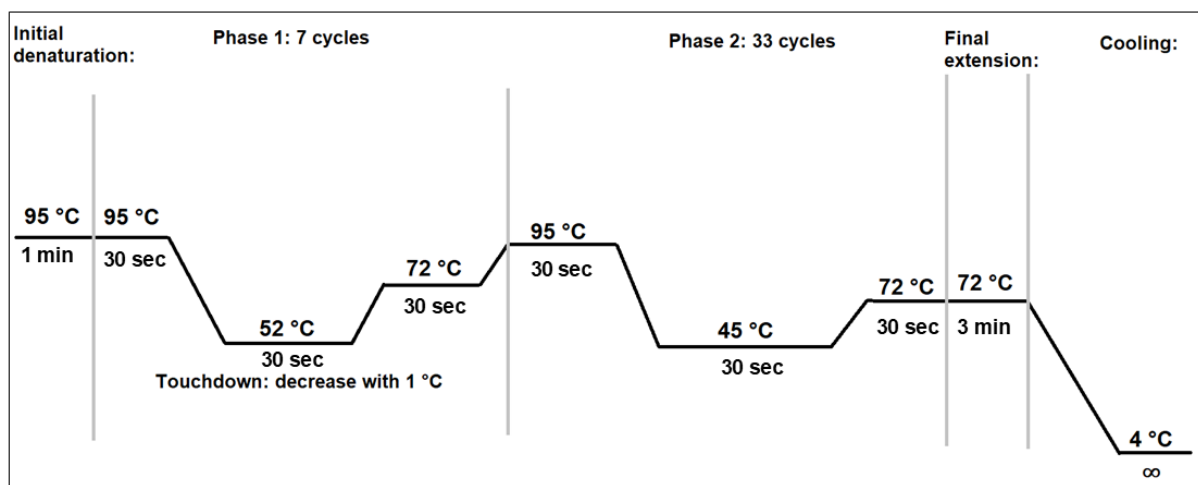


Figure 3.3 An illustration of the touchdown PCR used for the COI and 28S genes of sphaeriids.

The second protocol (Figure 3.4) had an initial denaturation of 94°C held for 10 min, followed by 30 cycles of 94°C for 30s, 48°C for 1 min, and 72°C for 1min. The final extension was at 72°C for 10 mins, and it was held at 12°C till further analysis (Hata et al., 2017).

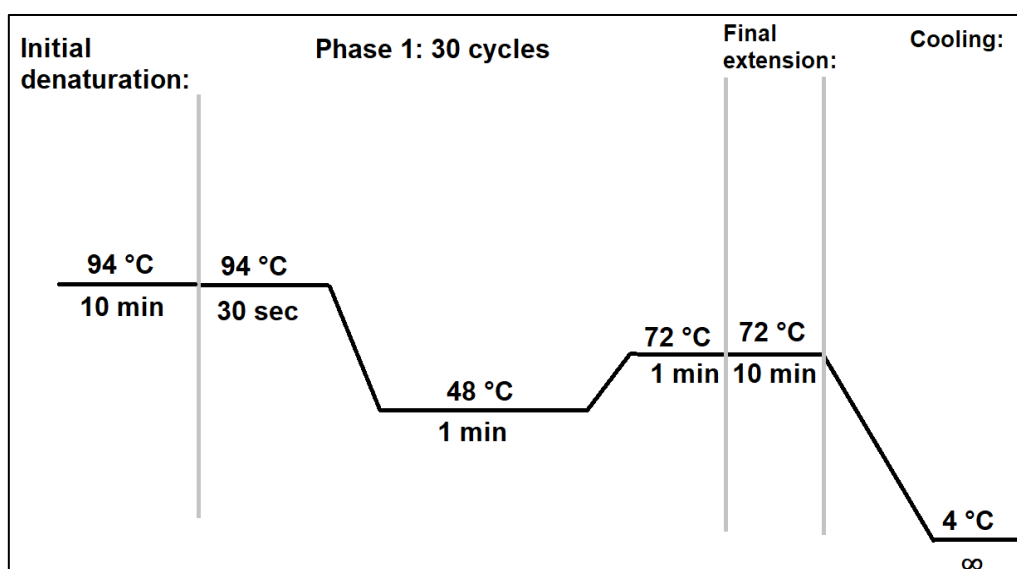


Figure 3.4 An illustration of the PCR protocol used for the 16S gene of sphaeriids.

3.2.4 Map creation and coordinate plotting

Quantum Geographic Information System (QGIS) was used to create maps for this chapter using coordinates from the NWU Snail Collection, GBIF, and from the current study to indicate an updated distribution (see Results). The coordinates of sites where bivalves were found during this study were added to QGIS in the form of a layer, together with the following layers: African border, South African border, provinces of South Africa, and a Google satellite image.

For the NWU Snail Collection (referred to on the map as Snail Collection), the National Science Collection Facility virtual facility (hereto further referred to as the virtual facility) was used in conjunction with QGIS as discussed in Chapter 2 (section 2.2). A description of the site in the form of a farm name, number of the farm, and town or district was provided by the virtual facility. This information was input into QGIS containing a myriad of layers that contain all the different farms and their respective numbers. Noteworthy layers also included: SA rivers, SA 1:50 topomap, 1:250 topomap, and protected areas. After the coordinate was found on QGIS, it was input in the virtual facility with the name of the farm, etc. Information also added to the georeference included the uncertainty parameter that is calculated by QGIS (level of confidence or accuracy associated with the data), what protocol was used (usually the 1:250 topomap), the source used to find the farm (usually SANBI gazetteer) the date, ORCID of the person doing the georeference, and any remarks that might be relevant to the specific georeference. These georeferences were stored in a database until all georeferences that could be done, were done

(5,910 georeferences). For each species map, the relevant species information was extracted from this database and added to a blank QGIS with the necessary layers.

3.3 Results

3.3.1 Morphological results

Currently, there are three genera in the family Sphaeriidae from South Africa: *Euglesa*, *Eupera*, and *Sphaerium*. Within this country, there are 10 species known from these genera. All these species will be revised below and updated where possible.

Taxonomic results – *Euglesa*:

Phylum Mollusca

Class Bivalvia

Order Sphaeriida

Family Sphaeriidae Deshayes, 1855

Subfamily Sphaeriinae Deshayes, 1855 (1820)

Genus *Euglesa* Jenyns, 1832

Notes: Appleton (2002) was used to place the bivalves in this section in the genus *Euglesa*, based on their size, umbo orientation, sculpture and teeth. *Euglesa* as a genus was only recently introduced into the family Sphaeriidae and consists of 90 species of which only eight are known from South Africa. According to Appleton (2002), it is difficult to distinguish between these eight species since all are small, whitish and usually smaller than 6 mm. During this project, no bivalve was found to be bigger than 3 mm. According to Mandahl-Barth (1988), most are smaller than 5 mm, and it is due to this small size that they are hard to identify. Their umbo orientation is posterior to the median vertical line. All of them have growth lines, but only one has defined sculpture: *Euglesa costulosa*. A red/brown deposit can usually be found on the posterior and dorsal areas and all of them have two cardinal teeth. Some species are widely distributed, possibly because of passive dispersal by insects and birds (Appleton, 2002).

3.3.1.1 *Euglesa casertana* (Poli, 1791)

Original name: *Cardium casertanum* Poli, 1791.

Type locality: Sicily, Italy (Mandahl-Barth, 1988).

Distribution: In South Africa, this species is known from Western Cape, KwaZulu-Natal, and Mpumalanga (Kuiper, 1964). It is the most cosmopolitan species in the family (Bespalaya, 2018), occurring on every continent according to GBIF (Global Biodiversity Information Facility). As mentioned in Chapter 2, the NWU Snail Collection was recently digitised in the form of each data point being georeferenced. In Figure 3.5 these points can be seen for *Euglesa casertana* in Mpumalanga. This species was not found in the present study.

Museum material: Some specimens can be found in South African Museums, including the Snail Collection at North-West University (Kuiper, 1964).

Size: The minimum recorded length is 2.8 mm and the maximum is 2.8 mm (Kuiper, 1964; Nesemann and Sharma, 2005).

GBIF: 17,446 occurrences (Figure 3.5).

Available sequences: 94 on GenBank (16S, 28S, COI); 354 on BOLD (COI) (Table 3.3).

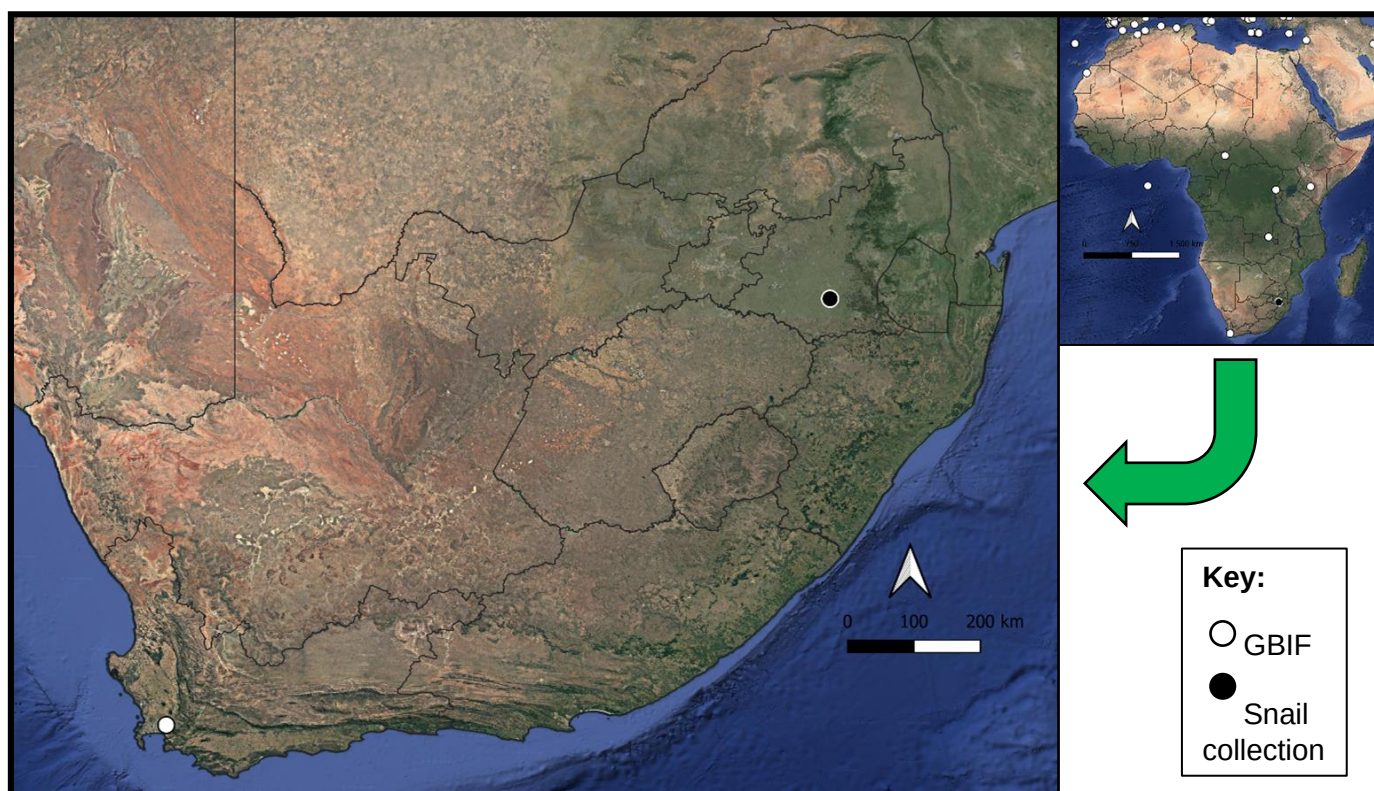


Figure 3.5 South African distribution of *Euglesa casertana* (Poli, 1791) based on data points from GBIF and georeferenced data from the NWU Snail Collection.

Photos: When searching for *Euglesa casertana* and *Pisidium casertana*, 102 images on GBIF and 16 on iNaturalist were labelled as *Euglesa casertana*.

Descriptive notes: Thick-shelled, surface with fine irregular striations. Periostracum is moderately dull to slightly glossy and yellowish to red-brown in the posterior half with a curved hinge. Thick anterior and posterior teeth. Cardinal teeth are distinctly curved. The umbo is prominent and shifted posteriorly. Their striae are fine and the periostracum is moderately dull to slightly glossy. Dorsal and ventral margins are parallel and gently curved, and the dorsal margin frequently connects with slight angles. The anterior end is moderately long and rounded with the posterior end is truncate. The hinge-plate is also moderately long, broad, and its lateral teeth are distinct, but not long. The cusps on their laterals are quite sharp (Figure 3.6).

Remarks: According to Mandahl-Barth (1988), *E. casertana* is the largest of all *Euglesa* species, with one record in Africa being 5.8 mm in length. Smaller specimens are usually identified by eliminating other species as they are quite variable. It is a useful indicator species because of its high abundance and long lifespan. *Euglesa casertana* are found abundantly in very small natural cold streams. They are wide-spread and thus used for life history studies. This species has the most cosmopolitan distribution (Bespalaya, 2018); however, this is questioned because it has few distinguishing characteristics which means some of the bivalves could be misidentified as something else (Mandahl-Barth, 1988). Their abundance is strongly related to substrate type and macroion content. They favour silty-sand or sand-gravel substrate type and lower macroion content (mainly SO_4^{2-} , Ca^{2+} and Mg^{2+}) (Bespalaya, 2018). According to the IUCN Red List, this species is of least concern, but the population is decreasing (Prie and Lopes-Lima, 2024). The conservation status was last assessed in May 2024.

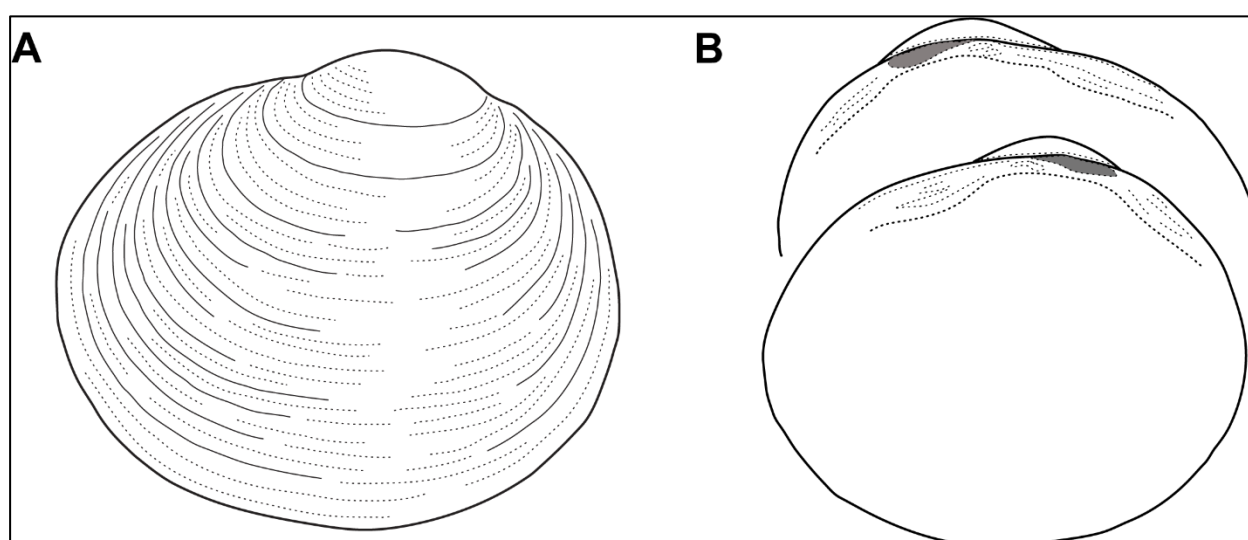


Figure 3.6 A redrawn image of *Euglesa casertana* (Poli, 1791), based on an illustration by Kuiper (1964).

3.3.1.2 *Euglesa costulosa* (Connolly, 1931)

Original name: *Pisidium costulosum* Connolly, 1931.

Type locality: Rhenoster River near Rustfontein, South Africa (Mandahl-Barth, 1988).

Distribution: This species is only known from South Africa. In the Western Cape area, this species has been found in the Berg River, Klein Berg River, and the dam in Tulbagh Kloof. In the Free State province, they are continuously spread in northeastern parts and along the eastern and southern border occurring in Rustfontein and the Rhenoster River (Kuiper, 1964). They are widely distributed in the Gauteng province in the Klein Jukskei River, Braamfontein stream, Zandfontein stream, Leeukop stream and two sites of the Vaal River: near Vereeniging and near Standerton. They have also been found in the Klip River, in KwaZulu-Natal. Kuiper (1964) mentions that they are distributed in the central/southern parts of Mpumalanga, sparsely distributed in the Northern Cape, and poorly represented in Limpopo. According to De Kock and Wolmarans (2008), this species has also been found in the North West province, in the Mooi River catchment area. Based on the georeferenced data, *E. costulosa* has been recorded in the Gauteng, Mpumalanga, Free State and Western Cape provinces (Figure 3.7). It was found during this study in the North West, Gauteng, and Mpumalanga provinces (Figure 3.7).

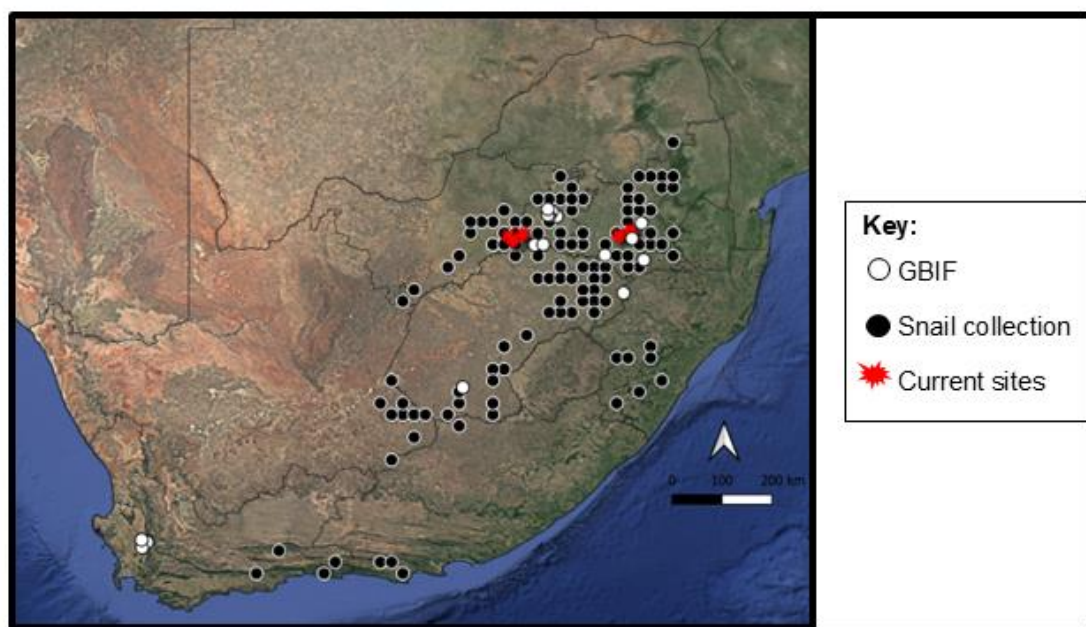


Figure 3.7 South African distribution of *Euglesa costulosa* (Connolly, 1931) based on data points from GBIF and georeferenced data from the NWU Snail Collection. The sites where it was found during the present study are indicated in red.

The current sites where *Euglesa costulosa* was found, are Muiskraal, Mooi River (Table 2.12), Mooi River Upstream (Table 2.14), Wonderfonteinspruit site 3 (Table 2.10), X-spruit site 1 (Table 2.15), X-spruit site 3 (Table 2.17), and X-spruit site 4 (Table 2.18).

Museum material: Unknown.

Size: The maximum length recorded for this species was 3.5 mm and the minimum length was 3 mm (Appleton, 2002; Kuiper, 1964; Mandahl-Barth, 1988). Specimens found in this study (Figure 3.8) had a maximum length of 3.6 mm and a minimum length of 2 mm.

GBIF: 30 occurrences.

Available sequences: 2 on GenBank (16S, COI) (Table 3.3). Sequences from this study were obtained for 16S (26) and COI (18).

Photos: None available online.

Descriptive notes: *Euglesa costulosa* can be distinguished from the other *Euglesa* species by its regular sculpture of concentric narrow grooves. The shape of the bivalve itself does not vary much, but the sculpture can vary (Figure 3.8). The grooves are widely spaced from each other on the lower part of the shell compared to the part closer to the umbo where the grooves become closer to each other. Juvenile shells can sometimes present prominent, more widely spaced grooves. Lastly, the ligament is long and internal (Kuiper, 1964).

Remarks: *Euglesa costulosa* resembles *E. pirothi* but can be distinguished by its external ligament (Mandahl-Barth, 1988). This species is listed as Least Concern on the IUCN Red List but was last assessed in 2007. The population trend is currently unknown (Kristensen et al., 2010a).

3.3.1.3 *Euglesa harrisoni* (Kuiper, 1964)

Original name: *Pisidium harrisoni* Kuiper, 1964.

Type locality: Vaal River between Morgenzon and Amersfoort, South Africa (Kuiper, 1964). Mandahl-Barth (1988) however states that its type locality is in the Vaal River and that it is only known from this locality.

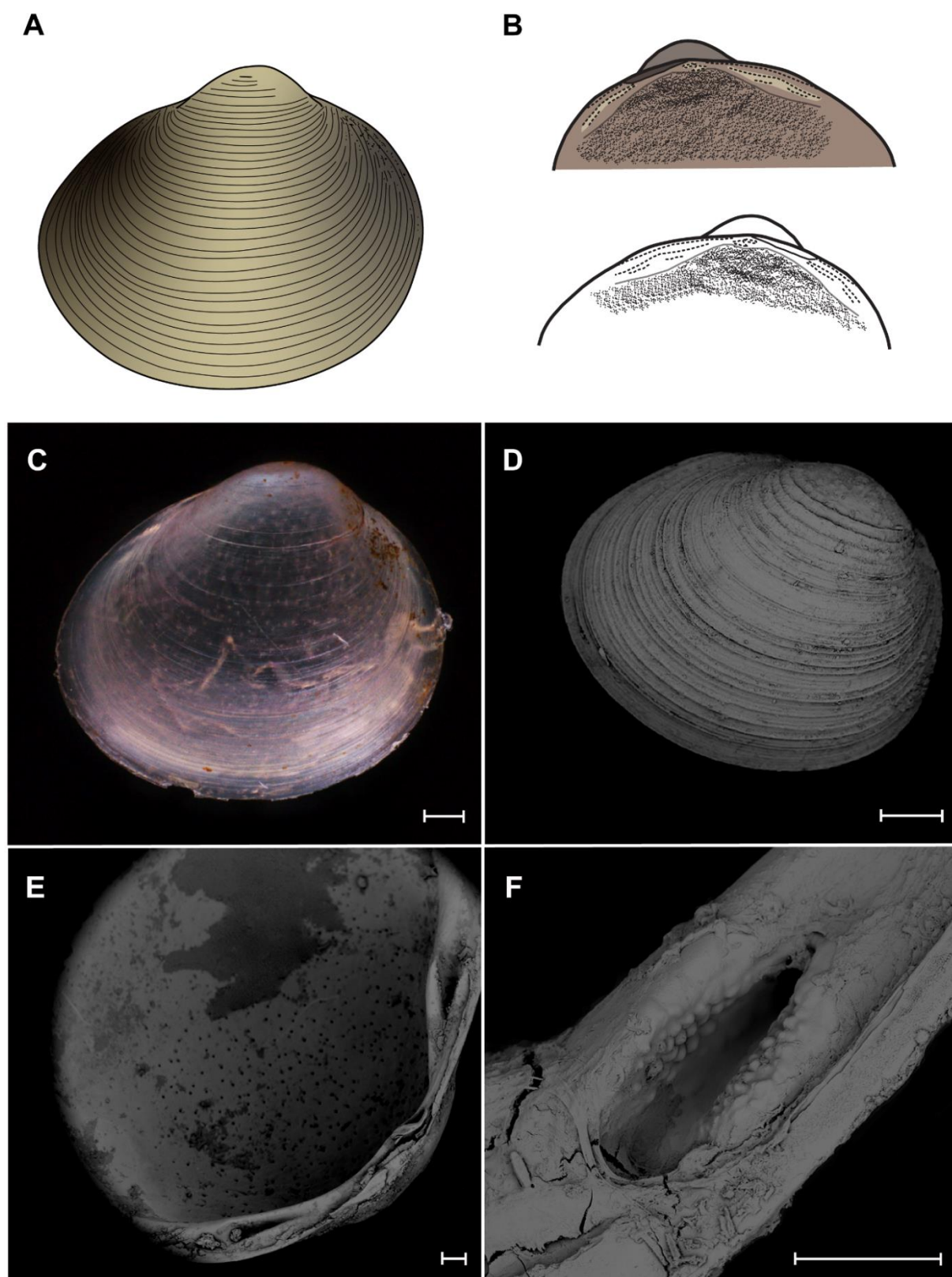


Figure 3.8 *Euglesa costulosa* (Connolly, 1931) visualised by different means: A and B – redrawn images based on Kuiper's (1964) illustration of (A) external features and (B) internal features. C – light photomicrograph of *E. costulosa* found during this study. D to F – Scanning Electron Microscopy (SEM) images of (D) external features, (E) cardinal and lateral teeth, and (F) a closeup of a lateral tooth. Scale bars: C & D – 250 μm ; E & F – 100 μm .

Distribution: This species is endemic to South Africa (De Kock and Wolmarans, 2008a). It is known from the Vaal River near Vereeniging and at the Vaal Barrage (De Kock and Wolmarans, 2008). This is confirmed by the distribution on GBIF (Figure 3.9). There were no reports in the NWU Snail Collection of this species, and it was not found in this study.

Size: Varies from a length of 3.4 mm to 2.35 mm according to Kuiper (1964). It was not examined during this study.

GBIF: 7 occurrences.

Available sequences: None.

Photos: None available online.

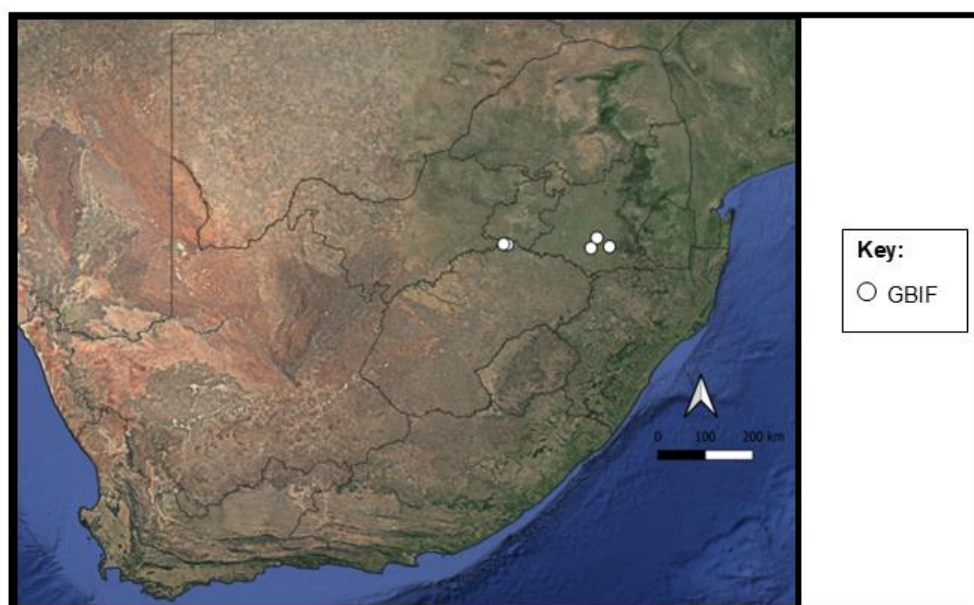


Figure 3.9 South African distribution of *Euglesa harrisoni* (Kuiper, 1964) based on data points from GBIF. No georeferenced data was available from the NWU Snail Collection.

Descriptive notes: This species is identified by having an inequilateral, thin, elongate, ovate shell with low and scarcely prominent umbones, placed far back at one-quarter of the shell length. The dorsal margin is half the shell length and slightly curved, and the ventral margin is broadly rounded. The anterior end is very elongate and blindly pointed, and the upper side is flattened. The posterior end is perpendicular and slightly curved (Figure 3. 10). There are irregular fine concentric striae (approximately 10–15) in the middle of the shell, 0.5 mm apart. It has an internal ligament (Kuiper, 1964).

Remarks: This species is different to the preceding because of its elongate shell. This species is data deficient and was last assessed in 2007. The population trend is currently unknown (Kristensen et al., 2010).

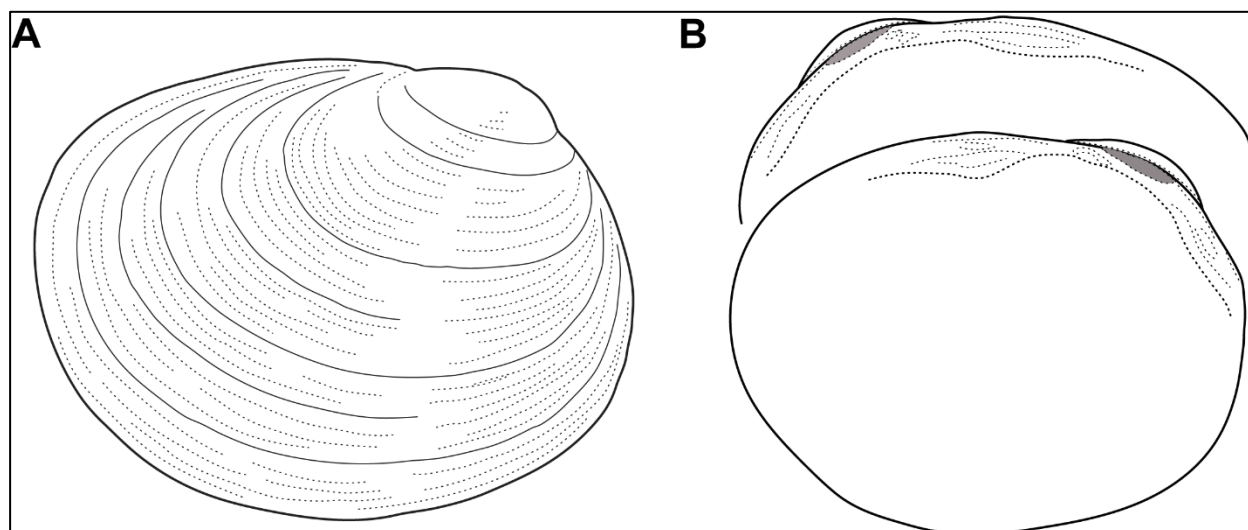


Figure 3.10 A redrawn image of *Euglesa harrisoni* (Kuiper, 1964), based on an illustration by Kuiper (1964).

3.3.1.4 *Euglesa langleyana* (Melvill & Ponsoby, 1891)

Original name: *Pisidium langleyanum* Melvill & Ponsoby, 1891

Type locality: The type locality is Port Elizabeth, South Africa (Mandahl-Barth, 1988; Melvill and Ponsoby, 1891).

Distribution: This species is endemic to southern Africa (De Kock and Wolmarans, 2008a) and has a relatively continuous distribution in southern Gauteng, the north-eastern Free State, and the central area of Mpumalanga. It is discontinuously spread through KwaZulu-Natal and the Eastern Cape. It is nearly absent in the Northern Cape and Western Cape and completely absent from Limpopo (De Kock and Wolmarans, 2008a). They are poorly represented in the North West but can be found in the Mooi River catchment area (De Kock and Wolmarans, 2008a). Based on the georeferenced data, they have been reported from Gauteng, Mpumalanga, Limpopo, KwaZulu-Natal, and the Eastern Cape (Figure 3.11) but were not found in this study.

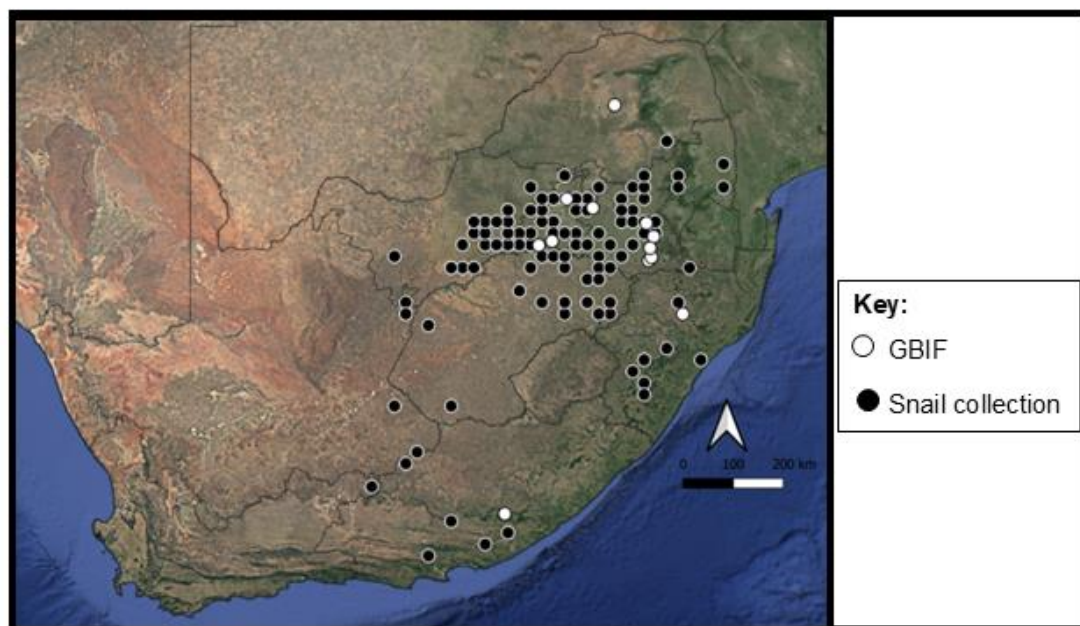


Figure 3.11 South African distribution of *Euglesa langleyana* (Melvill & Ponsoby, 1891) based on data points from GBIF and georeferenced data from the NWU Snail Collection.

Museum material: The Natural History Museum, London. Not examined.

Size: It ranges from a length of 3.9 mm to 2.2 mm (Kuiper, 1964). When this species was first described by Melvill and Ponsoby (1891), it was thought that this was the smallest species in the group. It was not examined in this study.

GBIF: 25 occurrences.

Available sequences: None.

Photos: When searching for *Euglesa langleyana* and *Pisidium langeleyanum*, one image was labelled as *Euglesa langleyana* on iNaturalist (Gert Sibande, Mpumalanga).

Descriptive notes: *Euglesa langleyana* has prominent umbones to the back of the shell and regular, concentric striae. Its shell is thin with a narrow hinge and has a sub-trigonal shape and is densely porous. The juvenile shells may have a few sharp striae. The dorsal margin is strongly rounded compared to the ventral margin. Its ligament is also internal but can sometimes be visible externally due to a slight gap in the valves behind the umbones (Figure 3.12)

Remarks: *Euglesa langleyana* is unique because of its elongate, prominent umbo, and can survive in a wide variety of waterbodies. This makes it an ideal species to monitor the accumulation of metals. Temperature and altitude are determining factors of where they end up

residing (De Kock, 2008). A high density of organisms usually resides close to one another because of their relatively small size. *Euglesa langleyana* has the largest number of records in the NWU Snail Collection of all the *Euglesa* species. This species is listed as Least Concern on the IUCN Red List and was last assessed in 2009. The population trend is currently unknown (Appleton et al., 2010).

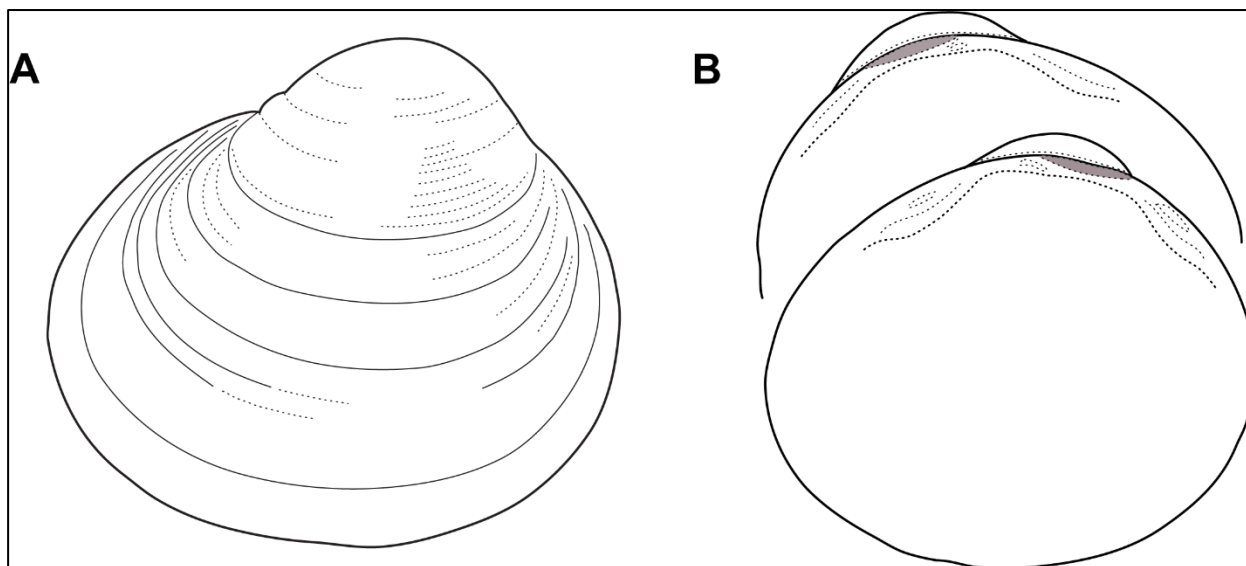


Figure 3.12 A redrawn image of *Euglesa langleyana* (Melvill & Ponsoby, 1891), based on an illustration by Kuiper (1964).

3.3.1.5 *Euglesa ovampica* (Ancey, 1890)

Original name: *Pisidium ovampicum* Ancey, 1890.

Type locality: According to Kuiper (1964), this species was originally found in the Western Cape Province, South Africa at the Gwang River near George and in Jonkershoek near Stellenbosch in fishponds.

Distribution: *Euglesa ovampica* is not very widely distributed and only known from South Africa. According to GBIF, *E. ovampica* can be found in the Western Cape and KwaZulu-Natal provinces in South Africa, but according to the georeferenced data, it has only been found in the North West (Figure 3.13). This species was not found in this study.

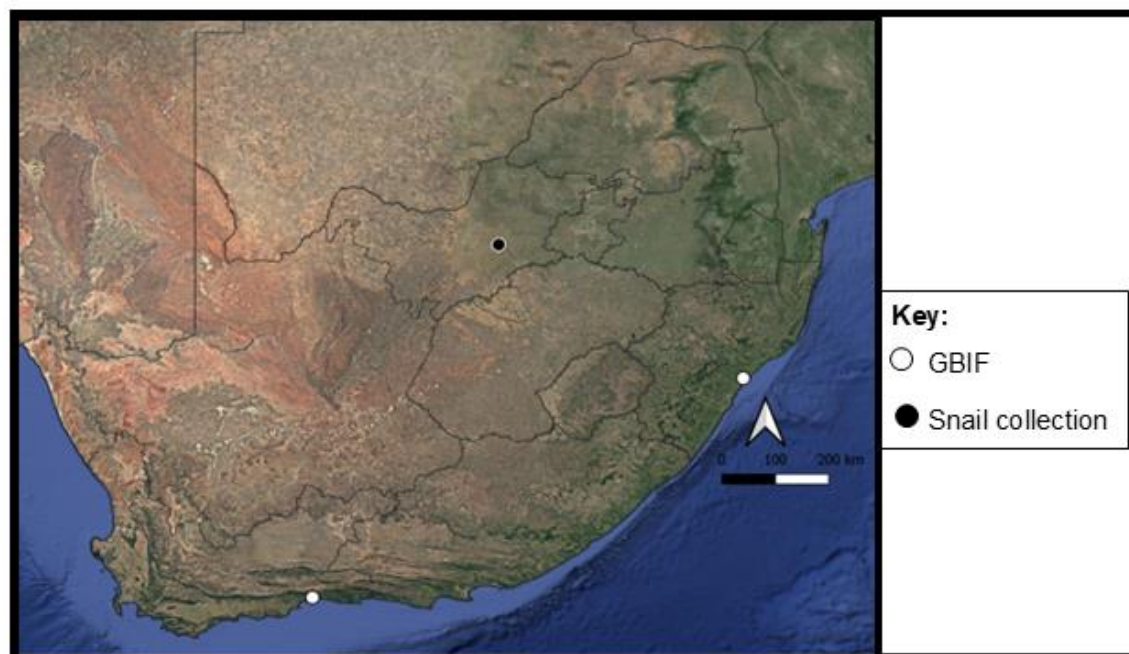


Figure 3.13 South African distribution of *Euglesa ovampica* (Ancey, 1890) based on data points from GBIF and georeferenced data from the NWU Snail Collection.

Museum material: Natural History Museum, London. Not examined.

Size: It has a length of 2.6 mm to 3 mm (Kuiper, 1964; Mandahl-Barth, 1988). The specimen was not examined in this study.

GBIF: 7 occurrences.

Available sequences: None.

Photos: None available online.

Descriptive notes: *Euglesa ovampica* has a thin, nearly equilateral, ovate, opaque, glossy shell with no pores visible. Its umbones are smooth, in the middle, not prominent and hardly rise above the dorsal shell margin. Very fine and irregular striae with two light growth-lines can be observed on the shell. Its ligament is also internal and has a dark brown colour. The hinge plate is narrow. The cardinal teeth are thin, short, and straight; C3 is the longest; C2 resides on the ventral margin of the hinge plate and is seen as the short tooth; C4 is the same length as C2 and stands parallel to each other (Figure 3.14). The posterior adductor can be distinguished from the anterior adductor because it is a bit bigger and lower (Kuiper, 1964).

Remarks: *Euglesa ovampica* is unique because of its opaque and glossy shell. It is listed as Least Concern on the IUCN Red List and was last assessed in 2016. The population trend is currently unknown (Van Damme, 2016).

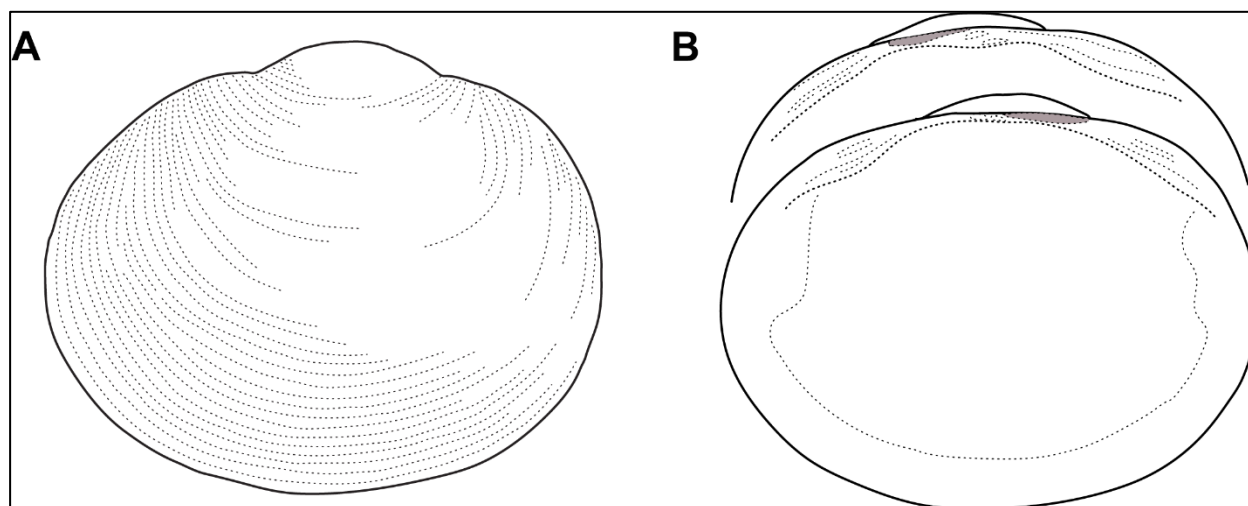


Figure 3.14 A redrawn image of *Euglesa ovampica* (Ancey, 1890), based on an illustration by Kuiper (1964).

3.3.1.6 *Euglesa pirothi* (Jickeli, 1881)

Original name: *Pisidium pirothi* Jickeli, 1881.

Type locality: Originally collected in South Africa, in the Great Letaba River near Letaba camp in the Kruger National Park and Phongolo River Floodplain (Kuiper, 1964; Schultzei, 2011).

Distribution: *Euglesa pirothi* is known from Africa and specifically in KwaZulu-Natal and Mpumalanga in South Africa (Kuiper, 1964; Kuiper 1966). However, according to GBIF, they have only been reported from Limpopo and based on the georeferenced data they have been found in Limpopo and Mpumalanga (Figure 3.15). According to GBIF, it has also been reported from Uganda (Figure 3.15) but was not found in this study.

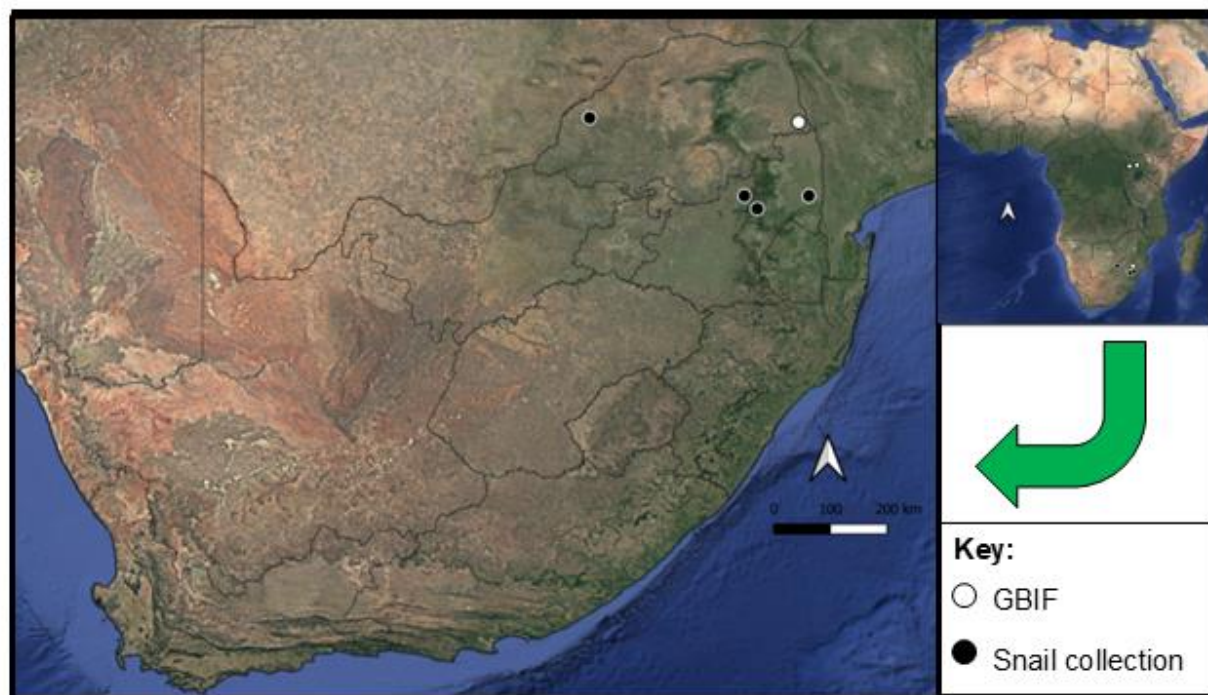


Figure 3.15 South African distribution of *Euglesa pirothi* (Jickeli, 1881) based on data points from GBIF and georeferenced data from the NWU Snail Collection.

Museum material: Natural History Museum, London. Not examined.

Size: Approximately 3.5 mm in length (Mandahl-Barth, 1988). Not examined in this study.

GBIF: 7 occurrences.

Available sequences: 1 on GenBank (COI) (Table 3.3).

Photos: There are two photos in Schultheiß et al. (2009).

Descriptive notes: *Euglesa pirothi* has a prominent external ligament with a long and very narrow ligament-pit which is bordered ventrally by a kind of callosity (Kuiper, 1964). It varies between a finer or rougher texture. The shell is thin, sub-transparent, and inequilateral with regular concentric fine striae (Figure 3.16).

Remarks: *Euglesa pirothi* can be classified as a pea clam and is distinguished from the other *Euglesa* species by its unique ligament (Kuiper, 1964; Mandahl-Barth, 1988). The species has an external ligament and callosity present. There is a large variability of shell characteristics (Schultheiß et al, 2009) and it is associated with higher climatic conditions (De Kock and

Wolmarans, 2008a). It is listed as Least Concern on the IUCN Red List and was last assessed in 2018. The population trend is currently unknown (Seddon, et al. 2018).

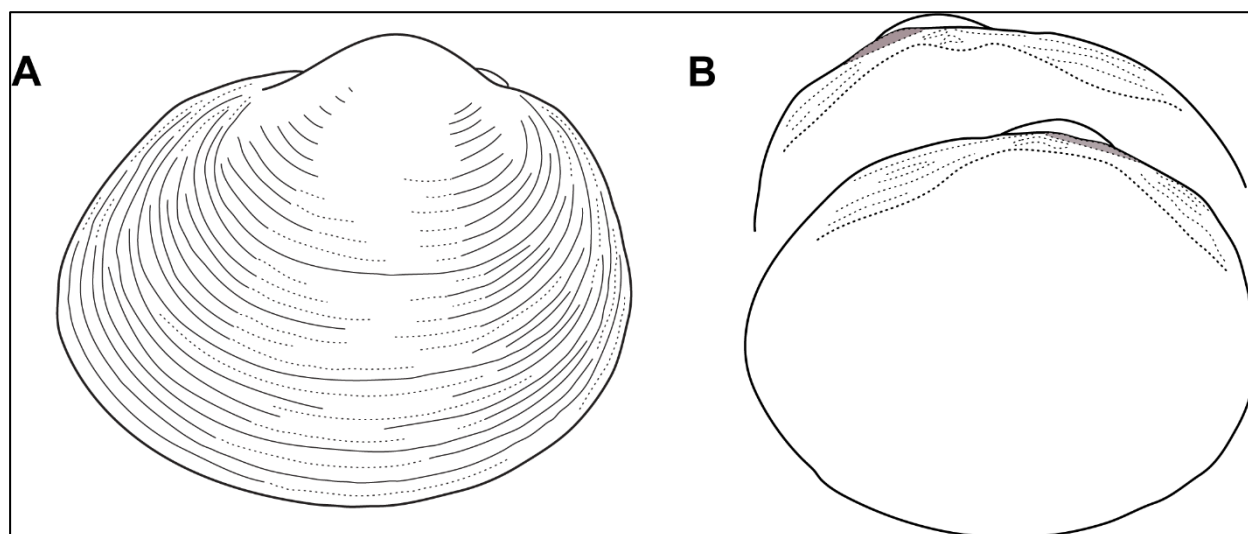


Figure 3.16 A redrawn image of *Euglesa pirothi* (Jickeli, 1881), based on an illustration by Kuiper (1964).

3.3.1.7 *Euglesa viridaria* (Kuiper, 1956)

Original name: *Pisidium viridarium* Kuiper, 1956.

Type locality: Karura River northwest of Nairobi, Kenya (Mandahl-Barth, 1988).

Distribution: In South Africa, this species can be found in the Gwang River near George in the Western Cape province, South Africa, and Maseru Mission Station in Lesotho (Kuiper, 1964). They have a relatively continuous distribution in the south-eastern part of the North West Province, the northern part of Gauteng, and central part of Mpumalanga. They are unevenly distributed in the Eastern Cape and Free State, uncommon in the northern and Western Cape and completely absent from Limpopo (De Kock and Wolmarans, 2008). According to GBIF, only one specimen has been recorded from Lesotho, but the georeferenced information confirms that they have been reported from North West, Gauteng, Free State, Northern Cape, Eastern Cape, KwaZulu-Natal, and Limpopo provinces in South Africa (Figure 3.14). It has also been reported from Ethiopia according to GBIF (Figure 3.17). This species was collected in this study in the North West and Limpopo provinces.

The current sites where *Euglesa viridaria* was found, are Wonderfontainspruit Site 10 (Table 2.11), Mooi River Upstream (Table 2.14), and Sabie River (Table 2.5).

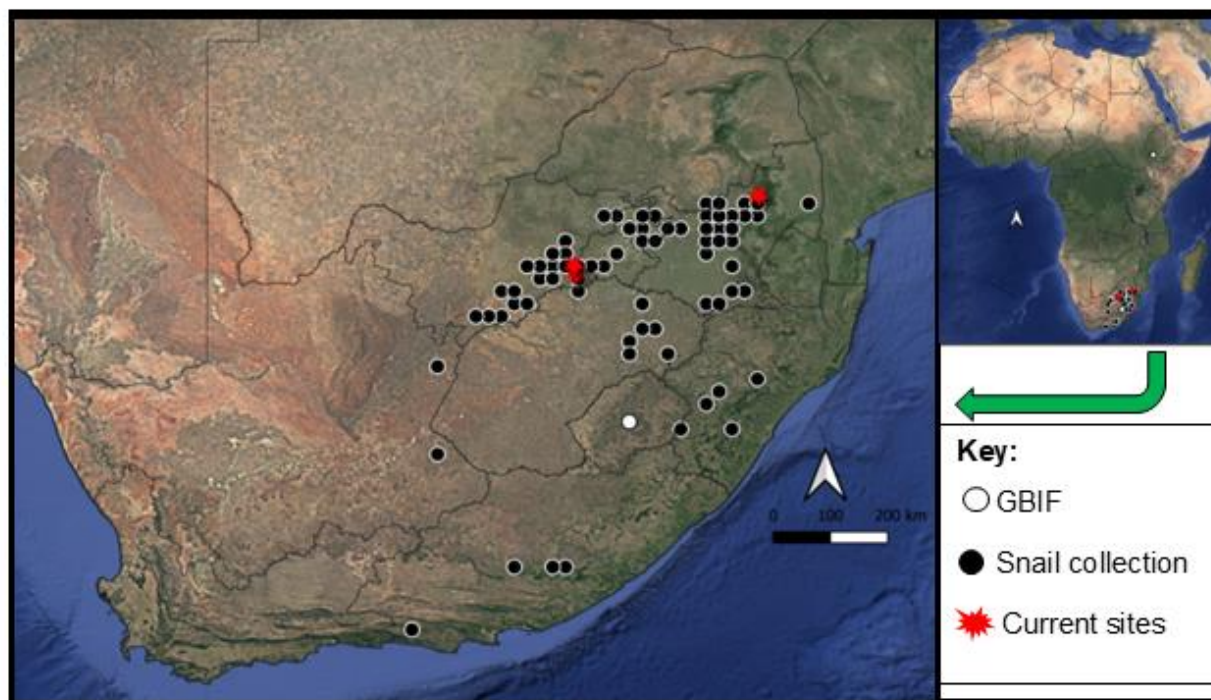


Figure 3.17 South African distribution of *Euglesa viridaria* (Kuiper, 1956) based on data points from GBIF and georeferenced data from the NWU Snail Collection.

Museum material: Natural History Museum, London. Not examined.

Size: The recorded length of this species ranges from 3.5 mm to 2.6 mm (Kuiper, 1956; Piechoki, 1993; Mandahl-Barth, 1988). *Euglesa viridaria* found during this study ranges from a length of 2.7 mm to 2.2 mm.

GBIF: 9 occurrences.

Available sequences: 1 on GenBank (COI) (Table 3.3). Sequences of this species were generated in this study for 16S (6) and COI (6).

Photos: None available online.

Descriptive notes: It has a thin, ovate shell with sub-median umbones. They have whitish shells with delicate, equally distant concentric striae especially in the lower parts. The shell is densely porous, i.e. they have several, dense canaliculi perforating the shell which are visible on the shell in the form of pores. The shell convexity increases proportionally with the shell size. The hinge plate is thin, and the most characteristic feature is the callosity (a typical hinge characteristic) found at the inner end of the lateral tooth (Figure 3.18).

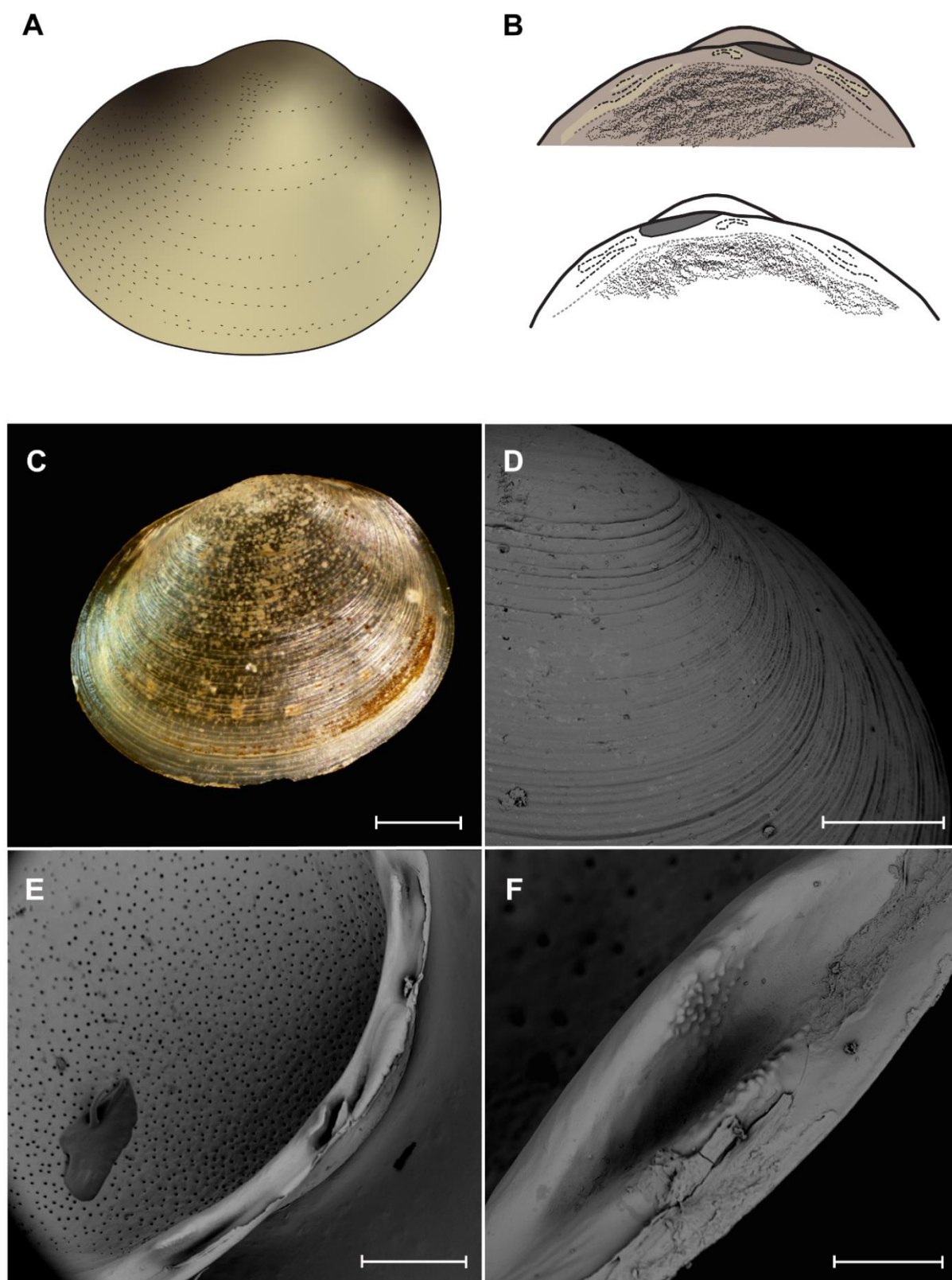


Figure 3.18 *Euglesa viridaria* (Kuiper, 1956) visualised by different means: A and B – redrawn images based on Kuiper's (1964) illustration of (A) external features and (B) internal features. C – light photomicrograph of *E. viridaria* found during this study. D to F – Scanning Electron Microscopy (SEM) images of (D) external features such as the umbo and grooves, (E) cardinal and lateral teeth, as well as pores, and (F) a closeup of a lateral tooth. Scale bars: C – 500 μm ; D & E – 300 μm ; F – 100 μm .

Remarks: *Euglesa viridaria* can be identified based on the canaliculi perforating the shell. Young specimens could resemble *E. ovampica*. The regular sculpture of fine, equally distant concentric striae is the same as in *E. langleyana*. According to Mandahl-Barth (1988), it can be distinguished from *E. langleyana* because of its ovate shape, and a small nodule in front of the upper posterior lateral tooth on the right valve. Temperature, altitude, type of water body and substratum influence their geographical distribution greatly. Their absence in certain areas is due to unfavourable environmental conditions rather than a lack of opportunity to disperse (De Kock & Wolmarans, 2008). De Kock and Wolmarans (2008) suggested that the potential of *E. viridaria* to act as an intermediate host for harmful helminth parasites should be evaluated. There has been debate about whether this species is valid or not based on the discrepancies in the morphological characteristics and that this species might be *Euglesa keniana* (Kuiper, 1964; Mandahl-Barth, 1988). *Euglesa viridaria* is listed as Least Concern on the IUCN Red List and was last assessed in 2016. The population trend is currently unknown (Van Damme, 2016).

Taxonomic results – Eupera:

Phylum Mollusca

Class Bivalvia

Order Sphaeriida

Family Sphaeriidae Deshayes, 1855

Subfamily Euperinae Heard, 1965

Genus *Eupera* Bourguignat, 1854

Notes: *Eupera* only occurs in Africa. The type specimen, *E. moquiniana* is from South America and was first described as *Pisidium*. There are differing opinions concerning where these species can be found. Pilsbry and Bequaert (1927) mention that they can be found in the valves of *Etheria* (oysters), but Mandahl-Barth (1988) strongly disagrees with them, mentioning that they are found free-living at the bottom of vegetation, similar to *Sphaerium*. Two species were known from South Africa: *Eupera parasitica* (Deshayes, 1854) and *Eupera ferruginea* (Krauss, 1848). Recently, *E. parasitica* was synonymised with *Eupera ferruginea* and thus the only *Eupera* species in South Africa is *E. ferruginea*.

3.3.1.8 *Eupera ferruginea* (Krauss, 1848)

Original name: *Cyclas ferruginea* Krauss, 1848.

Type locality: Knysna River, South Africa (Mandahl-Barth, 1988).

Distribution: According to GBIF, this species is recorded from KwaZulu-Natal, Limpopo and Mpumalanga in South Africa, but the georeferencing data only recorded them from the latter two provinces (Figure 3.19). They have also been reported from Botswana, Mozambique, Uganda, and the Democratic Republic of Congo (GBIF) (Figure 3.19). *Eupera ferruginea* was collected in this study from KwaZulu-Natal (Figure 3.19–3.20).

The present site where *Eupera ferruginea* was found, was in the Phongolo River (Table 2.19).

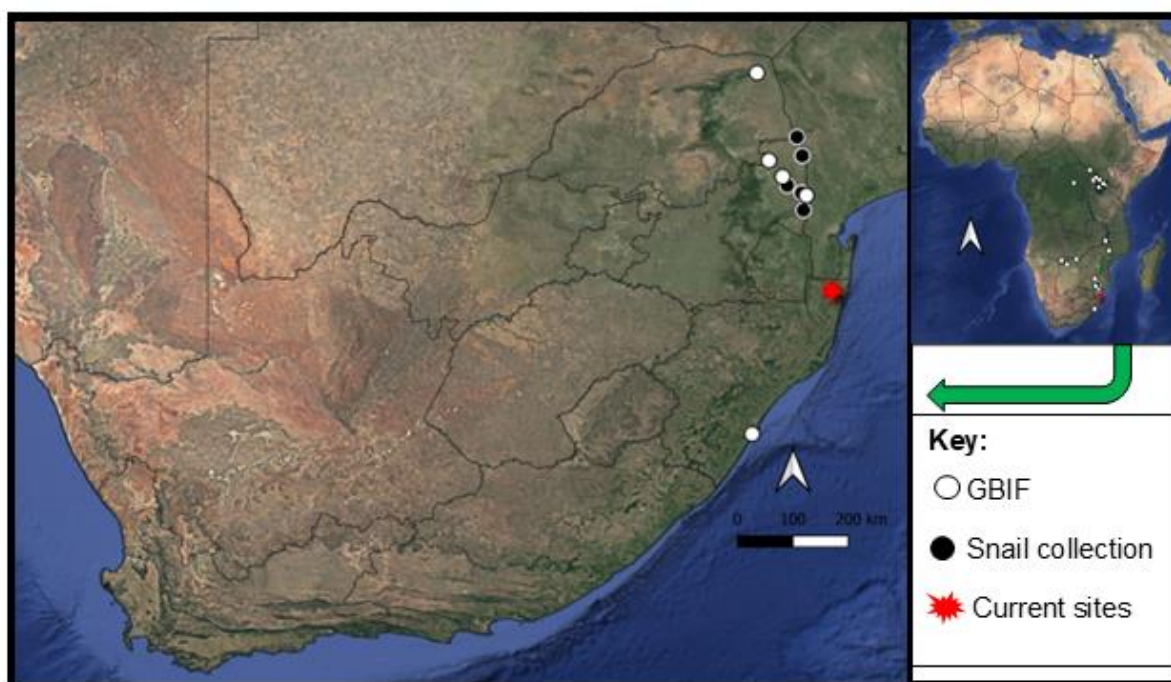


Figure 3.19 South African distribution of *Eupera ferruginea* (Krauss, 1848) based on data points from GBIF and georeferenced data from the NWU Snail Collection.

Museum material: Delaware Museum of Nature and Science. Not examined.

Size: According to Mandahl-Barth (1988), a specimen might have the following measurements: 7.5 mm (L) x 5 mm (W) x 3.2 mm (H). The specimen found in this study had a length of 4.6 mm.

GBIF: 470 occurrences.

Available sequences: 1 on GenBank (COI) (Table 3.3).

Photos: None available online.

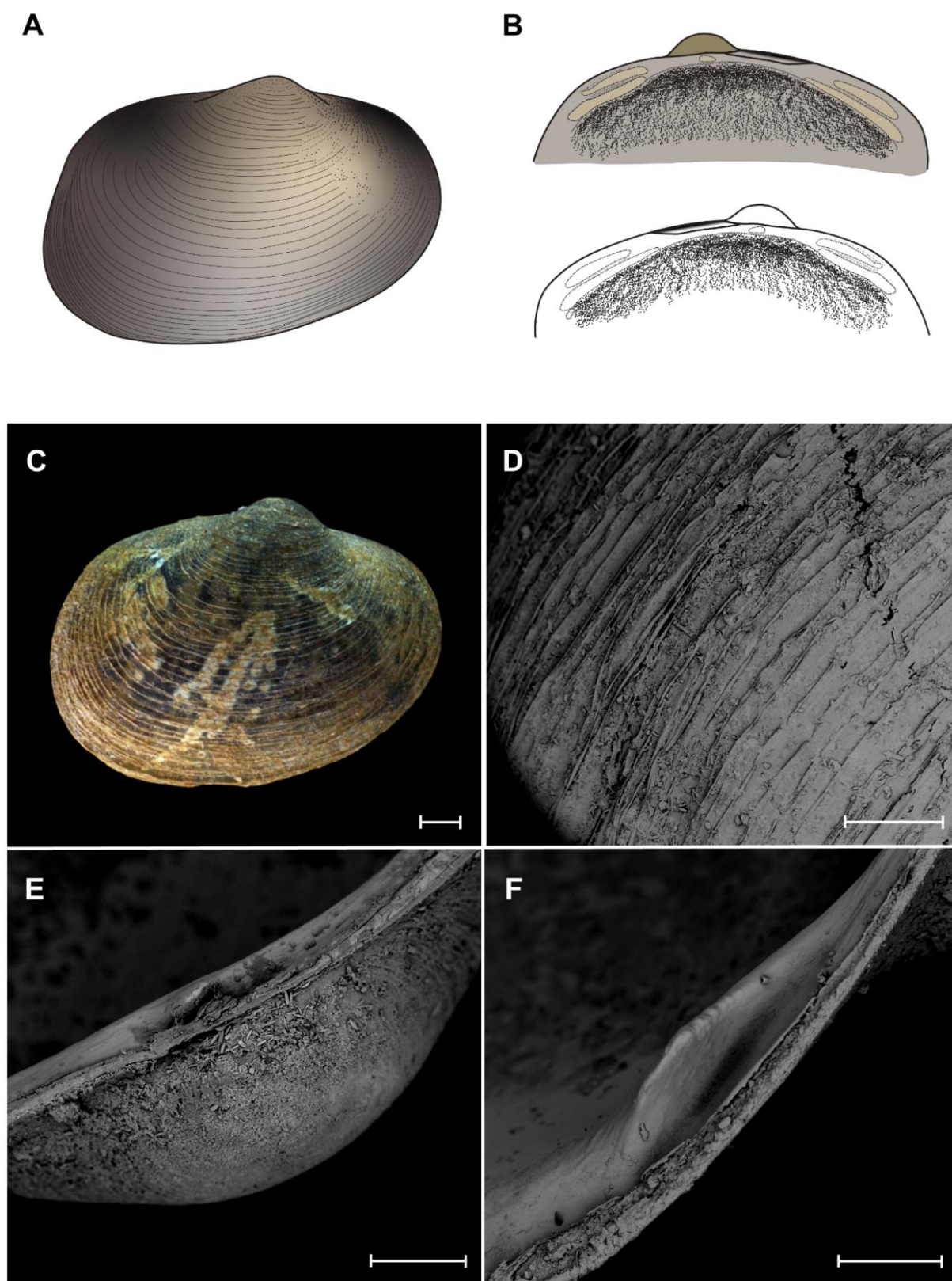


Figure 3.20 *Eupera ferruginea* (Krauss, 1848) visualised by different means: A and B – redrawn images based on Kuiper's (1964) illustration of (A) external features and (B) internal features. C – light photomicrograph of *E. ferruginea* found during this study. D to F – Scanning Electron Microscopy (SEM) images of (D) external features such as grooves, (E) a closeup of a cardinal tooth, and (F) a closeup of a lateral tooth. Scale bars: C – 500 μm ; D – 300 μm ; E & F – 200 μm .

Descriptive notes: This species has angular, rhomboidal valves, and the umbo orientation is anterior to the median vertical line. It has a single, small cardinal tooth on each valve. It is light brown with darker radiating flecks, and the dorsal and ventral margins are almost parallel (Figure 3.20).

Remarks: Like *Sphaerium*, this species occurs near submerged plants, but they attach by means of byssus threads (Appleton, 2002). *Eupera ferruginea* is listed as Least Concern on the IUCN Red List and was last assessed in 2018. The population trend is currently unknown (Seddon and Van Damme, 2018a).

Taxonomic results – *Sphaerium*:

Phylum Mollusca

Class Bivalvia

Order Sphaeriida

Family Sphaeriidae Deshayes, 1855

Subfamily Sphaeriinae Deshayes, 1855 (1820)

Genus *Sphaerium* Scopoli, 1777

Notes: *Sphaerium* has less available information but according to Appleton (2002), both species that occur in South Africa are whitish, small, grow up to 9 mm, and have two cardinal teeth. The two species are usually found together. They can be found on rhizomes of plants. They look similar to *Euglesa*. The African species of this genus are variable and thus challenging to distinguish. Many of the species in this group can be subdivided into geographical races, and this causes further challenges concerning identification up to species level (Mandahl-Barth, 1988).

3.3.1.9 *Sphaerium capense* (Krauss, 1848)

Original name: *Cyclas capensis* Krauss, 1848.

Type locality: Knysna River, South Africa (Mandahl-Barth, 1988).

Distribution: Based on human observation data collected from GBIF, *S. capense* distribution records have been recorded in Mpumalanga and Limpopo in South Africa. Distribution records based on the NWU snail database logged *S. capense* in the above-mentioned provinces as well as Western Cape, Eastern Cape, and the North West provinces. The georeferencing data shows

they were recorded from the Western Cape, Eastern Cape, KwaZulu-Natal, Limpopo, and Mpumalanga (Figure 3.18). Distribution was also recorded in the following KwaZulu-Natal freshwater systems: the western shore of Lake St. Lucia, Wewe River at Tongaat, Lake Futululu, small lakes about 6.5 km southeast of Empangeni (Brown, 1967), Mgobezeleni Lake (Bruton and Appleton, 1975), Lake Sibaya, Lake Zilonde, Sibaya ponds, Gezisa stream and Shazibe stream (Appleton, 1977). Mandahl-Barth (1988) states that they are distributed in southern Africa from Zambia and Zimbabwe to the Cape. They are also found in Madagascar because it is hypothesised that *Sphaerium madagascariense* Tristram 1863 and *S. capense* are the same species. It has also been found in the following countries: Botswana, Eswatini, Mozambique, Namibia, Zambia, and Zimbabwe (Van Damme et al., 2016). However, based on GBIF, they have been reported from Botswana, Zambia, and Uganda (Figure 3.21) and were not found in this study.

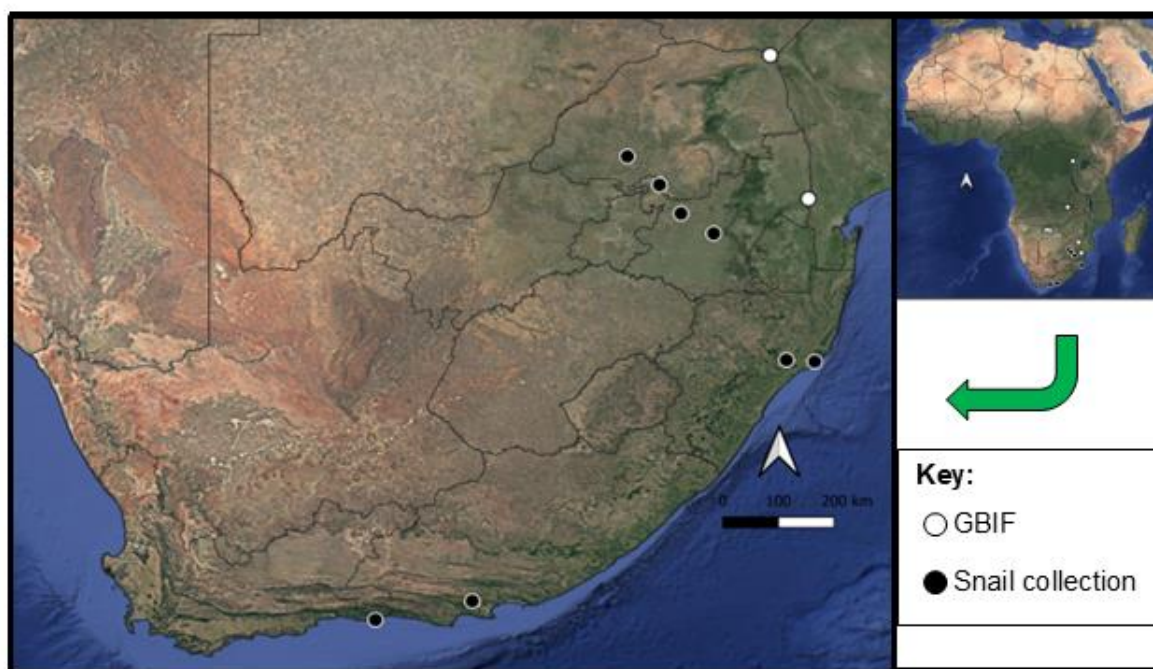


Figure 3.21 South African distribution of *Sphaerium capense* (Krauss, 1848) based on data points from GBIF and georeferenced data from the NWU Snail Collection.

Museum material: The Natural History Museum (Museum für Naturkunde), Berlin. Not examined.

Size: Ranges between 8 mm to 10 mm (Mandahl-Barth, 1988).

GBIF: 18 occurrences.

Available sequences: None.

Photos: None available online.

Descriptive notes: This species is rounded in shape and has lateral and two cardinal teeth. It has inflated valves and rounded anterior margins. The umbones are situated on the median vertical line, placed behind the middle and with a more angular posterior end, and have no sculpture except for growth lines (Figure 3.22).

Remarks: *Sphaerium capense* can be distinguished by its two cardinal teeth. They are viviparous and the embryos develop in broodsacs that are formed by gill filaments and are known to be hermaphroditic (Appleton, 2002). This species is listed as Least Concern on the IUCN and was last assessed in 2016. The population trend is stable (Van Damme et al., 2016).

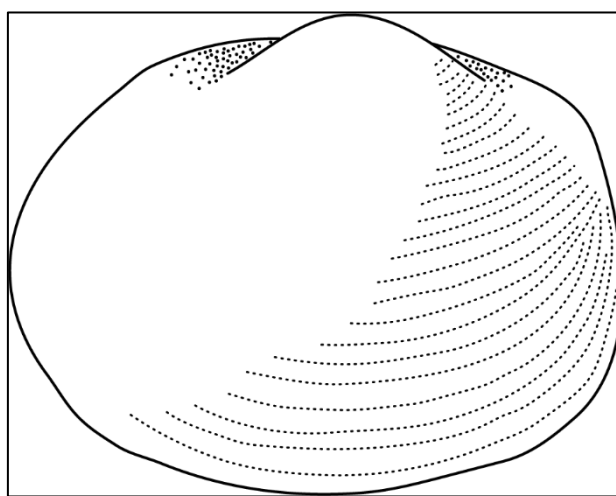


Figure 3.22 A redrawn image of *Sphaerium capense* (Krauss, 1848), based on an illustration by Appleton (2002).

3.3.1.10 *Sphaerium incomitatum* (Kuiper, 1966)

Original name: *Pisidium incomitatum* Kuiper, 1966.

Type locality: Lake Mcllwaine outside Harare, Zimbabwe (Mandahl-Barth, 1988).

Distribution: They have been found in KwaZulu-Natal at Lake Mgobezeleni (Bruton and Appleton, 1975), Lake Zilonde, and Mbazwana stream (Appleton, 1977). According to Appleton (1977), these are the only known occurrences of *S. incomitatum* in South Africa. They have been reported from Botswana, Namibia, Zambia and Zimbabwe (Appleton et al., 2010). Based on GBIF, they have been found in the KwaZulu-Natal province of South Africa, as well as Botswana (Figure 3.23). They were not reported from the NWU Snail Collection and were not found in this study.

Museum material: Unavailable.

Size: Ranges between 10 mm to 11 mm (Mandahl-Barth, 1988).

GBIF: 7 occurrences.

Available sequences: None.

Photos: None available online.

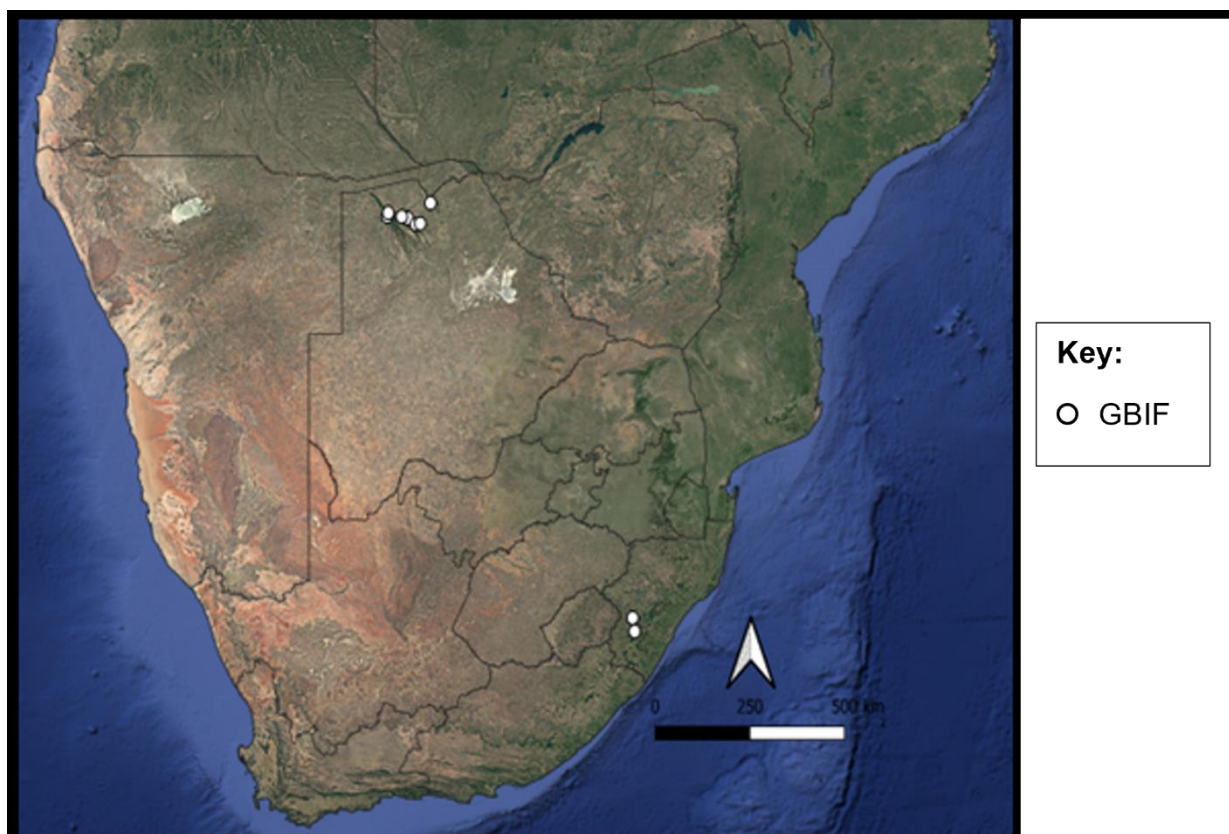


Figure 3.23 South African distribution of *Sphaerium incomitatum* (Kuiper, 1966) based on data points from GBIF. No georeferenced data from the NWU Snail Collection is available.

Descriptive notes: This species has a small, whitish oval-shaped shell, with a lateral and two cardinal teeth. It has inflated valves but less inflated than *S. capense* (Appleton, 2002; Mandahl-Barth, 1988). Umbones are situated slightly more posterior to the median vertical lines and have a pointed anterior margin. They have growth lines, but no sculpture (Figure 3.24).

Remarks: This species is different to the preceding as their shells are less inflated. This species is thought to be part of the *Euglesa* genus according to Mandahl-Barth (1988) as he mentions that Kuiper was only able to study young specimens whose umbones were clearly behind the middle. They are hermaphroditic and viviparous where the embryos develop in broodsacs that

are formed by gill filaments (Day and de Moor, 2002). *Sphaerium incomitatum* is listed as Least Concern on the IUCN and was last assessed in 2006. The population trend is currently unknown (Kristensen et al., 2010b).

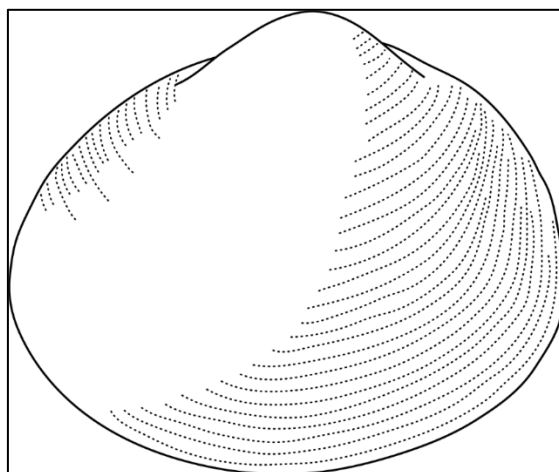


Figure 3.24 A redrawn image of *Sphaerium capense* (Krauss, 1848) based on an illustration by Appleton (2002).

3.3.2 Morphometrics of bivalves found

The bivalves were measured using the ImageJ/Fiji app (Table 3.4). The scale on the Z-stacking photos was used to reset the scale on the app for each image to accurately measure the width and length of each bivalve relative to the scale on the image.

Table 3.4 The average width and length as well as the minimum and maximum width and length of bivalves at each site where they were found (SE = standard error; - = only one bivalve).

Sites	Width (mm) ± SE	Width range (min – max)	Length (mm) ± SE	Length range (min – max)
<i>Euglesa</i> (SE = 0.12)				
Muiskraal	2.1	1.8 – 2.5	1.8	1.5 – 2.2
Groot Letaba	1.6	1.5 – 1.7	1.4	1.2 – 1.5
Luvuvhu Bridge	2.4	-	2.0	-
Luvuvhu River	2.0	1.5 – 2.3	1.7	1.2 – 2
Mac Mac River	2.4	2.6 – 2	2.0	1.7 – 2.2
Steelpoort Up	2.6	-	2.1	-
Sabie River	2.2	-	1.8	-
Wilge River	2.0	-	1.6	-
Wonderfonteinspruit 3	2.5	2.4 – 2.6	2.1	2 – 2.3
Wonderfonteinspruit 10	2.6	2.6 – 2.7	2.2	2.1 – 2.3
Mooi River Up	2.5	2.2 – 2.6	2.1	1.8 – 2.3
X-spruit 1	2	1.7 – 2.3	1.6	1.3 – 2.1
X-spruit 2	2.4	-	2.1	-

X-spruit 3	3.7	3.9 – 3.6	3.3	3.5 – 3.1
X-spruit 4	2.4	2.2 – 2.5	2.1	1.9 – 2.3
<i>Eupera</i>				
Phongolo Bridge	4.6	-	3.6	-

3.3.3 Phylogenetic results

A total of 76 new sequences were generated from this study (34 COI and 42 16S). They belong to two different genera: *Euglesa* (33 COI and 15 16S) and *Eupera* (one COI and one 16S). All relevant sequences from these genera from across the globe were added to the alignment with the novel sequences to construct a phylogenetic tree (Table 3.3).

For partial COI analysis, the alignment comprised 98 sequences, which included 34 novel sequences alongside 64 COI sequences retrieved from GenBank and BOLD (Table 3.3), resulting in a total length of 377 base pairs (bp). The GenBank and BOLD sequences for *Euglesa* were generally longer than the novel sequences, ranging from 500–650 bp. This is because the target region of the novel sequences was shorter due to the use of an internal primer: COI PisInt-F (Table 3.2). The novel sequence length ranged from 310–370 bp. For *Eupera*, general COI primers were used (Table 3.2), resulting in a longer sequence of 680 bp compared to the 600 bp sequence from GenBank. A phylogenetic tree for this gene can be seen in Figure 3.25.

For the partial analysis of the 16S region, 42 new sequences were generated. The alignment had 89 sequences with the novel sequences and 47 GenBank sequences (Table 3.3). The length of the alignment was 380 bp. The general 16S primers were used for targeting this region (Table 3.2), thus the GenBank and novel sequences ranged from approximately the same length: 400 bp to 500 bp. A phylogenetic tree for this gene can be seen in Figure 3.26.

A portion of the 28S gene region was also targeted using general primers (Table 3.2), but most sequences were unsuccessful in forming contigs. Only seven out of the 40 samples were successful in this regard. It is unclear why these sequences could form contigs and not the others. The unsuccessful sequences formed indels, which caused a lot of non-sense sequences. Due to this, the forward region did not have enough overlap with the reverse region to form a combined sequence. Thus, these sequences, and subsequently the 28S sequences were left out of the rest of the analyses and no phylogenetic tree was compiled for this gene.

3.3.3.1 Phylogenetics and species diversity

A tree for each of the two remaining genes, 16S and COI, was compiled during this project. Tables indicating the base pair differences and percentage similarities were also added (Tables 3.5–3.8).

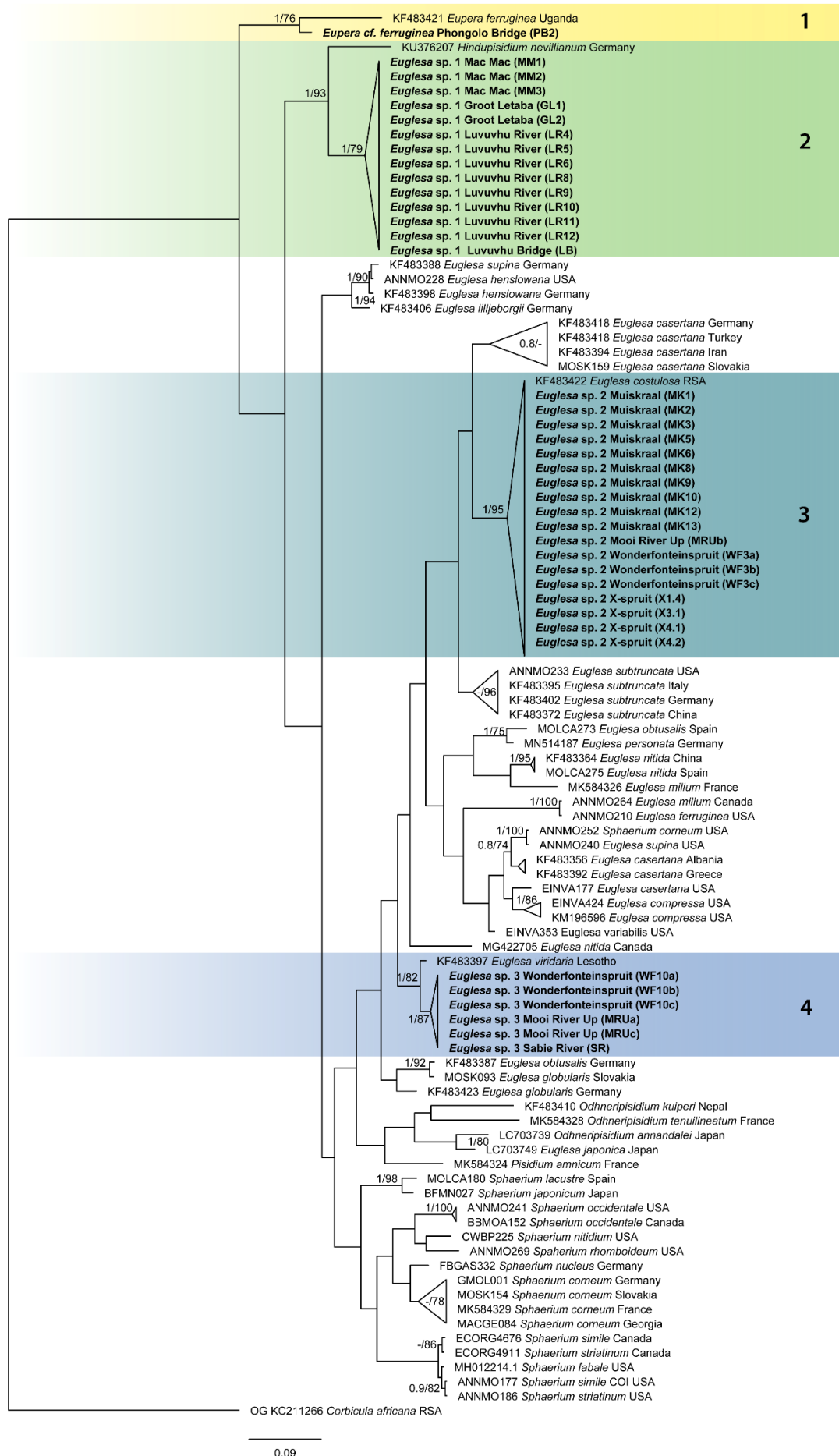


Figure 3.25 A COI phylogenetic tree of sphaeriids containing 34 novel sequences (in bold), sequences from GenBank and Barcode Of Life Database (BOLD), and the Bayesian inference (BI) and maximum likelihood (ML) values.

Based on this tree, three possibly different species are identified. They are labelled as *Euglesa* sp. 1, *Euglesa* sp. 2, and *Euglesa* sp. 3. *Eupera* forms its own clade, as was expected since it is a different genus. The BI and ML values indicated high support for these clades. Bayesian inference values below 0.7, as well as maximum likelihood values below 70 were excluded.

The base pair differences and percentage similarity between each species were calculated using Geneious and compiled into a table to provide a more detailed reasoning as to why sequences fell in certain clades. The table was separated into two, with the first table comparing the novel sequences (Table 3.5), and the second table comparing the GenBank/BOLD sequences compared to representatives of the novel sequences (Table 3.6). Sequences with the same values were removed as they are redundant and unnecessary in this comparison. Bayesian Inference and maximum likelihood values below 0.7 and 70% were excluded from the trees.

Table 3.5 Base pair differences (below the diagonal) and percentage similarity (above the diagonal) values for representatives of the COI gene novel sequences for *Euglesa*. Similar sequences are listed in the footnote. Abbreviations: MK = Muiskraal, MRU = Mooi River Up, WF = Wonderfonteinspruit, MM = Mac Mac, LR = Luvuvhu River, LB = Luvuvhu Bridge, X = X-spruit, GL = Groot Letaba River, SR = Sabie River, PB = Phongolo Bridge.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	MK1*		99	92	100	100	92	88	88	88	88	100	100	100	100	88	88	92	84
2	MK13	2		92	99	99	92	87	88	87	87	99	99	99	99	87	88	92	84
3	MRUa*	30	31		92	92	100	90	90	90	90	92	92	92	92	90	90	100	85
4	MRUb	0	2	30		100	92	88	88	88	88	100	100	100	100	88	88	92	84
5	WF3a*	0	2	30	0		92	88	88	88	88	100	100	100	100	88	88	92	84
6	WF10a*	30	31	0	30	30		90	90	90	90	92	92	92	92	90	90	100	85
7	MM1*	44	46	38	44	44	38		100	99	99	88	87	87	88	100	100	90	87
8	MM3	44	46	37	44	44	37	0		99	99	88	87	88	88	100	100	90	88
9	LR4*	45	47	38	45	45	38	2	2		99	88	87	87	88	99	99	90	87
10	LB	44	46	37	44	44	37	2	2	2		88	87	87	88	99	99	90	87
11	X1.4	0	2	29	0	0	29	44	44	45	44		100	100	100	88	88	92	85
12	X3.1	0	2	27	0	0	27	42	42	43	42	0		100	100	87	87	92	84
13	X4.1	2	3	28	2	2	28	42	42	43	42	2	2		100	87	88	92	85
14	X4.2	0	2	30	0	0	30	44	44	45	44	0	0	2		88	88	92	84
15	GL1	45	47	38	45	45	38	1	1	3	3	45	43	43	45		100	90	88
16	GL2	44	46	37	44	44	37	0	0	2	2	44	42	42	44	1		90	88
17	SR	30	31	0	30	30	0	38	37	38	37	29	27	28	31	38	37		85
18	PB2	58	59	56	58	58	56	47	46	47	48	57	52	50	60	47	46	56	

*MK1 represents MK1 to MK12, MRUa represents MRUa to MRUc, WF3a represents WF3a to WF3c. WF10a represents WF10a to WF10c, MM1 represents MM1 and MM2, LR4 represents LR4 to LR12.

For the second table (Table 3.6), replicate sequences were removed (as done with Table 3.5). Additionally, all *Sphaerium* sequences were removed as they are from a different genus and would not be comparable to *Euglesa* sequences. Only one of each species was included for the GenBank sequences due to spatial constraints.

The 16S gene is a conservative gene, providing valuable information for higher taxon phylogenies. The same results as the COI tree can be observed, where there are possibly three species in the *Euglesa* genus identified from this study: *Euglesa costulosa* found in the North West and Mpumalanga region; *Euglesa viridaria* also found in the North West and Mpumalanga but at different sites; and *Hindupisidium nevilleianum* which could possibly be *Euglesa langleyana* found in the Limpopo and Mpumalanga province. Tables 3.7 and 3.8 provides further support as to why the sequences grouped as they did. Bayesian inference values below 0.7, as well as maximum likelihood values below 70 were excluded.

3.3.4 Epibionts of Sphaeriidae

Diatoms were found on the *Eupera ferruginea* found in the Phongolo River in KwaZulu-Natal during this study. Nine different species of diatoms were found, and they were most abundant on the umbo and observed under the scanning electron microscope (SEM) (Figure 3.27). Some species, such as those belonging to *Amphora*, attached to *Eupera ferruginea* on site as the raphe of these species are on the ventral side (Taylor et al, 2007) and all the diatoms were facing downwards on the SEM images (Figure 3.27).

Some of the species identified included: *Amphora copulata* (Kützinger) Schoeman & Archibald, 1986, *Amphora veneta* Kützinger 1844, *Amphora* Ehrenberg ex Kützinger, 1844sp., *Capartogramma crucicula* (Grunow) Ross 1963, *Gomphonema* Ehrenberg, 1832 sp., *Navicula radiosa* Kützinger, 1844, *Navicula vandamii*, *Nitzschia* sp. and *Sellaphora* Mereschkowsky, 1902 sp.

Table 3.6 Base pair differences (below the diagonal) and percentage similarity (above the diagonal) values for representatives of the COI gene novel sequences for Euglesa and sequences from GenBank and Barcode Of Life Database (BOLD). Similar sequences are listed in the footnote. Abbreviations: MK = Muiskraal, MRU = Mooi River Up, WF = Wonderfonteinspruit, MM = Mac Mac, LR = Luvuvhu River, LB = Luvuvhu Bridge, X = X-spruit, GL = Groot Letaba River, SR = Sabie River, PB = Phongolo Bridge.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1 MK1		92	100	100	92	88	88	88	100	88	92	84	92	90	92	91	93	100	92	91	90	90	92	89	88	89	88	87	87	88	89	84	87	82	72
2 MRUa	30		92	92	100	90	90	90	92	90	100	85	93	91	93	92	92	92	93	93	91	91	98	89	92	91	90	88	86	88	88	84	86	82	72
3 MRUb	0	30		100	92	88	88	88	100	88	92	84	92	90	92	91	93	100	92	91	90	90	92	89	88	89	88	87	87	88	89	84	87	82	72
4 WF3a	0	30	0		92	88	88	88	100	88	92	84	92	90	92	91	93	100	92	91	90	90	92	89	88	89	88	87	87	88	89	84	87	82	72
5 WF10a	30	0	30	30		90	90	90	92	90	100	85	93	91	93	92	92	92	93	93	91	91	98	89	92	91	90	88	86	88	88	84	86	82	72
6 MM1	44	38	44	44	38		99	99	88	100	90	87	87	86	87	85	87	88	88	88	87	86	89	87	87	87	87	90	83	87	88	83	85	83	72
7 LR4	45	38	45	45	38	2		99	88	99	90	87	87	85	86	85	87	88	88	88	87	86	89	87	87	87	87	90	82	86	87	83	85	83	71
8 LB	44	37	44	44	37	2	2		88	99	90	87	87	86	87	86	87	88	88	88	87	86	90	87	87	87	87	90	83	86	87	83	85	83	71
9 X1.4	0	29	0	0	29	44	45	44		88	92	85	93	90	92	91	93	100	92	92	90	90	92	89	89	89	87	88	88	88	89	84	87	82	72
10 GL1	45	38	45	45	38	1	3	3	45		90	88	87	85	87	85	87	88	88	88	87	86	89	87	87	87	87	90	82	86	87	83	85	83	72
11 SR	30	0	30	30	0	38	38	37	29	38		85	93	91	93	92	91	92	92	92	90	91	98	88	92	91	89	88	86	88	88	84	87	82	71
12 PB2	58	56	58	58	56	47	47	48	57	47	56		85	85	85	85	84	83	86	86	85	87	84	85	87	88	82	85	82	84	85	81	85	90	70
13 KF483356 <i>E. casertana</i> Albania	24	22	24	24	22	40	40	39	23	40	23	48		98	98	97	91	92	91	91	91	91	93	90	89	89	91	87	88	84	88	87	86	81	73
14 ANNMO240 <i>E. supina</i> USA	36	34	36	36	34	53	57	52	39	57	35	59	7		97	97	91	91	93	92	90	92	92	90	89	89	90	87	86	84	88	87	87	82	72
15 EINVA353 <i>E. variabilis</i> USA	28	25	28	28	25	47	47	46	27	47	26	54	7	11		97	92	92	93	92	91	92	93	89	90	90	90	87	87	83	87	86	86	81	72
16 EINVA424 <i>E. compressa</i> USA	32	28	32	32	28	51	51	50	31	51	29	54	11	11	12		91	91	92	92	89	92	92	89	90	90	89	87	86	84	87	86	86	81	72
17 ANNMO233 <i>E. subtruncata</i> USA	23	29	23	23	29	44	45	44	23	45	30	57	29	31	27	33		94	92	92	90	91	92	89	90	90	89	86	88	88	88	83	86	82	72
18 KF483422 <i>E. costulosa</i> South Africa	0	25	0	0	25	37	38	37	0	38	26	54	24	28	24	29	20		91	91	91	90	92	89	88	88	88	87	87	88	89	84	86	82	73
19 KF483387 <i>E. obtusalis</i> Germany	26	23	26	26	23	38	38	37	25	38	24	45	28	24	24	25	27	27		99	92	93	93	89	91	91	89	88	86	88	88	86	88	84	72
20 MOSK093 <i>E. globularis</i> Slovakia	30	26	30	30	26	43	43	42	29	43	27	49	28	29	28	30	30	27	2		91	93	93	89	91	91	89	88	86	87	88	86	88	84	72
21 MG422705 <i>E. nitida</i> Canada	35	33	35	35	33	45	45	45	34	45	34	54	28	37	33	39	34	28	26	32		90	92	88	90	91	88	86	86	86	87	83	87	83	71
22 MN514187 <i>E. personata</i> Germany	31	28	31	31	28	43	43	43	30	43	28	43	27	25	25	25	28	33	24	24	31		91	90	92	93	88	86	85	85	87	83	90	83	73
23 KF483397 <i>E. viridaria</i> Lesotho	26	5	26	26	5	33	33	32	25	33	6	51	23	25	23	24	26	27	21	21	25	28		89	91	91	89	88	86	87	89	85	86	83	73
24 MK584326 <i>E. milium</i> France	32	33	32	32	33	38	38	37	31	38	34	44	29	30	31	32	32	33	33	33	35	29	33		87	88	86	83	86	87	89	84	88	83	72
25 ANNMO228 <i>E. henslowana</i> USA	36	25	36	36	25	40	40	39	35	40	25	41	35	35	31	32	31	38	27	27	31	26	29	38		97	89	88	84	86	85	83	88	85	72
26 KF483406 <i>E. lilljeborgii</i> Germany	35	28	35	35	28	40	40	40	34	40	28	40	35	35	33	32	32	37	29	29	29	24	30	34	11		89	88	86	86	86	83	88	84	73
27 ANNMO210 <i>E. ferruginea</i> USA	43	36	43	43	36	45	46	45	43	46	37	62	30	35	34	38	40	38	34	39	43	38	34	40	36	36		86	85	83	85	85	87	81	71
28 KU376207 <i>H. nevillianum</i> Germany	40	39	40	40	39	31	31	31	39	31	40	50	42	42	41	41	47	42	40	40	45	45	38	50	39	40	46		83	83	85	84	84	81	71
29 KF483410 <i>O. kuiperi</i> Nepal	39	44	39	39	44	54	54	53	38	54	45	58	38	43	41	45	39	40	45	45	44	47	45	41	49	45	47	55		86	88	84	86	80	71
30 LC703739 <i>O. annandalei</i> Japan	30	31	30	30	31	34	35	34	30	35	31	42	41	41	43	42	32	32	32	33	35	39	33	33	37	36	44	44	36		94	84	87	83	69
31 LC703749 <i>E. japonica</i> Japan	39	42	39	39	42	44	45	45	39	45	43	55	37	43	46	47	41	36	39	43	46	41	36	32	47	45	52	49	38	15		86	87	84	73
32 MK584328 <i>O. tenuilineatum</i> France	52	50	52	52	50	55	54	54	51	55	51	63	44	43	47	47	54	54	47	45	54	55	51	50	56	55	48	53	52	44	46		86	79	70
33 MK584324 <i>P. amnicum</i> France	30	31	30	30	31	33	34	33	30	33	31	35	32	31	33	33	33	32	28	29	31	24	32	29	29	29	31	39	32	30	31	35		83	68
34 KF483421 <i>Eup. ferruginea</i> Uganda	57	56	57	57	56	53	54	54	57	54	56	31	60	58	62	60	57	58	52	50	56	55	55	50	48	52	61	62	63	44	51	68	40	71	
35 OG KC211266 <i>C. africana</i> South Africa	93	94	93	93	94	92	94	94	93	93	95	100	86	95	96	96	96	85	89	96	99	85	86	84	89	88	97	92	92	80	92	97	76	93	

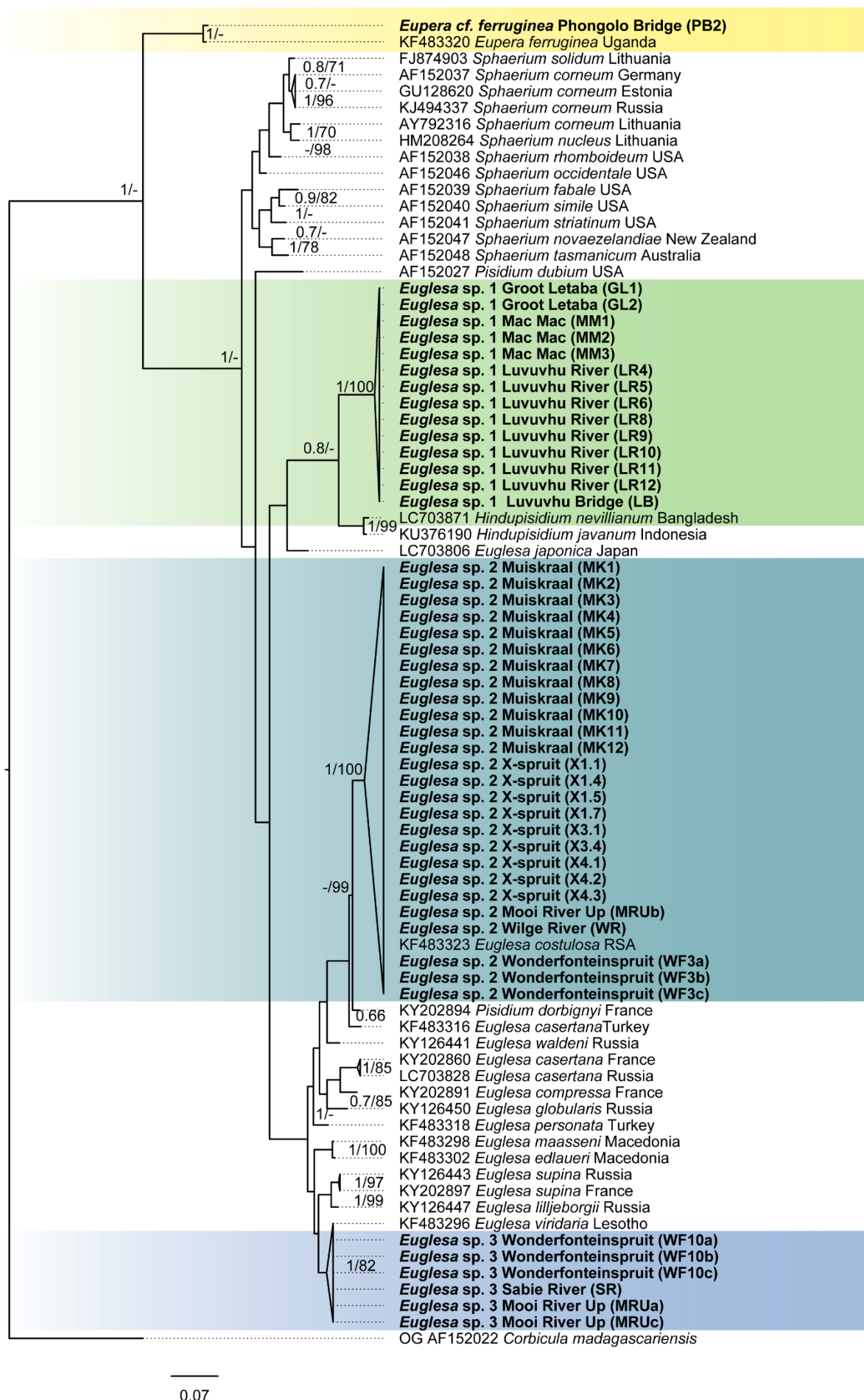


Figure 3.26 A 16S phylogenetic tree of sphaeriids containing 42 novel sequences, sequences from GenBank and Barcode Of Life Database (BOLD), and the Bayesian inference (BI) and maximum likelihood (ML) values.

Table 3.7 Base pair differences (below the diagonal) and percentage similarity (above the diagonal) values for representatives of the 16S gene novel sequences for sphaeriids. Similar sequences are listed in the footnote. Abbreviations: MK = Muiskraal, MRU = Mooi Up, WF = Wonderfonteinspruit, MM = Mac Mac, LR = Luvuvhu River, LB = Luvuvhu Bridge, X = X-spruit, GL = Groot Letaba River, SR = Sabie River, PB = Phongolo Bridge.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	MK1 *		94	100	100	94	100	100	99	100	100	100	100	84	84	94	100	84	84	80
2	MRUa*	30		94	94	100	94	94	94	94	94	94	94	86	86	100	94	86	86	81
3	MRUb	0	30		100	94	100	100	99	100	100	100	100	84	84	94	100	84	84	80
4	WF3a*	0	30	0		94	100	100	99	100	100	100	100	84	84	94	100	84	84	80
5	WF10a	31	1	31	31		94	94	94	94	94	93	94	86	86	100	94	86	86	80
6	X1.1*	4	33	4	4	34		100	99	100	100	99	100	84	84	94	100	84	83	79
7	X1.4	1	31	1	1	32	3		100	100	100	100	100	84	84	94	100	84	83	79
8	X3.1	3	33	3	3	34	5	2		100	100	100	100	83	83	94	99	83	83	79
9	X3.4*	0	30	0	0	31	3	0	0		100	100	100	84	84	94	100	84	84	79
10	X4.1	0	28	0	0	29	3	0	0	0		100	100	84	84	94	100	84	84	79
11	X4.2	1	28	1	1	29	4	1	1	1	1		100	84	84	94	100	84	84	79
12	X4.3	1	31	1	1	32	3	0	2	0	0	1		83	83	94	100	83	83	80
13	GL1*	87	76	87	87	77	90	88	90	87	74	70	86		100	86	84	100	100	77
14	MM1*	87	76	87	87	77	90	88	90	87	74	70	86	0		86	84	100	100	77
15	SR	31	1	31	31	2	34	32	34	31	28	28	31	75	75		94	86	86	81
16	WR	0	30	0	0	31	4	1	3	0	0	1	1	87	87	31		84	84	80
17	LR4*	87	76	87	87	77	90	88	90	87	74	70	86	0	0	75	87		100	77
18	LB	88	77	88	88	78	91	89	91	87	74	70	87	1	1	76	88	1		77
19	PB2	102	98	102	102	99	105	103	105	102	96	94	103	113	113	98	102	113	114	

*MK1 represents MK1 to MK12, MRUa represents MRUa and MRUc, WF3a represents WF3a to WF3c, X1.1 represents X1.1 to X1.7, X1.4 represents X1.4 and X1.5, GL1 represents GL1 and GL2, MM1 represents MM1 to MM3, and LR4 represents LR4 to LR12.

Table 3.8 Base pair differences (below the diagonal) and percentage similarity (above the diagonal) values for representatives of the 16S gene novel sequences for sphaeriids and sequences from GenBank. Similar sequences are listed in the footnote. Abbreviations: MK = Muiskraal, MRU = Mooi River Up, WF = Wonderfonteinspruit, MM = Mac Mac, LR = Luvuvhu River, LB = Luvuvhu Bridge, X = X-spruit, GL = Groot Letaba River, SR = Sabie River, PB = Phongolo Bridge.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1 MK1		94	100	100	94	100	84	84	94	100	84	84	80	88	94	94	94	94	93	94	93	93	93	94	100	98	91	83	84	80	67
2 MRUa	30		94	94	100	94	86	86	100	94	86	86	81	90	100	96	96	95	94	94	96	96	95	95	94	94	92	85	85	80	68
3 MRUb	0	30		100	94	100	84	84	94	100	84	84	80	88	94	94	94	94	93	94	93	93	93	94	100	98	91	83	84	80	67
4 WF3a	0	30	0		94	100	84	84	94	100	84	84	80	88	94	94	94	94	93	94	93	93	93	94	100	98	91	83	84	80	67
5 WF10a	31	1	31	31		94	86	86	100	94	86	86	80	90	99	96	96	95	94	94	96	96	94	95	94	94	92	85	85	80	68
6 X1.1	4	33	4	4	34		84	84	94	100	84	83	79	88	94	94	93	94	93	93	93	93	93	94	100	98	91	83	83	80	66
7 GL1	87	76	87	87	77	90		100	86	84	100	100	77	86	85	86	84	84	87	86	84	85	84	85	83	85	88	91	92	78	68
8 MM1	87	76	87	87	77	90	0		86	84	100	100	77	86	85	86	84	84	87	86	84	85	84	85	83	85	88	91	92	78	68
9 SR	31	1	31	31	2	34	75	75		94	86	86	81	90	99	96	96	95	94	94	96	96	94	95	94	93	92	85	85	80	68
10 WR	0	30	0	0	31	4	87	87	31		84	84	80	88	94	94	94	94	93	94	93	93	93	94	100	98	91	83	84	80	67
11 LR4	87	76	87	87	77	90	0	0	75	87		100	77	86	85	86	84	84	87	86	84	85	84	85	83	85	88	91	92	78	68
12 LB	88	77	88	88	78	91	1	1	76	88	1		77	86	85	86	84	84	87	86	84	85	84	85	83	85	88	91	92	78	68
13 PB2	102	98	102	102	99	105	113	113	98	102	113	114		79	80	80	79	79	80	80	79	80	79	80	79	80	80	76	77	99	69
14 AF152027 <i>P. dubium</i> USA	55	47	55	55	47	57	68	68	47	55	68	68	98		90	88	88	90	87	89	89	88	88	90	88	90	91	86	86	80	66
15 KF483296 <i>E. viridaria</i> Lesotho	30	2	30	30	3	32	74	74	3	30	74	74	98	47		96	96	95	94	94	96	96	95	95	94	94	92	85	85	80	69
16 KF483298 <i>E. maasseni</i> Macedonia	29	18	29	29	19	32	66	66	18	29	66	66	93	57	18		100	96	95	94	96	95	94	95	94	93	91	86	86	80	68
17 KF483302 <i>E. edlaueri</i> Macedonia	32	21	32	32	22	35	79	79	22	32	79	79	101	58	21	1		95	95	94	96	95	94	95	94	93	91	84	85	80	68
18 KY126441 <i>E. waldeni</i> Russia	30	25	30	30	26	33	81	81	26	30	81	81	104	47	25	21	25		94	95	94	94	95	95	94	95	92	85	85	79	67
19 KY202860 <i>E. casertana</i> France	34	29	34	34	30	36	64	64	30	34	64	64	93	59	29	25	25	27		95	94	95	94	94	93	93	90	86	86	80	67
20 KY202891 <i>E. compressa</i> France	31	28	31	31	27	33	68	68	29	31	68	68	92	51	28	26	27	24	22		94	94	94	94	94	94	91	86	86	80	67
21 KY126443 <i>E. supina</i> Russia	35	19	35	35	20	38	79	79	20	35	79	79	101	53	19	18	20	32	29	29		98	94	94	93	93	92	84	85	80	67
22 KY126447 <i>E. lilljeborgii</i> Russia	36	19	36	36	20	39	77	77	20	36	77	77	98	55	19	24	25	32	25	31	10		94	95	93	93	92	84	84	80	67
23 KY126450 <i>E. globularis</i> Russia	34	27	34	34	28	37	81	81	28	34	81	81	104	56	27	26	29	24	29	27	31	32		94	93	93	92	84	84	79	67
24 KF483318 <i>E. personata</i> Turkey	31	24	31	31	25	34	76	76	25	31	76	76	97	49	24	25	27	27	31	28	28	27	28		94	94	91	84	85	80	67
25 KF483323 <i>E. costulosa</i> RSA	0	30	0	0	31	3	85	85	31	0	85	85	102	55	30	29	32	30	34	31	35	36	34	31		98	91	83	84	80	67
26 KY202894 <i>P. dorbignyi</i> France	10	30	10	10	29	12	71	71	31	10	71	71	87	46	29	30	31	23	34	28	31	32	31	26	9		91	86	86	81	66
27 LC703806 <i>E. japonica</i> Japan	34	30	34	34	30	36	45	45	30	34	45	45	76	35	31	33	33	30	37	35	31	30	29	33	34	33		89	90	80	69
28 LC703871 <i>H. nevilleianum</i> Bangladesh	87	79	87	87	78	89	45	45	78	87	45	45	116	69	76	65	80	78	68	67	80	81	82	79	84	64	40		99	77	67
29 KU376190 <i>H. javanum</i> Indonesia	83	75	83	83	74	85	40	40	74	83	40	40	112	69	75	65	77	76	69	68	77	78	80	76	83	65	38	5		78	67
30 KF483320 <i>Eup. ferruginea</i> Uganda	103	100	103	103	101	105	110	110	101	103	110	110	4	97	102	94	103	106	95	94	103	100	106	99	103	88	74	115	113		70
31 OG AF152022 <i>C. madagascariensis</i>	159	150	159	159	151	161	150	150	150	159	150	150	141	160	149	153	153	155	155	154	155	155	156	155	159	150	116	157	156	143	

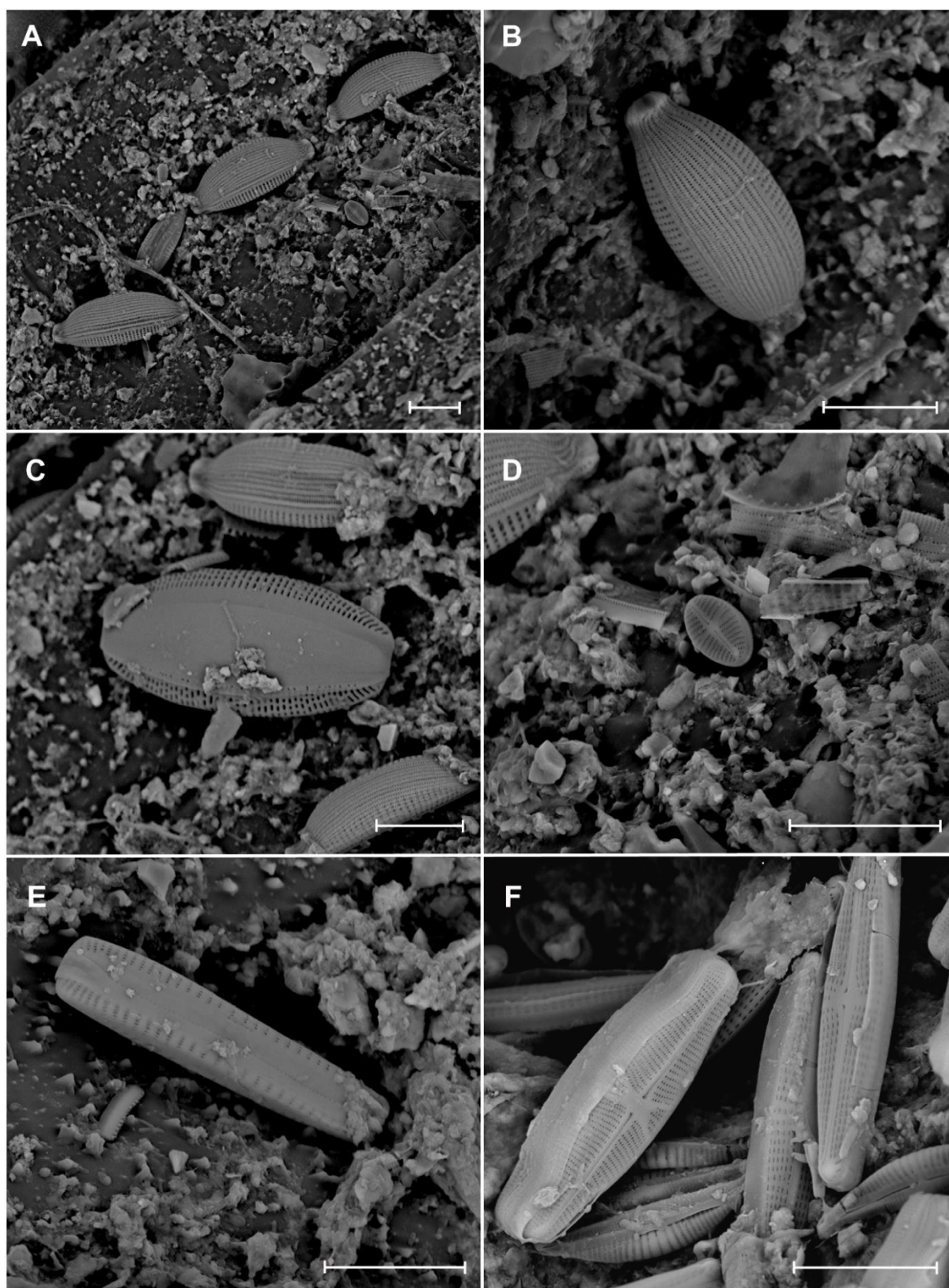


Figure 3.27 Different species of diatoms found on *Eupera ferruginea* from the Phongolo River: A & B – *Amphora veneta*, Kützing 1844. C – *Amphora copulate* (Kützing) Schoeman & R.E.M. Archibald, 1986. D – *Sellaphora* C. Mereschkowsky, 1902 sp. E - *Gomphonema* C.G. Ehrenberg, 1832 sp. (girdle view). F - *Capartogramma crucicula* (Grunow) R.Ross 1963. Scale bar A – F = 10 µm.

3.3.5 Distribution of bivalves found based on ecological parameters

In Chapter 2, the distribution of all the sample sites was portrayed on maps containing the following data: Level 1 ecoregions, mean annual rainfall, and elevation. Figures 3.28–3.30 portray where sphaeriids were found based on these parameters.

Elevation was considered the first ecological parameter (Figure 3.28). *Eupera ferruginea* was the only species that was found at low altitude, with *Euglesa costulosa* and *Euglesa viridaria* being found at higher altitude sites.

Level 1 ecoregions were also considered as another ecological parameter (Figure 3.29). *Eupera ferruginea* is the only one that was found in the North-Eastern Highlands. *Euglesa viridaria* occurred in two different ecoregions: North escarpment mountains, and the Highveld. *Euglesa costulosa* was only found in the Highveld.

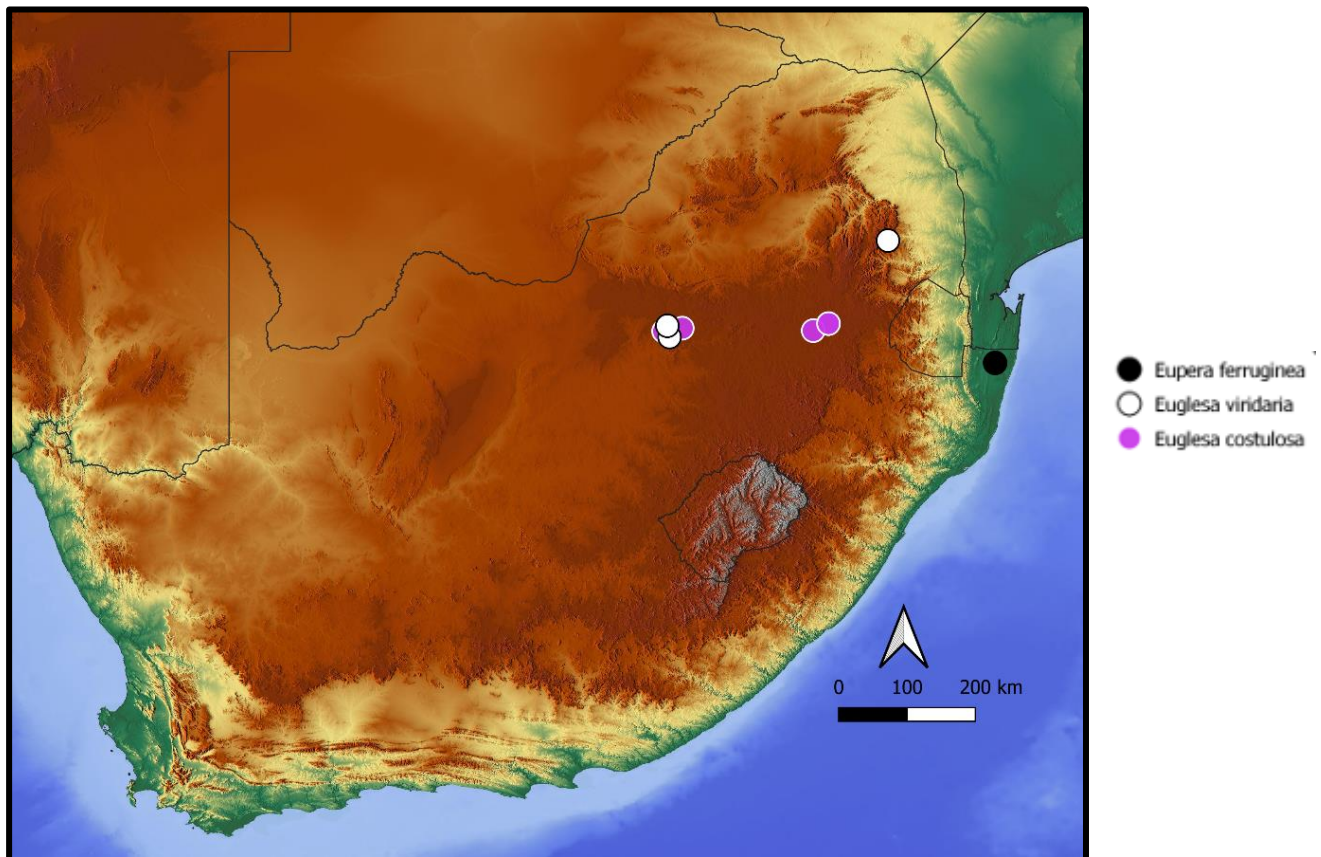


Figure 3.28 South African map indicating at which altitudes the sphaeriids found during this study, was found.

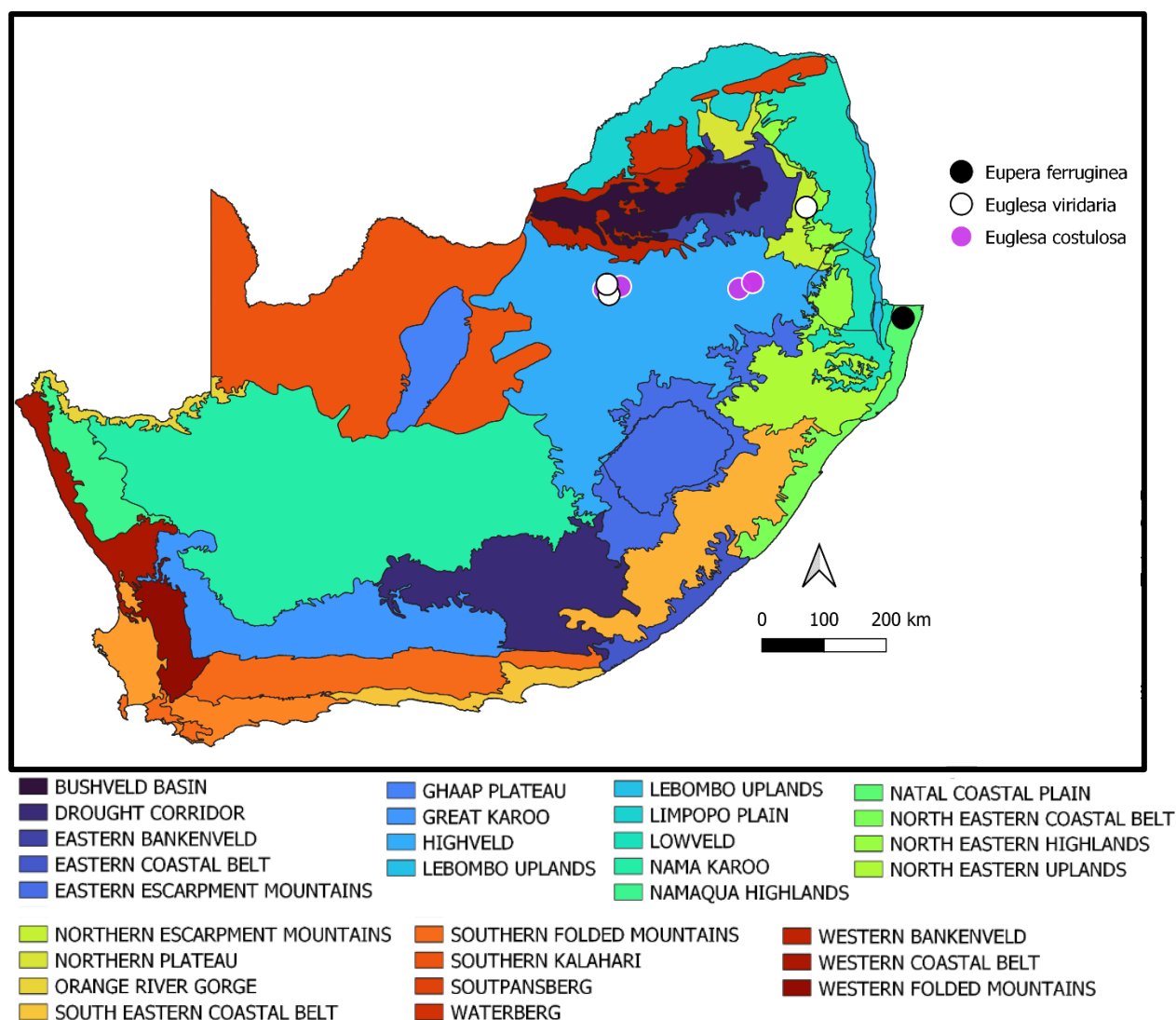


Figure 3.29 South African map indicating in what level 1 ecoregions sphaeriids found in this project were found.

The last ecological parameter was rainfall (Figure 3.30). All three species, *Eupera ferruginea*, *Eupera viridaria*, and *Euglesa costulosa* were found in the 601 – 800 mm region, with one site where *Euglesa viridaria* was found in the > 1000 mm region.

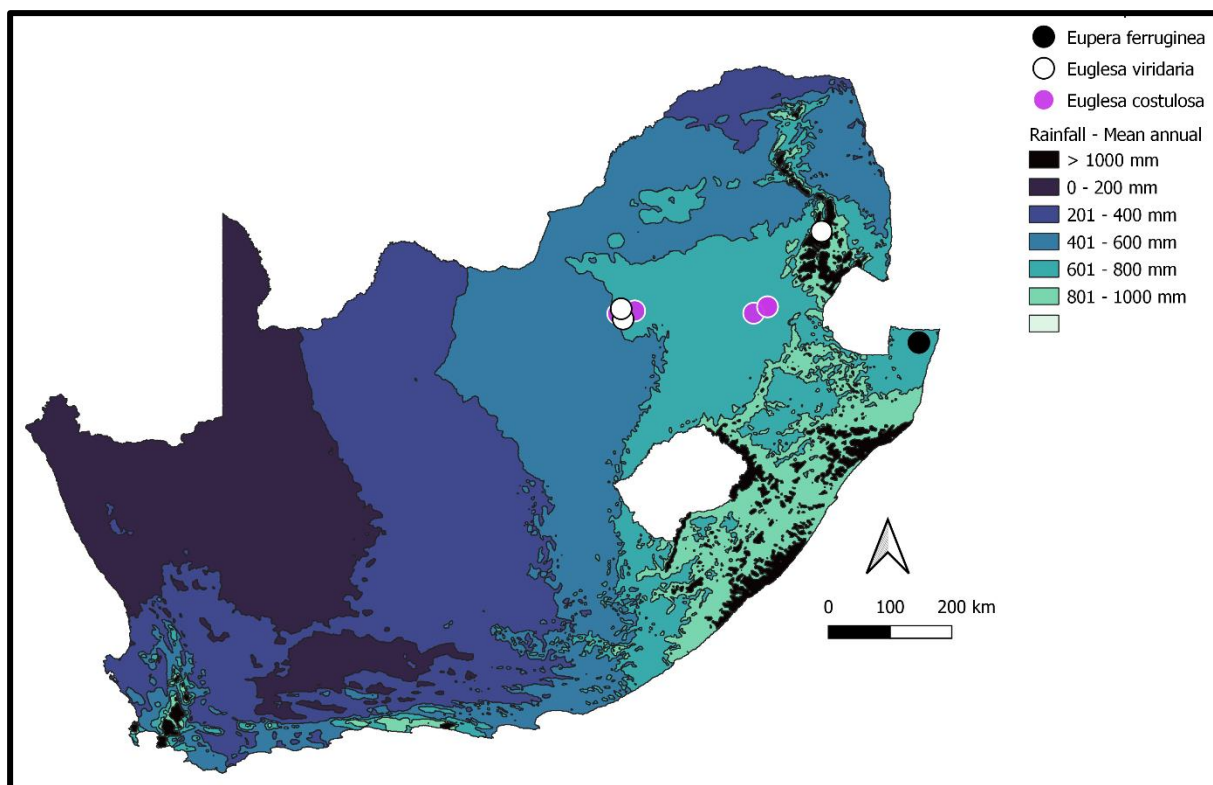


Figure 3.29 South African map of the mean annual rainfall indicating where the sphaeriids found in this project were found.

3.4 Discussion

Based on existing literature, approximately 1,335 sphaeriid bivalve species have been identified worldwide since 1795, up to date. Currently there are approximately 200 species present worldwide, where 11 are described from South Africa, but three that are thought to be most abundant: *Euglesa costulosa*, *Euglesa langleyana*, and *Euglesa viridaria* (De Kock and Wolmarans, 2008). In the context of this study, a dataset of 34 COI sequences, and 42 16S rRNA sequences of four different biological species have been analysed. Three of these species: *Euglesa* sp. 2, *Euglesa* sp. 3, and *Eupera* cf. *ferruginea* had analogues in GenBank, and were thus confirmed molecularly and morphologically to be *Euglesa viridaria*, *Euglesa costulosa*, and *Eupera ferruginea* respectively. *Euglesa* sp. 1 however, didn't have analogues in GenBank.

Sequences for both the 16S and COI phylogenetic trees were selected from GenBank and BOLD based on certain criteria to best portray accurate results. The first criterion was that sequences had to be longer than 500 bp, to ensure that the novel sequences had sufficient base pairs to align with as the primers used for the novel sequences were internal primers (Table 3.2) and targeted a shorter piece of the COI gene than those found on GenBank and BOLD. The bivalves

included in this study were unsuccessful in producing sequences with the universal primers, thus the internal primers were used. Sequences that have not been published in a journal were excluded as they are less reliable as the information cannot be verified with an image, measurements, or molecular analysis of the species being sequenced. A representative collection of sequences from all over the world was added for the relevant genera, i.e. not every single sequence of the genera in Sphaeriidae was added but at least one sequence of each species from each country according to GenBank and BOLD. Sequences were extracted from these databases up to 31 October 2024, thus any sequences added to the database after this date were not included in this study. Sequences were also excluded when they did not have a species identity assigned to it, for example, *Pisidium* sp. or *Euglesa* sp. These sequences, while they might be published, are not an accurate representation of what species in this family are present in South Africa. Sequences that had the abbreviation “cf.” were also excluded as this abbreviation implies that the author was not sure whether it was the correct species name or not. Again, while some of these were published sequences, there were enough sequences with a solid identification to exclude these ones that were less accurate. Bernal et al. (2024) published many sequences recently, but they were also excluded from this study because they were 16S and 28S concatenated sequences. Thus, the novel 16S sequences could not align with them to form a workable alignment. Concerning the alignment of both 16S and COI genes, sequences with big gaps were eliminated as they didn't align enough with the other sequences in the alignment and would be the cause of low maximum likelihood and Bayesian inference supports in the phylogenetic tree.

The phylogenetic trees in Figure 3.25 (COI) and Figure 3.26 (16S) divide into two main clades because *Eupera* and *Euglesa* are two separate genera under the same family. Clade 1 contains the only newly generated sequence of *Eupera ferruginea* and a sequence from GenBank. Based on the high support, it is confirmed that this sample was correctly identified. There are 31 base pair differences between *Eupera cf. ferruginea* (PB2) and the *Eupera* sequence from GenBank based on the COI gene (Table 3.6). However, according to the 16S gene there are only 4 base pair differences (Table 3.8). This suggests that the novel sequence was correctly identified up to genus level, but it may be a different species. However, only this one species occurs in South Africa, thus the amount of base pair differences could also be attributed to the fact that the GenBank sequence was found in another country (Uganda). This bivalve was collected from the northern parts of KwaZulu-Natal, however, according to GBIF and the NWU Snail Collection they were reported from the southern parts of KwaZulu-Natal, Mpumalanga and Limpopo (Figure 3.19). There have only been ten accumulated reports of this species, signifying its limited distribution in South Africa, and can serve as an explanation as to why only one *Eupera ferruginea*

was found during this study. According to Mandahl-Barth (1988), the type locality of this species is in the Knysna River in the Western Cape, but this has not been reported by the GBIF or the National Freshwater Snail Collection. The possibility exists that there are other distribution records that are not on the map in Figure 3.19. The size of the *Eupera ferruginea* found here is much smaller compared to what has been reported in literature by Mandahl-Barth (1988), but because only one was found it is not clear whether this is a trend or if this was just a young specimen.

Clade 2 matched with *Hindupisidium nevilleianum* Vinarski & Bernal, (2023). Recently, the taxonomy has changed extensively within the family Sphaeriidae. The current species in *Euglesa* were previously known as *Pisidium*, and *Hindupisidium* was also previously known as *Pisidium*. This change took place at the end of 2023 according to Molluscabase. There has also been mention of *Hindupisidium* and *Euglesa* being closely related in the literature (Bernal et al., 2024). With this said, it is important to note that not all the South African sphaeriid species are on GenBank and BOLD, so the chances are good that this clade might correspond to *Hindupisidium* because it had the least base pair differences in comparison to the others. Concerning morphology, all of the representative bivalves in this clade have fine grooves, GL2 and LR11 have bald spots, and MM3 has an umbo which is a bit more prominent than the other two. Only MM3 and LR11 have pores visible but all of them have growth rings. The sizes of these bivalves are quite variable, with the width ranging from 1.5 mm to 2.6 and the length ranging from 1.5 mm to 2.2 mm. All seven species in South Africa were considered when identifying the bivalves in this clade. It cannot be *Euglesa viridaria* or *Euglesa costulosa* because they formed other clades in the tree. It also cannot be *Euglesa casertana* as there are multiple sequences from GenBank and BOLD in the tree and they did not fall in this clade. When considering the morphological key characteristics of this clade *Euglesa harrisoni* can be eliminated as the umbo orientation and sculpture do not match with the bivalves found. That leaves *Euglesa langleyana*, *Euglesa ovampica* and *Euglesa pirothi*. *Euglesa pirothi* makes more sense because its type locality is the Groot Letaba River and some samples from this clade were found there. Bernal et al. (2024) further support this as they placed the species known as *Euglesa pirothi*, previously *Afropisidium pirothi*, in the genus *Hindupisidium*, and *H. nevilleianum* was also previously known as *Afropisidium nevilleianum*. The genus *Hindupisidium* was created to include these species that previously belonged to *Afropisidium*. The morphological characteristics of *E. pirothi* provided in literature are very inconspicuous, and the only steadfast thing to distinguish them from the rest of the species is their ligament but it is only visible when they are alive. However, a sequence of *Euglesa pirothi* was added to the phylogenetic trees and it did not fall into this clade. Thus, *Euglesa pirothi* can be ruled out for Clade 1. That leaves *Euglesa langleyana* and *Euglesa ovampica* as options. Between them, *Euglesa langleyana* makes more sense as the recorded distribution (Figure 3.11)

is in the same areas as where the bivalves in this clade were found, and it has been previously reported that *Euglesa langleyana* is abundant in South Africa (De Kock and Wolmarans, 2008). The new sequences have 0 bp differences between each other, but 29 bp differences compared to *H. nevillianum*. In the 16S tree the small clade that matched with clade 2, contains two sequences from GenBank that are both in the genus *Hindupisidium*. There are only 5 base pair differences between these two sequences, suggesting that they may be the same species, but unfortunately, there is not COI sequences available for these species, so this assumption cannot be validated. But it is further supported that this clade 2 might fall under the genus *Hindupisidium*. *Euglesa japonica* from Japan also seems to be closer related to this clade than the others. It had the least amount of base pair differences compared to some of the *Sphaerium* species. Again, this could be a misidentification, but nothing can be said for certain without a COI sequence.

The samples in clade 3 are *Euglesa costulosa* based on the strong maximum likelihood and Bayesian inference support. It is interesting that the X-spruit (Mpumalanga) samples from Mpumalanga formed in the same clade as the Muiskraal, Wonderfonteinspruit, and Mooi River samples which are all in the North West. A common characteristic for this group is that their umbo is more prominent and skewed a bit, and their grooves are inconsistent throughout, ranging from fine grooves to more prominent grooves, and concentric to inconspicuous grooves. However, MK2 and X4.2 have more prominent grooves than WF3a. This can be explained by interspecies variation. MK2 and WF3a have characteristics that match with Appleton (2002) and Kuiper (1964) *E. costulosa* descriptions, which these samples clade with. They also share characteristics of the redrawn *E. costulosa* (Figure 3.8). Their width ranges from 1.9 mm to 3.6 mm and their length ranges from 1.6 mm to 3.1 mm. The 3.6 mm in width was the outlier though, as the second largest width was 2.6 mm. Only the outlier (X3.1) correlates with the sizes mentioned by Kuiper (1964), Appleton (2002), and Mandahl-Barth (1988). The base pair differences table of *Euglesa* sp. 3 further supports the fact these new sequences are in fact *E. costulosa* as there are 0 bp differences between each new sequence and the *E. costulosa* from GenBank. The bivalves found in this clade were found in five different sites in North West and Mpumalanga, which correlates to the distribution as recorded by the NWU Snail Collection and GBIF. This indicated that the distribution of these bivalves has not changed.

According to Clewing et al. (2022), *Euglesa casertana* formed a sister clade with *Euglesa costulosa*. The same can be seen for this study's *Euglesa costulosa* in Clade 3 in Figure 3.1 and Figure 3.2, where the *Euglesa casertana* sequences from GenBank formed a sister clade to Clade 3. This is further support of correct identification for Clade 3. *Euglesa casertana* has different forms on different continents, making it more difficult to identify because of all the variability as illustrated by Clewing et al. (2022). It was demonstrated that *Euglesa casertana* in its European

form varies from that on other continents, and a specimen found in that study that resembled *Euglesa casertana* originated from southern Africa, however, in the phylogeny it was shown to be very distant from the European form. This species has been proven to be paraphyletic by a few authors (Lee and O Foighil, 2003; Clewing et al. 2013, 2020; Clewing et al, 2022;). Clewing et al. (2022) further demonstrated that *Euglesa casertana* might represent another species, *Euglesa ethiopica* (Korniushin, 1994). It was thought that *Euglesa ethiopica* was critically endangered, but after discovering that some *Euglesa casertana* might be *Euglesa ethiopica*, it might not be as endangered as originally thought. The wide distribution of this species (Rassam, 2023) makes it possible that there have been misidentifications similar to the example mentioned here, but more research will need to be done to establish this.

The Bayesian inference and maximum likelihood values of Clade 4 indicated high support that *Euglesa* sp. 4 is *Euglesa viridaria* which can be seen at the base of the clade. What is interesting about this clade, is that the sample from the Sabie River formed in the same clade as the samples found from the Mooi River and Wonderfonteinspruit which are both close to one another. This is interesting because usually samples from the same area tend to clade together as can be seen with the other two clades and from the sequences from GenBank and BOLD. However, this does bring into question whether SR is in fact *E. viridaria*, there are 0 bp differences between this novel sequence and the *E. viridaria* from GenBank. The morphology of this clade is represented by Z-stacking photos and SEM photos of three representatives. The grooves are fine and concentric, and the umbo is wide and situated more towards the middle for all. This fits the description of the Appleton (2002) key, for *E. viridaria* which is what this group clades with. Kuiper (1964) and Piechocki (1993) mention a nodule that is present at the base of the lateral tooth of *E. viridaria*, which is supposedly a key feature for this species, but this is not clear in the SEM photos. The Sabie River (SR) specimen seems to have more prominent grooves, but this could be explained by interspecies variation or the fact that it was found in another locality, the Sabie River in Mpumalanga. These characteristics also correlate with the redrawn bivalve of *E. viridaria* (Figure 3.18). There are 0 bp differences between all the new sequences in this clade, and 5 bp differences between each of the new sequences and the *E. viridaria* from GenBank. This is further evidence that all the bivalves in Clade 4 are *E. viridaria*. The bivalves in this clade are all of a similar size (Table 3.4) with a width ranging from 2.6 mm to 2.7 mm and length ranging from 2.1 mm to 2.3 mm. The SR specimen did not fall in this range, and was significantly smaller with a width of 2.2 mm and a length of 1.8 mm. These measurements correlate more with Piechocki (1993) than with Kuiper (1964) and Mandahl-Barth (1988) that stated that *E. viridaria* was much bigger. Considering their measurements and the ones found in this study, it seems that the size of *E. viridaria* has decreased over the years. This could indicate a shorter lifespan, or these

bivalves may have evolved to better survive in their habitats. The sites where the bivalves from this clade include North West, Gauteng, and Mpumalanga and it correlates to those reported from the NWU Snail Collection (Figure 3.17). Thus, it does not seem that this species distribution has changed for these parts of the country. The sequence this clade matched with is from Lesotho (Clewing et al., 2013) (see Table 3.3).

According to Clewing et al. (2022), *Euglesa viridaria* has been found in Ethiopia and Rwanda, and according to GBIF, it has also been reported from Ethiopia (Figure 3.17). The species found from Ethiopia and Rwanda formed a strong but unresolved clade with *Euglesa pirothi* found in Ethiopia and Malawi. However, the sequence of the *Euglesa viridaria* found in Lesotho, which is also included in this study (Table 3.3), formed a weakly supported, polytomic group with *Euglesa* species found in Rwanda, Ethiopia, and Kenya. *Euglesa pirothi* has also been found in Malawi and Ethiopia (Clewing et al., 2022). Although there are no sequences for this species, it is the type species for the genus *Afropisidium*. According to Clewing et al. (2022), *Euglesa ovampica* cannot be identified based on geography, as specimens from different areas look the same, which is not the case for other *Euglesa* species.

Euglesa and *Eupera* sequences, while different genera, were included in the same tree as there is not sufficient information for *Eupera* to make one tree for this genus alone. The highlighted sections are the clades containing the novel sequences (bolded) as well as what they matched with. All other sequences in the clades are from the family Sphaeriidae from across the globe. Out of the two trees, the COI tree portrays a more accurate illustration of identification up to species level, whereas 16S provides a better idea of what genera the novel sequences would be in, thus it can be said with some certainty that the novel sequences in Clade 2, for example, belong to the genus *Hindupisidium*. It is also noteworthy that the following genera were once all collectively in the *Pisidium* genus and thus included in the trees: *Euglesa*, *Odhneripisidium*, *Afropisidium*, *Hindupisidium*. *Sphaerium* species were also added to the tree following the same criteria as for the other species, i.e., one species from each country, published, exact known species. By adding this genus, it provides a more complete picture of the family and how all the genera are related to one another. For example, clade 2 is separate from the rest, further supporting the fact that it may be a different genus. Whereas the other clade containing clades 3 and 4 also contain the *Sphaerium* clade, suggesting that they are closer related to Clade 3 and 4, i.e., *Euglesa viridaria* and *Euglesa costulosa* than it is to Clade 2. It is also interesting to see that *Eupera* is in a whole other clade than the rest, which is supported by their evident differences in morphology as well as the fact that different primers were used to sequence *Eupera cf. ferruginea* (PB2). It can also be deduced that *Euglesa* and *Eupera* are further related than *Euglesa* and *Sphaerium*.

Concerning the *Sphaerium* clade in the 16S tree, it divides into two subclades. The sequences from the first clade, when observed from top to bottom, mainly originated from Europe, however, there are two from the USA that fell in the same clade but are further related. Whereas the second clade splits into two again, separating the USA sequences from the island sequences from New Zealand and Australia which makes sense as there are only 16 base pair differences between the latter two sequences. Concerning the *Sphaerium* clade in the COI tree, it is very similar to that represented by the 16S tree, except for the fact that the *Sphaerium corneum* from the USA fell in a clade with the *Euglesa* species. This is highly likely due to misidentification because this species is very widespread.

There are clear differences between the *Sphaerium* distribution as reported by GBIF compared to that recorded by the NWU Snail Collection (Figure 3.21). There is some overlap with KwaZulu-Natal, Limpopo, and Mpumalanga, but they do not occur in the same areas in these provinces. *Sphaerium incomitatum* was not reported from the NWU Snail Collection, but they have been reported from GBIF. It could be because it was wrongfully identified as the morphology for this species is very vague and little differences exist between *S. capense* and *S. incomitatum*. For *S. capense*, the distribution is quite scattered across Africa (Figure 3.21), and some sites do not make sense biogeographically as the altitude of these sites ranges from 920 m above sea level (Congo) to 1,710 m above sea level (Crocodile River). The type locality for this species is the Knysna River with an altitude of 72 m above sea level. Most of the Snail Collection sites are low-lying areas which could mean that these are possibly correctly identified. Although this comparison has made big strides for this genus, each species needs to be revised thoroughly and individually to determine whether they are still valid.

Interestingly, *Pisidium dubium* from the USA in the 16S tree fell into its own clade separate from the rest. It is also the only *Pisidium* in the tree that maintained its genus name and did not undergo change. This sequence has many base pair differences with the rest of the sequences but has the least amount of base pair differences with the GenBank sequence, *Sphaerium corneum* from Lithuania. The possibility exists that this species could have been misidentified.

Another noteworthy point is *Euglesa casertana* from Turkey in the 16S tree that did not fall in the same clade as the *Euglesa casertana* from France and Russia respectively, which could once again be a misidentification. The most widely distributed sphaeriid worldwide is *Euglesa casertana* (Bespalaya et al., 2024) which has been reported in South Africa according to Kuiper (1964) and Mandahl-Barth (1988), but it was not found during this study. Bespalaya et al. (2024) illustrated that this species represents a group of closely related organisms containing multiple genetic lineages, although the exact taxonomy still needs some clarification. There are a few sequences

of *Euglesa casertana* from all over the world represented in the COI tree, and not all of them fall in the same clade. Most European sequences of this species fell in one clade, but three others from Albania, Greece, and USA fell in a separate clade. Because this is the most widespread species in the world, the likelihood of misidentification is high.

There are some questions about the validity of some of the sequences' identifications and quality as illustrated by the following examples: In the 16S tree, *Euglesa maasseni* and *Euglesa edlaueri* both from Macedonia have one base pair difference between them, suggesting that they are the same species. It is interesting to see that *Euglesa henslowana* from USA in the COI tree fell within a clade with *Euglesa supina* from Germany, rather than with *Euglesa henslowana* from Germany. *Euglesa nitida* in the COI tree has sequences from China, Spain, and Canada. China and Spain fell in the same clade, but Canada is completely on its own, again questioning its validity. These together with a few examples mentioned when discussing each clade individually, highlight the importance of this family and its need for revision.

The ecological parameters considered in this study showed a trend where *Eupera ferruginea* was found in very different areas than the *Euglesa* species. Although more samples will be needed to confirm this trend. *Euglesa costulosa* and *Euglesa viridaria* were mostly found in the same type of ecological parameters which has been confirmed in other literature where Clewing et al. (2013) states that these species prefer high mountain areas, and that is where most specimens were found: Highveld, and consequently high altitude. De Kock and Wolmarans (2008) further supports this by mentioning that these species usually occur together.

Epibionts were observed on some bivalve species in this study. This is the first time epibionts in the form of diatoms have been found on small freshwater bivalves. Epibionts are organisms that flourish on the surface of other organisms that act as their host, known as basibionts (Rex and Ravi, 2023). The most popular examples are barnacles (epibiont) on whales (basibiont). Epibiosis is a form of symbiosis, which can be facultative if there is a tropical exchange between the epibiont and basibiont (Rex and Ravi, 2023). They have the potential to be very ecologically informative if the relationship is known and understood. Epibionts, in the form of diatoms, were found on sphaeriid bivalves during this study. Diatoms are sensitive to changes in the environment, especially insecticides (Kock, 2020). Bivalves are also sensitive to changes in the environment thus, they could potentially form a great bioindicator together.

Kock (2020) supports the fact that the following species were attached on-site as they have been reported from the same river as where the *Eupera ferruginea* was found in: *Amphora veneta*, *Capartogramma crucicula*, *Gomphonema* sp., *Navicula radiosa*, and *Sellaphora* sp. These

species differ ecologically as some can be found in heavily polluted waters, such as *Amphora veneta*, and others are sensitive to pollution, such as *Gomphonema* (see Taylor et al., 2007; Figure 3.27). The other species that were found on *Eupera ferruginea* and were not reported in Kock (2020) are *Amphora copulata*, *Amphora vandammi*, and *Navicula subcostulata* var. *avittata* Cholnoky, 1959.

According to the IUCN, only two out of the eleven South African sphaeriid species population trend is known: *Euglesa casertana* which is decreasing, and *Sphaerium capense* which is stable. There have been no reports of epibionts in the form of diatoms on these bivalves, that were encountered in this study, but it could potentially be a new field to explore, possibly assisting in biomonitoring. These are just a few knowledge gaps to show how much there is still to learn about these bivalves.

3.5 Conclusion

To conclude, novel sequences of three different sphaeriid species were generated and there are possibly four different species present in the 74 sites in the northern provinces of South Africa that were sampled. From the genus *Euglesa*, the following were found: *E. costulosa*, *E. cf. langleyana* or species under the genus name, *Hindupisidium*, and *E. viridaria*, From the genus *Eupera*, *E. ferruginea* was found. The information from the IUCN, GBIF, and the NWU Snail Collection provide a comprehensive overview of the state of the distribution of sphaeriid bivalves in South Africa, however the IUCN data could be updated since the population trend of only two out of the ten species discussed here are known. The sphaeriids that were found were found in similar areas to that reported in literature and based on GBIF and the NWU Snail Collection. This confirms that the distribution remains unchanged, but the species diversity has decreased. The ecological parameters at which the sphaeriids were found also correlate to literature. The taxonomy for *Euglesa* has changed a lot over the years, but it seems to be stabilising with an increase of attention on this family around the world and some extra attention on South African sphaeriids could be advantageous in determining the validity of all species that have been reported in the area. The first step would be to obtain more sequences for all species and compile an updated morphological key with more comprehensive and sure-fire morphological characteristics. This work contributes to the current knowledge on South African sphaeriids as there are only two COI sequences, and the outcome of this work is 34 COI sequences.

CHAPTER 4 AN OVERVIEW OF FRESHWATER BIVALVES IN THE FAMILY IRIDINIDAE IN SOUTH AFRICA

4.1 Introduction

The family Iridinidae is a distinct group of bivalve molluscs known for its unique evolutionary adaptations and ecological roles. Their shell morphologies, which include various ridges and hinge mechanisms, are tailored to certain soil types and predatory stresses (Cowie, 1998). Furthermore, their feeding techniques, which frequently include filter feeding, are important for nutrient cycling and sediment stability (Cohen and Weyl, 2007). Ecologically these clams play an important role in the dynamics of freshwater environments. Their presence affects sediment composition and stability, and their interactions with other organisms highlight their importance in complex food webs (Buckland et al., 2010). Appleton (2002) states that iridinids occur in large numbers in lakes, dams and perennial rivers. Some species have been known to persist in densities of 30–50 / m². They have also been known to aestivate (Appleton and Miranda, 2005). In the Iron Age, these bivalves were thought to be an important but unsustainable food source because of how long it took them to grow to a reasonable size. It is also thought that the shells might have been used to make clay pots and beads (Appleton, 2002).

According to Mandahl-Barth (1988), Iridinidae shells (previously Mutelidae Gray, 1847) look very similar to Unionidae shells, and the most reliable determining factor is that iridinid bivalves are bigger. Some iridinid species have undefined sculptures on their shell, which is another character distinguishing them from unionids. However, internally, an important distinguishing feature is that iridinids do not have lateral or cardinal teeth (Mandahl-Barth, 1988). In some cases, the bivalves are pseudotaxodont, which indicates that their hinge plate has a series of tubercles present. The alternative is that the shell is completely smooth. Iridinid eggs are also smaller than unionid eggs, which is strange as adult iridinid bivalves are, as a rule, bigger than adult unionid bivalves. According to Mandahl-Barth (1988), the eggs measure up to 0.1 mm in diameter.

Iridinid bivalves are found as separate sexes, or they can be hermaphroditic (Appleton, 2002). Their gonads are in the foot where ducts discharge ripe gametes into the mantle, from where they are released into the water. Egg cells attach themselves to the brood sacs inside the chamber of the bivalve, whereas sperm is released into the water. The eggs are fertilised when the bivalve draws in sperm from the water. Here they form into haustorial larvae, where they are equipped with the necessary tools to attach themselves as parasites to fish. They

usually attach to the fins, gill filaments or skin of the fish. They mimic food for fish as they are covered with mucus, and if they do not attach to a fish, they will die. However, if they do manage to attach on time, they become physiologically dependent on the fish and stay there for approximately a week to a few months. Thereafter, they will drop to the substratum and live out their lives as adult bivalves (Appleton, 2002; De Kock et al., 2017).

Some species appear to be host-specific, whereas others are not at all, but this needs further research (Appleton, 2002). Some species in this family become encysted in the fish skin as they pinch off a piece of tissue, whereas others, e.g., *Mutela zambesiensis*, develop special stalks whereby they attach themselves to the host fish (Appleton, 2002). In either case, if too many larvae attach to one fish, the fish may die. This mode of reproduction also acts as a mode of distribution seeing as these bivalves are otherwise quite stationary (Appleton, 2002). They may lay thousands of eggs in their lifetime, spanning between 10,000 and 26,000 depending on the size of the bivalve. This may continue annually for several years, and the mortality rate is highest amongst juveniles.

The family Iridinidae only occurs in Africa (Van Bocxlaer and Van Damme, 2009) and developed when the African continent was entirely secluded during the Tertiary epoch (Van Damme, 1984). Its monophyly is supported by the following: their well-developed pedal elevator scars under the umbo, the mantle that closes dorsally to the excurrent opening and with a tubular excurrent siphon, a haustorium that is formed when the parasitic lasidium latches to the host using tubular appendages, and the larvae that are released in an egg capsule (Graf and Cummings, 2006b). In more recent literature, this family is supported as non-monophyletic (Combosch et al., 2017). According to Van Bocxlaer and Van Damme (2009), the number of genera in this family remains undetermined but there are currently nine recognised on MolluscaBase. Four of those genera (Dentaspitharia Modell, 1964, Itaimbeia Mezzalira, 1974, Pseudodiplodon W. Adam, 1957, and Taxodontites Simone & Mezzalira, 1997) are fossils only. The remaining five are still extant and are found only in Africa. These are *Aspatharia* Bourguignat, 1885, *Chambardia* Bourguignat, 1891, *Chelidonopsis* Ancey, 1887, *Mutela* Scopoli, 1777, and *Pleiodon* Conrad, 1834 (see Table 4.4).

The family's classification has been significantly revised to reflect advances in molecular phylogenetics and comparative anatomy (Glover and Taylor, 2001). Understanding the taxonomic framework of Iridinidae is critical for determining their evolutionary history and biogeographic distribution. It is also crucial for their conservation, although according to the IUCN all are of least concern, but based on findings from this study the species might be more threatened than originally thought.

Mutela was originally created for “*Mutel*” Adanson, a form of *Mutela dubia* (Gmelin, 1791) (previously *Mytilus dubius* Gmelin, 1791). Thereafter, Lamarck (1819) created a subgenus *Iridina* Lamarck, 1819 and allocated pseudotaxodont iridinid bivalves to *Mutela*. In 1823, another *Mutela* species was described by Swainson, however, it was questioned by Conrad (1834) who redescribed this species under another genus name i.e. *Pleiodon*. Woodward (1859) described a third species that led to the third subgenus being formed by Bourguignat (1879). Simpson (1914) indicated that *Iridina* was not a valid genus, and merged *Iridina* taxa with species from *Mutela*. Despite this synonymy, Pilsbry and Bequaert (1927) disagreed and declared *Iridina* a valid genus and retained the original three subgenera. Pain and Woodward (1964) did an extensive overview of this genus and made concluding remarks indicating that *Iridina* and *Mutela* were synonymous with each other, with *Mutela* taking precedence.

Before this thorough overview from Pain and Woodward (1964), there were many uncertainties as iridinids were frequently synonymised with *Pleiodon*. The family name, *Iridinidae* Swainson, 1840, was first established by Bourguignat (1885), but all other authors from Adams and Adams (1857), placed *Pleidon* and *Iridina* in the family Mutelidae Gray, 1847 (currently Iridinidae). To date, *Pleiodon* is still regarded as a valid genus within the family Iridinidae. Mandahl-Barth (1988) suggested that iridinids are distributed throughout South America, Africa, and Australia. The scope of this study was limited to the genera found in southern Africa, i.e. *Aspatharia*, *Chambardia*, and *Mutela* (Table 4.1).

There has been some confusion around the genus, *Chambardia*, as it was referred to as *Spathopsis* Simpson, 1900 in the late 1990s based on the work of Mandahl-Barth (1988). The confusion originated from the type species that was a subfossil and researchers at the time wanted to take the more conservative route. According to Seddon et al. (2011), the confusion should rather be reduced by the fact that *Spathopsis* and *Chambardia* are now considered interchangeable. Unfortunately, MolluscaBase does not recognise these two as synonyms and does not mention *Spathopsis* at all. The synonymisation of these two genera is however confirmed by Herbert (1998). This author provided a detailed account of the synonymy of the *C. wahlbergi* species, confirming that Daget (1998) regards *Spathopsis* and *Chambardia* as synonymous.

Based on the lack of newer work on the family, this chapter aimed to provide an overview of the family Iridinidae in South Africa concerning taxonomy and distribution as well as contributing to the existing knowledge with this study’s findings. Where available, the overview of the family is supported by both morphological and molecular results.

Table 4.1 All southern Africa Iridinidae species, accompanied by their synonyms, sequences and their IUCN category.

Species name	Synonyms	Sequences	IUCN red list category
<i>Aspatharia pfeifferiana</i> (Bernardi, 1860)	• <i>Anodon cochlearis</i> G. B. Sowerby II, 1870 • <i>Aspatharia sinuata</i> (E. von Martens, 1883) • <i>Aspatharia stuhlmanni</i> (E. von Martens, 1897) • <i>Margaritana pfeifferiana</i> Bernardi, 1860 • <i>Mutela lukululensis</i> Preston, 1913 • <i>Spatha (Leptospatha) stuhlmanni</i> E. von Martens, 1897 • <i>Spatha cryptoradiata</i> Putzeys, 1899 • <i>Spatha sinuata</i> E. von Martens, 1883 • <i>Spatha stuhlmanni</i> E. von Martens, 1897 • <i>Spathella protchei</i> Rochebrune, 1886	6 on GenBank (COI, 18S, 16S, 28S)	Least concern
<i>Chambardia petersi</i> (E. von Martens, 1860)	• <i>Aspatharia petersi</i> (E. von Martens, 1860) • <i>Spatha modesta</i> I. Lea, 1864 • <i>Spatha petersi</i> E. von Martens, 1860	None	Least concern
<i>Chambardia wahlbergi</i> (Krauss, 1848)	• <i>Anodon tabula</i> G. B. Sowerby II, 1867 • <i>Anodonta guillaini</i> Récluz, 1850 • <i>Aspatharia (Spathopsis) figulorum</i> Coen, 1935 • <i>Aspatharia (Spathopsis) hartmanni</i> (E. von Martens, 1866) • <i>Aspatharia (Spathopsis) hartmanni bourguignati</i> (Bourguignat, 1886) • <i>Aspatharia (Spathopsis) hartmanni guillaini</i> (Récluz, 1850) • <i>Aspatharia (Spathopsis) wahlbergi</i> (Krauss, 1848) • <i>Aspatharia wahlbergi</i> (Krauss, 1848) • <i>Iridina wahlbergi</i> Krauss, 1848 • <i>Spatha (Leptospatha) bourguignati</i> var. <i>major</i> Germain, 1906 • <i>Spatha (Spatha) wahlbergi</i> (Krauss, 1848) • <i>Spatha bertilloniana</i> Preston, 1910 • <i>Spatha hartmanni</i> E. von Martens, 1866 • <i>Spatha maitenguensis</i> Sturany, 1898 • <i>Spatha wahlbergi</i> (Krauss, 1848) • <i>Spatha wahlbergi</i> var. <i>dorsalis</i> E. von Martens, 1897 • <i>Spathella adansonii</i> Jousseaume, 1886	29 on GenBank (COI, 18S, 16S, 28S)	Least concern

	• <i>Spathella bozasi</i> Rochebrune & Germain, 1904 • <i>Spathella brumpti</i> Rochebrune & Germain, 1904		
<i>Mutela zambesiensis</i> Mandahl-Barth, 1988	• None	None	Least concern

4.2 Materials and methods

4.2.1 Collection

Seven iridinids were collected at the Klein Letaba River, and one was found in the Vaal River (see additional info on sites in Chapter 2). The Klein Letaba iridinids were found in a shallow pool and, bivalves were picked from the sediment by hand. Larger specimens such as the one found in the Vaal River can usually be found at the banks of waterbodies. In deeper water, a snail sampler was used to scrape the bottom after which bivalves were manually collected by sifting through the scooped sediment by hand. Specimens were placed into 96% ethanol for molecular analyses.

4.2.2 Morphological methods

As these bivalves are quite large, a dissection microscope could not be used to capture images as in the previous chapter. Instead, a phone camera (Samsung Galaxy, A54) was used and a vernier calliper was used to measure them. Appleton (2002) and Mandahl-Barth (1988) were mainly used to identify these bivalves based on key morphological characteristics. This included the shape, sculpture, and colour of their shell, where their umbo lies in relation to the median line, the colour of the inside of the shell, and the size of the bivalve (Figure 4.1). Illustrations of all newly collected and previously published specimens (from Kuiper, 1964 and Mandahl-Barth, 1988) were created using Adobe Illustrator (Creative Cloud, 2024) and a Wacom Intuos 4 Pro graphic tablet (PTK-640).

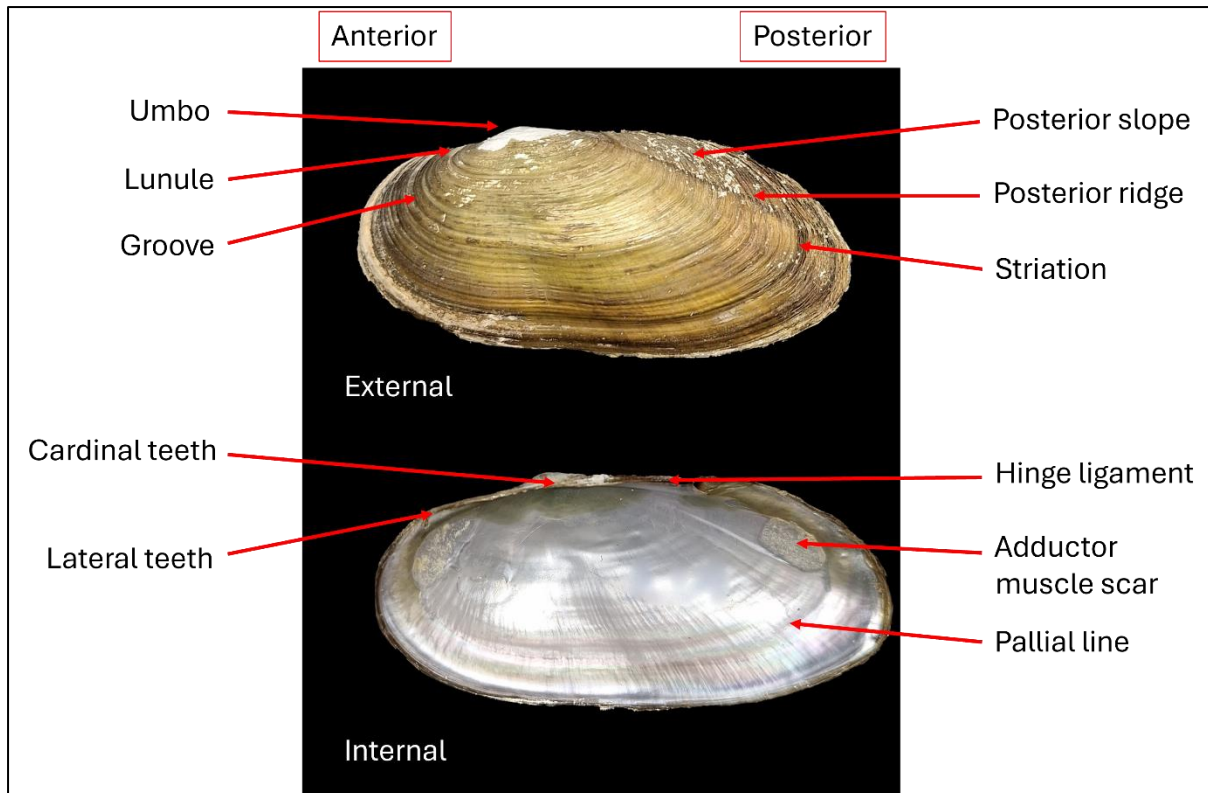


Figure 4.1 General morphological characteristics of mussels in the order Unionida (iridinids and unionids).

4.2.3 Molecular methods

The bivalves were opened by inserting a sterile blade in-between the valves and twisting it until the bivalve could be prised open. A piece of the foot or mantle tissue was cut off and placed in an autoclaved tube and placed in the freezer. The rest of the tissue was placed in a separate container with molecular-grade ethanol, and the shell was cleaned and marked for further morphological analysis. Chelex 10% was used to extract the DNA of each bivalve (see chapter 3.2.3 for more details). Three genes were targeted to identify the bivalve genetically: 16S, COI, and 28S. For each different primer combination (Table 4.2), the volumes remained the same throughout the extraction: double distilled water = 7 μL , forward primer = 1.25 μL , reverse primer = 1.25 μL , Kapa Hifi HotStart Ready Mix = 12.5 μL , DNA = 3 μL . A negative control was added to each run to ensure there was no contamination.

Table 4.2 Information of primers used for molecular analyses of iridinids.

Genetic marker	Primer	Sequence	Reference	PCR conditions (below)
COI	LCO1490	5' -GGNTCTGCN TAYGTNGARAG-3'	Folmer et al. (1994)	Figure 4.1
	modHCO2198	5'-TCAGGGTGACCAAAAATCA-3'	Clewing et al. (2013)	
16S	16Sar	5'-CGCCTGTTTAACAAAAACAT-3'	Hata et al. (2017)	Figure 4.2
	16Sbr	5'-CCGGTCTGAACTCAGATCATGT-3'		
28S	1274F	5'-GACCCGTCTTGAAACACG GA-3'	Markmann and Tautz (2005)	Figure 4.1
	1276R	5'-CTAGTTGCTTCGGCAGGTGAG-3'	Møller et al. (2008)	

The successfully extracted samples were sequenced at Inqaba Biotechnical Industries, whereafter all the same software and conditions as stated in Chapter 3 under section 3.2.3 were followed to compile the phylogenetic trees.

For phylogenetic analysis, sequences were first selected based on the genus name (Table 4.3). All species belonging to the relevant genera (*Aspatharia*, *Chambardia*, and *Mutela*) were added based on the following criteria: one species from each country, published sequences were prioritised, and sequences that have a country of origin were prioritised. Since there were not many sequences available, one sequence without a country of origin was added as it was a species of interest that occurs in South Africa (*Aspatharia pfeifferiana*). Two unpublished COI sequences of *Chambardia* sp. and *Chambardia wahlbergi* were also added as they were the only sequences available for this genus.

Two different PCR protocols were used for the amplification of DNA (Table 4.2). The first one (Figure 4.1) followed the following conditions: initial denaturation starts at 95°C for 1 minute; 7 cycles with a denaturation of 95°C for 30s, and an annealing temperature lasting for 30s starting at 52°C and decreasing with each cycle by 1°C, to end at 46°C. An extension of 72°C for 30s forms part of these 7 cycles. Then, 33 cycles of 95°C for 30s, 45°C for 30s, and 72°C for 30s. The final extension was at 72°C for 3 min and it was held at 4°C till further analysis. Bößneck et al. (2016) was used as a guideline for this modified method better suited for these bivalves (Figure 4.1).

Table 4.3 Sequences obtained from GenBank along with their name and authority, accession number, reference for sequences, country of origin, and gene. The outgroup is shaded in grey.

Sequence name and authority [#]	Accession number	Reference for sequence	Country of origin
16S gene			
<i>Aspatharia pfeifferiana</i> (Bernardi, 1860)	KC429264	Sharma et al. (2013)	
<i>Chambardia wahlbergi</i> (Krauss, 1848)	KP184845	Graf et al. (2015)	Zambia
<i>Mutela harger</i> E. A. Smith, 1908	KX713237	Combosch et al. (2016)	Zambia
<i>Etheria elliptica</i> Lamarck, 1807 (Outgroup)	KX713219	Combosch et al. (2016)	Zambia
COI gene			
<i>Mutela harger</i> E. A. Smith, 1908	KX713482	Combosch et al. (2016)	Zambia
<i>Mutela dubia</i> (Gmelin, 1791)	NC034844	Guerra et al. (2017)	Egypt
<i>Mutela rostrata</i> (Rang, 1835)	JN243884	Whelan et al. (2011)	Egypt
<i>Chambardia wahlbergi</i> (Krauss, 1848)	OR525253	Unpublished	South Africa
<i>Chambardia rubens</i> (Lamarck, 1819)	MK764010	Unpublished	Egypt
<i>Chambardia wahlbergi</i> (Krauss, 1848)	KX713448	Combosch et al. (2016)	Zambia
<i>Aspatharia pfeifferiana</i> (Bernardi, 1860)	JN243885	Whelan et al. (2011)	Zambia
<i>Etheria elliptica</i> Lamarck, 1807 (Outgroup)	KX713462	Combosch et al. (2016)	Zambia

[#]Up to date until 31 October 2024

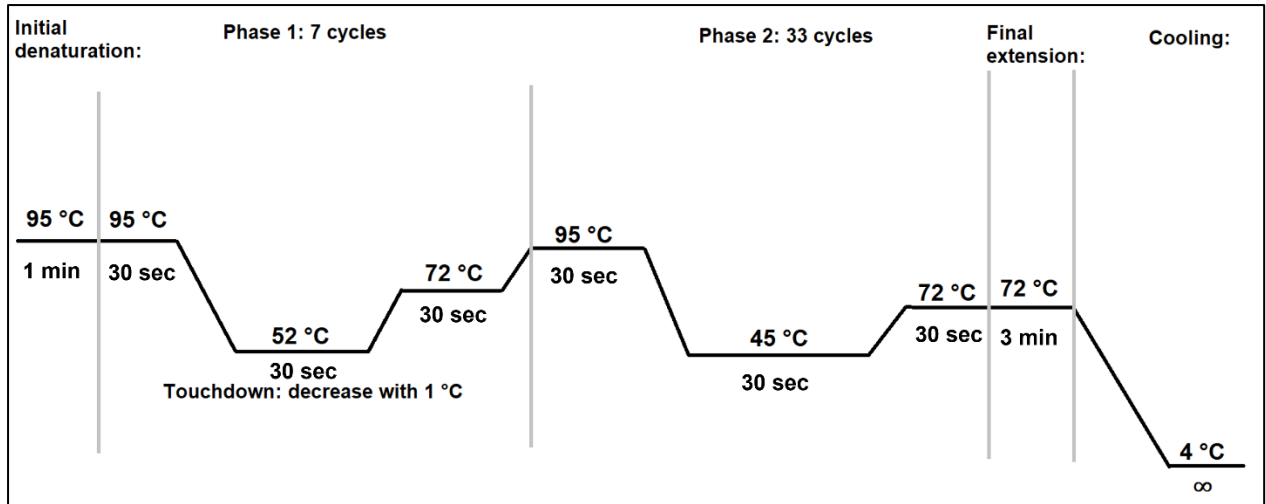


Figure 4.2 An illustration of the touchdown PCR used for COI genes of iridinids.

The second protocol (Figure 4.2) followed the following conditions: initial denaturation was at 95°C for 15 seconds, whereafter there were 34 cycles of denaturation at 95°C for 15s, annealing at 53°C for 15s, and extension at 72°C for 15s. A final extension period was at 72°C for 10 minutes, thereafter the samples were held at 12°C until further analysis (Prie and Puillandra, 2014) (see Figure 4.2).

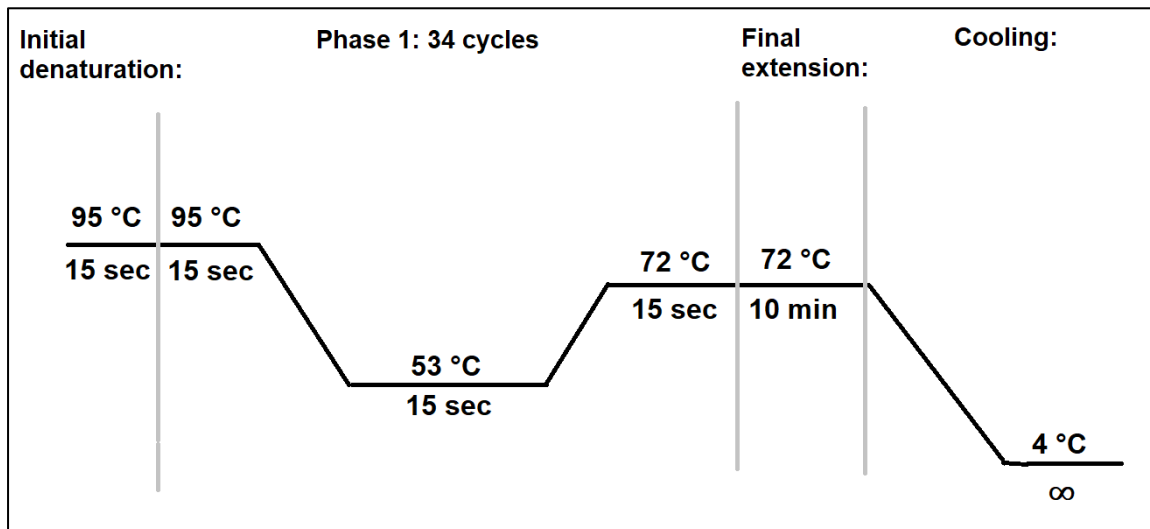


Figure 4.3 An illustration of the PCR conditions used for 16S genes of iridinids.

4.2.4 Map creation and coordinate plotting

The QGIS software (Version 3.22) was used to compile the various maps for this study. The coordinate points consist of GBIF data, NWU Snail Collection, and GPS coordinates from the current study. The same methods as in Chapter 3, under section 3.2.4, were followed for this section.

4.3 Results

There are only African species in the family Iridinidae (Table 4.4). Basic information about each of the species in the family can be found in Table 4.4, while detailed descriptions of all species occurring in South Africa are presented in the following section. All of the African species were included in Table 4.4 to provide a brief overview of where the South African species fit in with the rest of the African species, and to identify gaps in literature concerning this family. Furthermore, it can be seen in Table 4.4 that most species do not have sequences available on GenBank or BOLD and many are found within concerning IUCN conservation categories such as data deficient, endangered, and critically endangered.

Table 4.4 All species in the family Iridinidae from Africa along with their authority, IUCN status, GBIF occurrences, sequences, and type locality.

Species name	IUCN status	GBIF occurrences	Sequences	Type locality
<i>Aspatharia</i>				
<i>Aspatharia chaiziana</i> (Rang, 1835)	Data deficient	37	None	Senegal
<i>Aspatharia dahomeyensis</i> (Lea, 1859)	Data deficient	34	None	Dahomey
<i>Aspatharia divaricata</i> (E. von Martens, 1897)	Critically endangered	4	None	Victoria Lake
<i>Aspatharia droueti</i> (Chaper, 1885)	Vulnerable	15	None	Ivory Coast
<i>Aspatharia anciniensis</i> (Rochebrune, 1886)	Least Concern	8	None	Congo
<i>Aspatharia marnoi</i> (Jickeli, 1874)	Data deficient	15	None	Nile Basin
<i>Aspatharia pangallensis</i> (Rochebrune, 1882)	Vulnerable	3	None	Mali
<i>Aspatharia pfeifferiana</i> (Bernardi, 1860)	Least Concern	94	2 – COI 1 – 16S	Gabon
<i>Aspatharia rochebrunei</i> (Jousseume, 1886)	Vulnerable	3	None	Mali

Species name	IUCN status	GBIF occurrences	Sequences	Type locality
<i>Aspatharia rugifera</i> (Dunker, 1858)	Least Concern	55	None	Como River
<i>Aspatharia subreniformis</i> (Sowerby II, 1867)	Least Concern	29	None	Lake Nyassa
<i>Chambardia</i>				
<i>Chambardia bourguignati</i> (Bourguignat, 1886)	Least Concern	9	None	Lake Victoria
<i>Chambardia flava</i> (Pilsbry & Bequaert, 1927)	Unknown	15	None	Congo
<i>Chambardia moutai</i> (Darteville, 1939)	Unknown	8	None	Angola
<i>Chambardia nyassaensis</i> (Lea, 1864)	Least Concern	16	None	Lake Nyassa
<i>Chambardia petersi</i> (E. von Martens, 1860)	Least Concern	18	None	Mozambique
<i>Chambardia rubens</i> (Lamarck, 1819)	Least Concern	210	7 – COI	Senegal
<i>Chambardia trapezia</i> (E. von Martens, 1897)	Data deficient	19	None	Lake Victoria
<i>Chambardia wahlbergi</i> (Krauss, 1848)	Least Concern	178	23 – COI 3 – 16S 2 – 28S 1 – 18S	South Africa
<i>Chambardia welwitschii</i> (Morelet, 1867)	Unknown	5	None	Angola
<i>Chambardia wissmanni</i> (E. von Martens, 1883)	Least Concern	21	None	Congo
<i>Chelidonopsis</i>				
<i>Chelidonopsis hirundo</i> (E. von Martens, 1881)	Least Concern	29	None	Angola
<i>Mutela</i>				
<i>Mutela alata</i> (Lea, 1864)	Least Concern	38	None	Lake Nyassa
<i>Mutela alluaudi</i> Germain, 1910	Data deficient	2	None	Lake Albert
<i>Mutela bourguignati</i> Bourguignat, 1886	Near Threatened	2	None	Lake Victoria
<i>Mutela dubia</i> (Gmelin, 1791)	Least Concern	225	7 – COI 1 – 18S	Central Africa
<i>Mutela franci</i> Daget, 1964	Endangered	2	None	Niger River
<i>Mutela hageri</i> Smith, 1908	Least Concern	62	1 – COI 1 – 16S 1 – 28S 1 – 18S	Mweru Lake

Species name	IUCN status	GBIF occurrences	Sequences	Type locality
<i>Mutela joubini</i> (Germain, 1904)	Endangered	1	None	Congo
<i>Mutela langi</i> Pilsbry & Bequaert, 1927	Endangered	4	None	Zambia
<i>Mutela legumen</i> (Rochebrune, 1886)	Vulnerable	20	None	Congo
<i>Mutela mabilli</i> (Rochebrune, 1886)	Least Concern	12	None	Gabon
<i>Mutela rostrata</i> (Rang, 1835)	Least Concern	137	5 – COI 1 – 28S	Senegal
<i>Mutela soleniformis</i> Bourguignat, 1886	Data Deficient	12	None	Lake Tanganyika
<i>Mutela wistarmorrisi</i> Graf & Cummings, 2006	Data deficient	5	None	Cuanza River
<i>Mutela zambesiensis</i> Mandahl-Barth, 1988	Least Concern	15	None	Zambesi River
<i>Pleiodon</i>				
<i>Pleiodon spekii</i> (Woodward, 1859)	Least Concern	91	None	Lake Tanganyika
<i>Pleiodon ovatus</i> (Swainson, 1823)	Critically Endangered	91	None	Western Africa

4.3.1 Morphological results

There are three genera in the family Iridinidae, namely *Aspatharia*, *Chambardia*, and *Mutela*. In southern Africa, there are four species known from these genera that will be revised below.

Taxonomic results – *Aspatharia*

Phylum Mollusca

Class Bivalvia

Order Unionida

Family Iridinidae Swainson, 1840

Genus *Aspatharia* Bourguignat, 1885

Notes: According to Mandahl-Barth (1988), a defining feature of this genus is that it does not have a presiphonal suture, but it does have sculpture on the umbo in the form of parallel, broadly V-shaped ridges. This is easier seen in juveniles, as the umbo is prone to erosion as

the bivalves age. This species usually has a thin, smooth hinge plate that ends posteriorly in a triangular manner.

4.3.1.1 *Aspatharia pfeifferiana* (Bernardi, 1860)

Original name: *Spatha* (*Aspatharia*) Bourguignat, 1885.

Type locality: Gabon (MolluscaBase; Mandahl-Barth, 1988).

Distribution: *Aspatharia pfeifferiana* is believed to be an uncommon species. According to Appleton (2002), it is only known from the Kunene, Okavango, and upper Zambezi rivers. According to Mandahl-Barth (1988), it has a wide distribution from Chad to Zimbabwe and Namibia (Ovamboland). In East Africa, it is only known from one location in Uganda. There were no records of this species in the NWU Snail Collection, and according to GBIF they have only been reported from southern to central Africa in the following countries: Botswana, Zambia, Democratic Republic of Congo, Gabon, and Cameroon (Figure 4.3). This species was not found during this study.

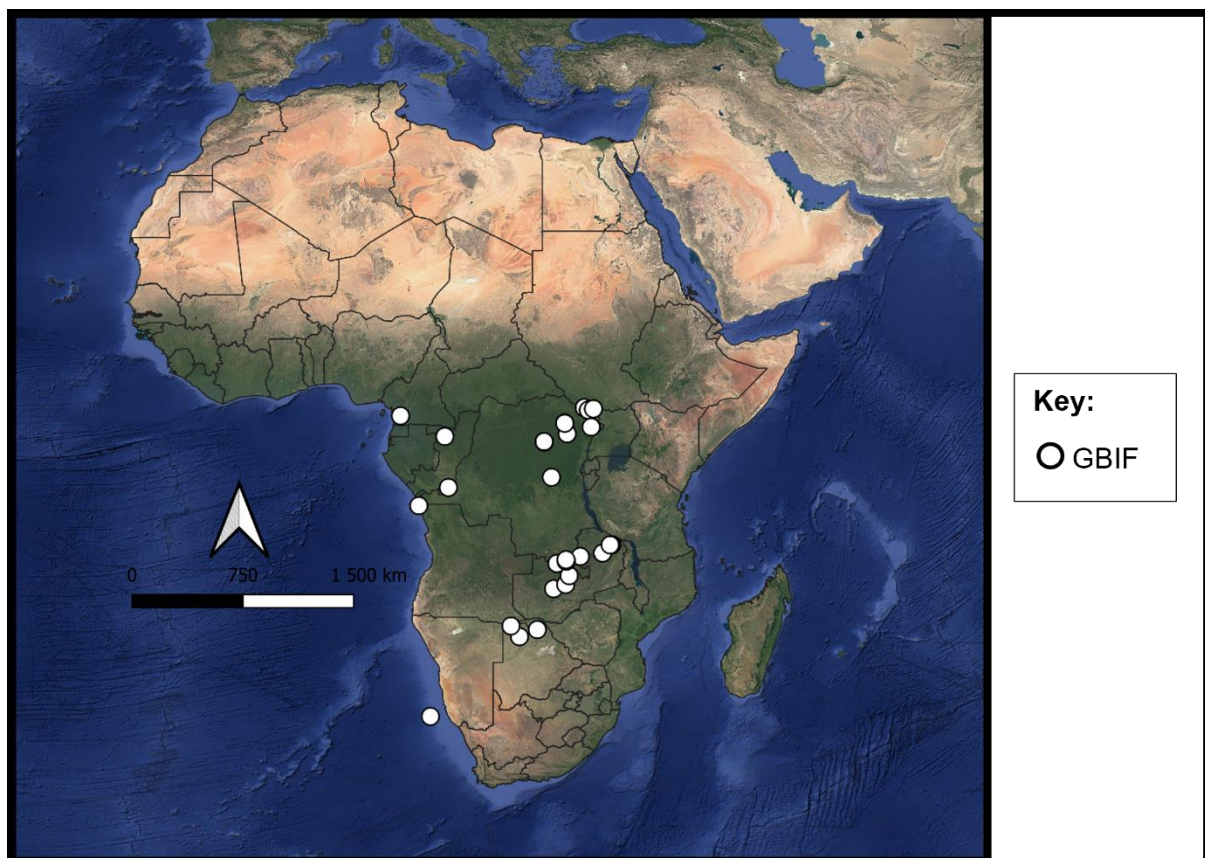


Figure 4.4 African distribution of *Aspatharia pfeifferiana* (Bernardi, 1860) (white dots) based on records from GBIF.

Size: According to Appleton (2002), they can grow up to 8.5 cm in length and 3.7 cm in width.

GBIF: 94 occurrences (Figure 4.3).

Available sequences: Two COI, and one 16S sequence on GenBank.

Photos: When searching for *Aspatharia pfeifferiana*, seven images were found on GBIF.

Descriptive notes: The dorsal and ventral margins are parallel to one another. As a rule, the height of the shell is a bit smaller than half the shell length, and the diameter of the shell is equal to two-thirds of the height. The dorsal margin is also usually slightly sloped (Figure 4.4).

Remarks: This species is of Least Concern based on the IUCN Red List, although their population trend is decreasing (Van Damme and Graf, 2020).

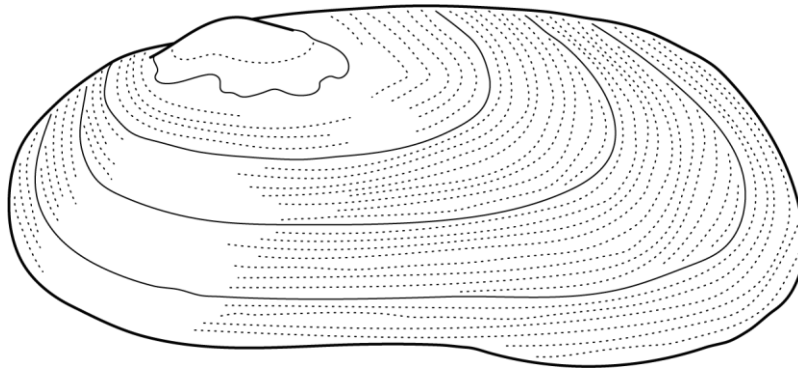


Figure 4.5 Redrawn image of *Aspatharia pfeifferiana* (Bernardi, 1860) based on an illustration done by Mandahl-Barth (1988).

Taxonomic results - *Chambardia*

Phylum Mollusca

Class Bivalvia

Order Unionida

Family Iridinidae Swainson, 1840

Genus *Chambardia* Bourguignat, 1891

Notes: *Chambardia* shells are usually bigger than *Aspatharia*. They have a lunula (sinulus) that is sharply triangular and large; they also have an edentulous (“toothless”) hinge and the same umbonal sculpture as can be seen in *Aspatharia* (Van Bocxlaer and Van Damme, 2009).

4.3.1.2 *Chambardia petersi* (E. von Martens, 1860)

Original name: *Spatha petersi* E. von Martens, 1860.

Type locality: Mozambique.

Distribution: According to Appleton (2002), this species has a limited distribution occurring in the lower Zambezi River and the eastward-flowing rivers of Zimbabwe, Mozambique and South Africa (in Limpopo province). Based on the NWU Snail Collection, this species was found in four provinces of South Africa: North West, Gauteng, Mpumalanga, and Limpopo (Figure 4.5). Some records of GBIF coincide with these results, where it was reported from Mpumalanga in the same area, but some were in a different area than that of the NWU Snail Collection (like those in Limpopo). The type locality of this species was also confirmed to be in Mozambique by GBIF. This species was not found during the present study's field collections.

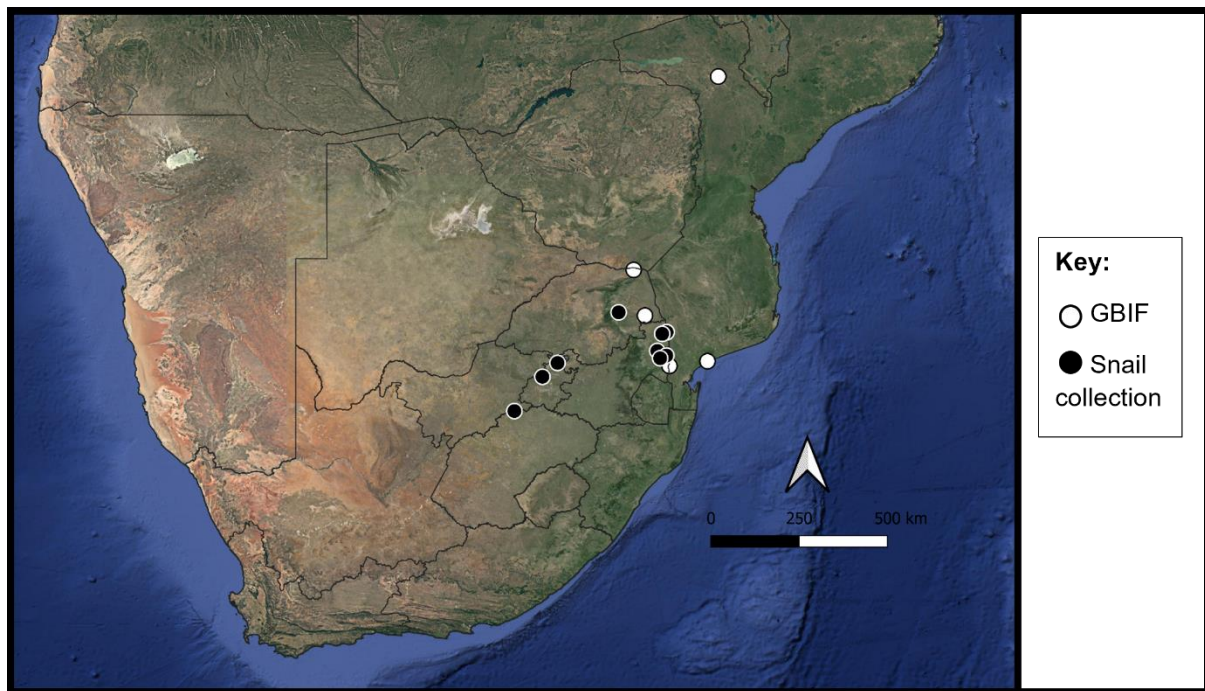


Figure 4.6 Southern Africa distribution of *Chambardia petersi* (E. von Martens, 1860) as recorded by GBIF (white dots) and the NWU Snail Collection (black dots).

Museum material: Smithsonian National Museum of Natural History. This specimen was not examined during this project.

Size: The size dimensions of this species are 7.2 x 3.0 cm (Appleton, 2002).

GBIF: 18 occurrences (Figure 4.4).

Photos: When searching for *Chambardia petersi*, one image was found on MolluscaBase, two on GBIF, and 17 on the MUSSELP database.

Descriptive notes: The bivalve is elongated with the dorsal and ventral margins almost parallel (Figure 4.6). They have growth lines, but they are finer than *Chambardia wahlbergi*.

Remarks: This species is small in comparison to the other species in this genus (Appleton, 2002). According to the IUCN, this species is of least concern, however, the population trend is unknown (Van Damme et al., 2018).

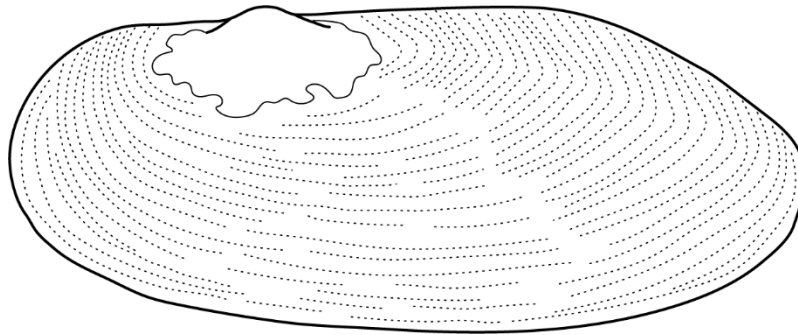


Figure 4.7 Redrawn image of *Chambardia petersi* (E. von Martens, 1860), based on an illustration done by Mandahl-Barth (1988).

4.3.1.3 *Chambardia wahlbergi* (Krauss, 1848)

Original name: *Iridina wahlbergi* Krauss, 1848.

Type locality: South Africa (Affen River in Limpopo).

Distribution: This species can be found distributed between the Kunene River, the rivers that flow eastward in the highveld, and the northern parts of KwaZulu-Natal (Appleton, 2002). According to Mandahl-Barth (1988), they are common in southern Africa, specifically from KwaZulu-Natal, Gauteng, northern Limpopo and southern Mpumalanga areas, and all the way to southern Tanzania. They have also been reported from other countries in Africa based on GBIF: Mozambique, Botswana, Namibia, Tanzania, Zimbabwe, Zambia, Malawi, Democratic Republic of Congo, Uganda, Sudan, and Ghana (Figure 4.7). In South Africa, they were reported in the Free State, North West, and Mpumalanga according to GBIF. The NWU Snail Collection has reports of this species from KwaZulu-Natal, Limpopo, and Mpumalanga. This species was found during this study from the Klein Letaba River in Limpopo and the Vaal River in the North West (Figure 4.7).

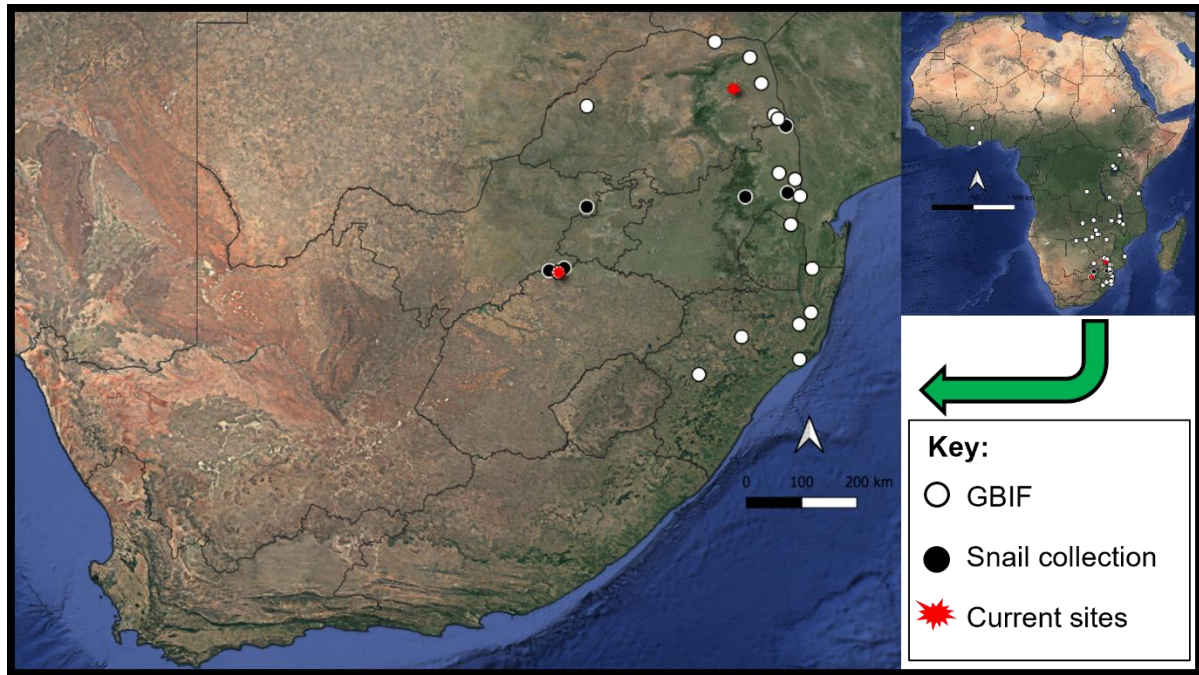


Figure 4.8 Southern Africa distribution of *Chambardia wahlbergi* (Krauss, 1848) as recorded by GBIF (white dots), Snail Collection (black dots), and this study (red stars).

Museum material: Museum National D'Histoire Naturelle (from synonym *Spatha hartmanni* (Roger, 1943)).

Size: It has been known to range from 13.6 cm to 11.5 cm in length and 6.7 cm to 5.4 cm in width (Appleton, 2002). This species had the following measurements in this study: 4.4 cm to 12.5 cm in length, and 1.5 cm to 7.1 cm in width.

GBIF: 178 occurrences (Figure 4.7).

Photos: When searching for *C. wahlbergi*, 28 images were found on GBIF, and over 100 on the MUSSELP database.

Descriptive notes: The inside of the shell is often pinkish or white. Their shell is long and slightly hard. The umbo is placed near the anterior end and the dorsal margin is usually straight but slopes a little bit toward the posterior (Figure 4.8).

Remarks: *Chambardia wahlbergi* is the largest freshwater mussel in southern Africa. In the past, baroque pearls were found in this species (Appleton, 2002). The species is of Least Concern according to the IUCN Red List and the population trend is stable (Seddon and Van Damme, 2018b).

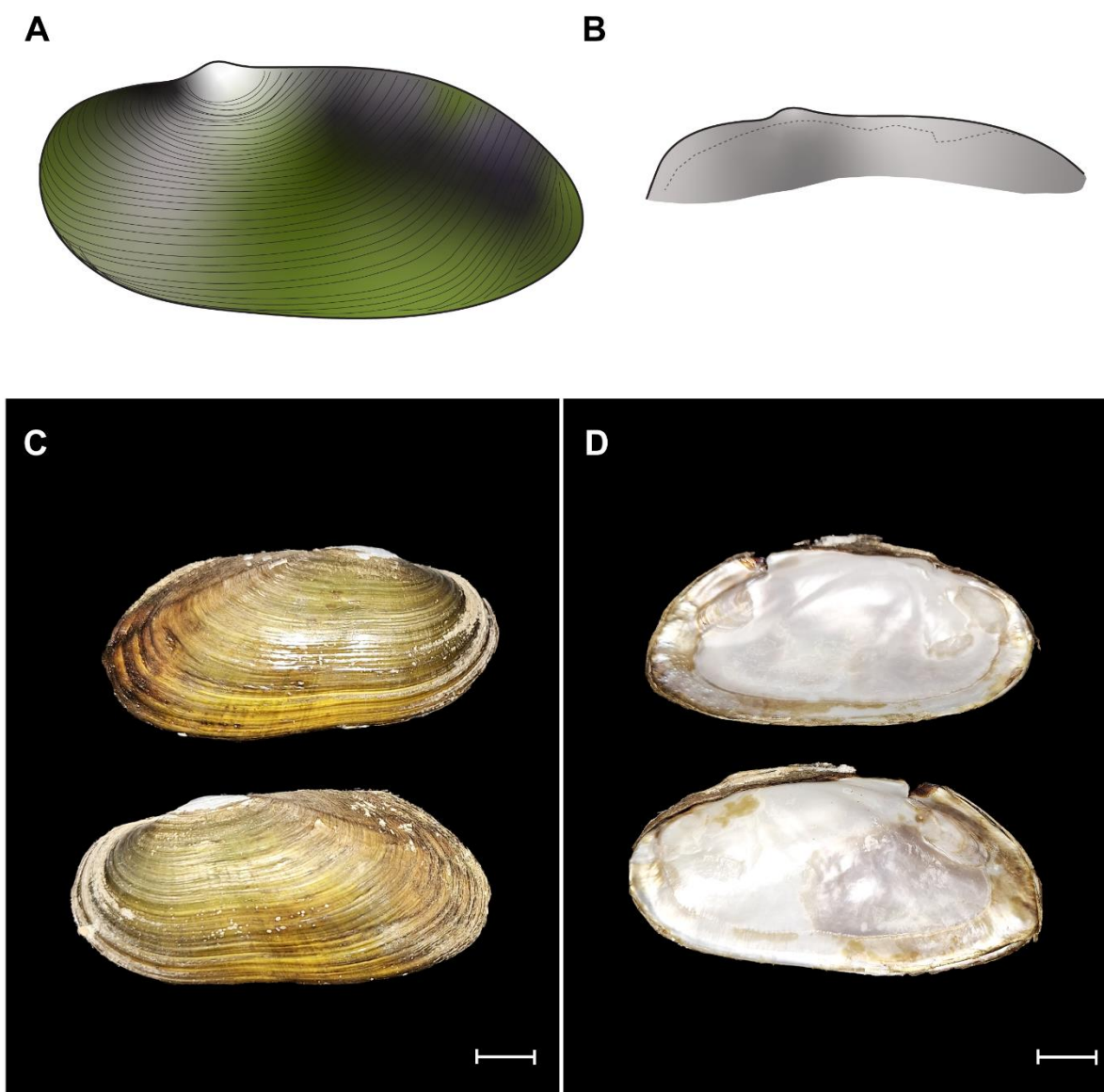


Figure 4.9 Redrawn image of *Chambardia wahlbergi* (Krauss, 1848) based on an illustration done by Mandahl-Barth (1988), as well as a representative bivalve that was found in this study.

Taxonomic results - *Mutela*

Phylum Mollusca

Class Bivalvia

Order Unionida

Family Iridinidae Swainson, 1840

Genus *Mutela* Scopoli, 1777

Notes: This genus is distinguished by the absence of lateral teeth on the posterior side of the valve, a feature referred to by Appleton (2002) as a small triangular lunule, which is present in the preceding genera.

4.3.1.4 *Mutela zambesiensis* Mandahl-Barth, 1988

Original name: *Mutela zambesiensis* Mandahl-Barth, 1988.

Type locality: Zambesi River between Kariba and Chirundu (MolluscaBase; Mandahl-Barth, 1988).

Distribution: This species is endemic to southern Africa and can be found in the Zambesi River and eastern Caprivi (Appleton, 2002). Based on reports from GBIF, this species has been found in Botswana, Zimbabwe and Zambia (Figure 4.9) but it has not been reported in South Africa by GBIF or the NWU Snail Collection. It was also not found during this study.

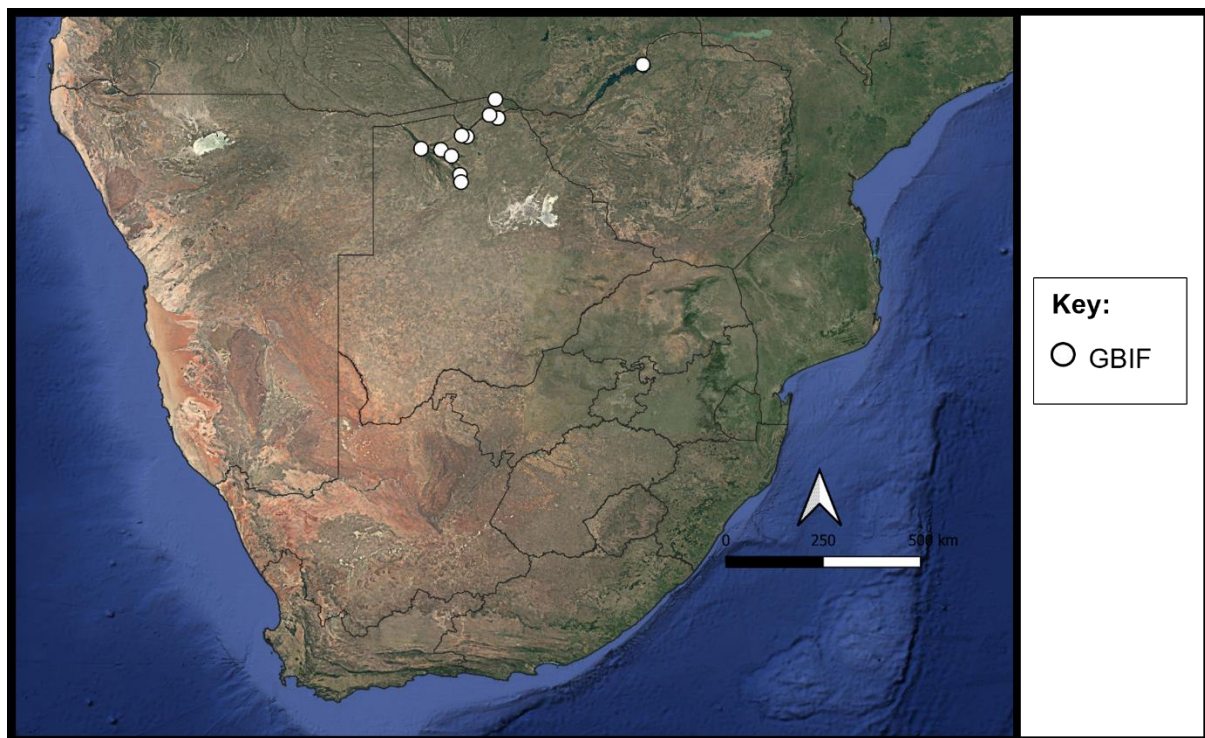


Figure 4.10 Southern Africa distribution of *Mutela zambesiensis* Mandahl-Barth, 1988 (white dots) as recorded by GBIF (white dots).

Size: The species can range from 11 cm to 8.5 cm in length and 4 cm to 3.2 cm in width (Appleton, 2002; Mandahl-Barth, 1988).

GBIF: 14 occurrences (Figure 4.9).

Photos: When searching for *Mutela zambesiensis*, three images were found on GBIF and 12 on the MUSSELP database.

Descriptive notes: The elongate shell is green/brown in colour, especially in young bivalves when the colour is sometimes iridescent. The shell is thin compared to the other species. The anterior end is broadly rounded, but the posterior end is more pointed. The dorsal margin is straight but sometimes can be sloped slightly with obscure angles towards the anterior and posterior margin. When the size increases of this species, a more sloped dorsal margin can be observed which means that the highest height of the shell will be more towards the posterior end. The inside is usually a pinkish/blueish colour, whereas the periostracum (outer layer of the shell) can range between a lighter to darker brownish colour (see Figure 4.10).

Remarks: Their larvae develop in brood sacs only in the inner pair of the demibranchs (v-shaped gills). This species is of Least Concern based on the IUCN Red List and the population trend is unknown (Kristensen and Stensgaard, 2010c).

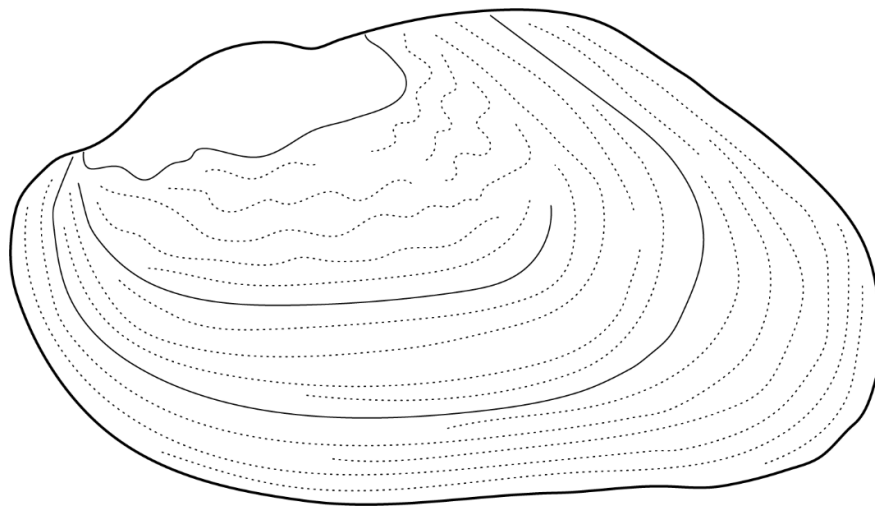


Figure 4.11 Redrawn image of *Mutela zambesiensis* Mandahl-Barth, 1988 based on an illustration done by Mandahl-Barth (1988).

4.3.2 Morphometrics

A vernier calliper was used to measure the width and length of each bivalve. For the length, the calliper was placed at the utmost ends from posterior to anterior, and for width, the measurement started at the top of the umbo and went straight down to where the shell ended (Table 4.5). Seven iridinid bivalves were found at Klein Letaba and all were measured, but there was only the one from Vaal River.

Table 4.5 The average width and length as well as the minimum and maximum width and length of iridinid bivalves at each site where they were found (SE = standard error (length = 2.5 cm, width = 1.7 cm); - = only one bivalve)

Sites	Length (cm) ± SE	Length range (min – max)	Width (cm) ± SE	Width range (min – max)
Klein Letaba River	5.5	4.4 – 7.8	2.1	1.5 – 3.2
Vaal River	12.5	-	7.1	-

4.3.3 Phylogenetic results

Four new sequences were generated from this study (one COI and three 16S). Based on Figure 4.11 and Figure 4.12 all the sequences matched with the same species, i.e. *Chambardia wahlbergi*.

For partial COI analysis, eight sequences were selected from GenBank (Table 4.3) to form an alignment with the sequence from the Vaal River with a length of 490 base pairs. Interestingly, all the sequences obtained from GenBank had a length of 490 base pairs, and the novel sequence had a length of 489 base pairs.

For partial 16S analysis, four sequences were selected from GenBank (Table 4.3) to form an alignment with the three novel sequences from Klein Letaba that had a length of 541 base pairs. The sequences from GenBank were generally shorter than the novel sequences, but there were no major differences. The sequence lengths ranged from 482 base pairs to 532 base pairs.

For the 28S gene, the samples were unsuccessful in producing sequences because they did not form contigs and subsequently could not align with other sequences. These were the same issues that were encountered with the Sphaeriidae in Chapter 3 under section 3.3.3 where the forward sequences did not align with the reverse sequences.

4.3.3.1 Phylogenetics and species diversity

Phylogenetic trees for the 16S and COI genes were constructed to illustrate the relationship between the novel sequences and the GenBank sequences (Figures 4.11 and 4.12). Base pair difference and percentage similarity tables were also compiled to provide a more comprehensive explanation of why these sequences grouped as they did (Tables 4.6 and 4.7). The other genera that occur in South Africa (*Mutela* and *Aspatharia*) are included with

Chambardia in the trees to indicate the relationship between the three genera and to determine the position of the novel sequence relative to the other species.

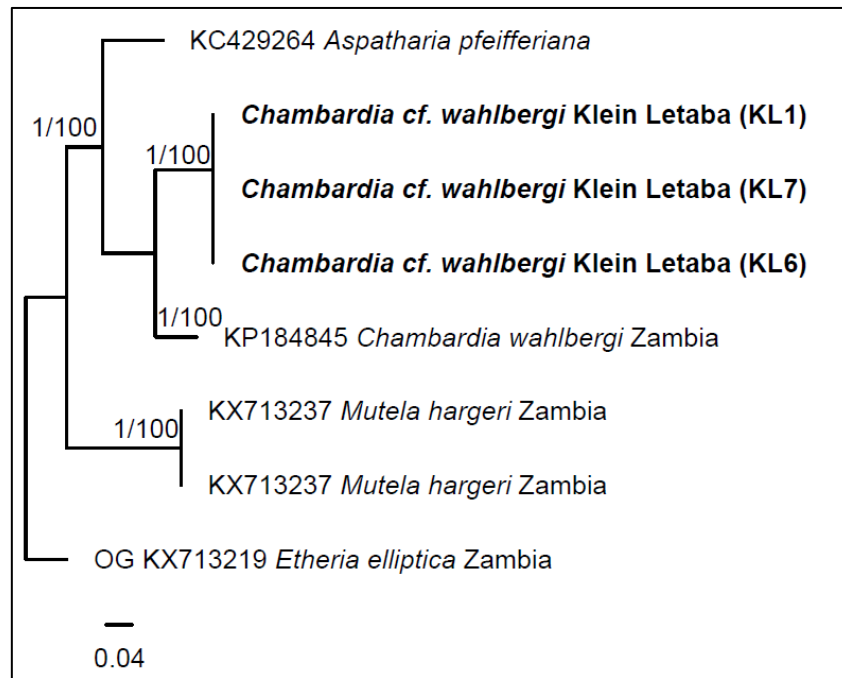


Figure 4.12 A 16S phylogenetic tree containing three novel sequences from Klein Letaba River (bolded), five sequences from GenBank and their Bayesian inference and maximum likelihood values.

Based on the strong Bayesian inference and maximum likelihood support (1/100), the novel sequences matched the best with *Chambardia wahlbergi*. Bayesian inference values below 0.7, as well as maximum likelihood values below 70 were excluded. As 16S is a conservative gene, only the genus name can be confirmed with this gene. It can also be seen that the three genera discussed in this chapter each form their own clade, with *Chambardia* being closer related to *Aspatharia* than with *Mutela*, as *Chambardia* and *Aspatharia* are sister taxa. For base pair differences and percentage similarity, Table 4.6 can be observed in conjunction with this tree to confirm the results.

Table 4.6 Base pair differences (below the diagonal) and percentage similarity (above the diagonal) values for representatives of the 16S gene novel sequences and GenBank sequences for iridinids.

		1	2	3	4	5	6	7
1	KL1 Klein Letaba		100	100	81	87	75	75
2	KL6 Klein Letaba	0		100	81	87	75	75
3	KL7 Klein Letaba	1	1		81	87	75	75
4	KC429264 <i>Aspatharia pfeifferiana</i>	100	100	100		82	78	78
5	KP184845 <i>Chambardia wahlbergi</i> Zambia	66	66	66	87		78	75
6	KX713237 <i>Mutela hargeri</i> Zambia	129	129	129	109	111		76
7	OG KX713219 <i>Etheria elliptica</i> Zambia	129	129	129	109	124	121	

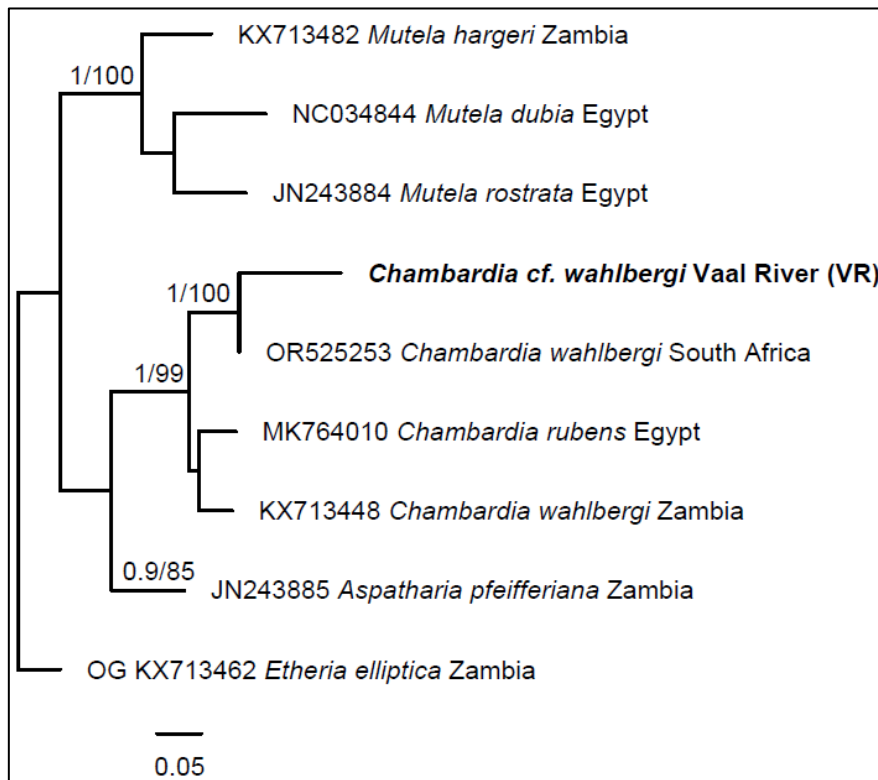


Figure 4.13 A COI phylogenetic tree containing one novel sequence from the Vaal River (bolded), and eight sequences from GenBank.

Based on the strong Bayesian inference and maximum likelihood values (1/100) of the branch containing the novel sequence, the bivalve found in this study can be phylogenetically identified as *Chambardia wahlbergi*. It is worth noting that the branch containing the novel sequence is longer than the *C. wahlbergi* from GenBank, indicating a slight difference in the base pairs which can be seen in Table 4.7.

Table 4.7 Base pair differences (below the diagonal) and percentage similarity (above the diagonal) values for representatives of the COI gene novel sequences and GenBank sequences for iridinids.

		1	2	3	4	5	6	7	8	9
1	VR Vaal River		76	76	77	79	84	92	85	75
2	JN243884 <i>Mutela rostrata</i> Egypt	117		88	88	83	84	82	83	81
3	NC_034844 <i>Mutela dubia</i> Egypt	117	61		87	83	83	82	83	81
4	KX713482 <i>Mutela harger</i> i Zambia	114	60	66		84	84	83	82	82
5	JN243885 <i>Aspatharia pfeifferiana</i> Zambia	102	81	82	79		87	87	88	83
6	KX713448 <i>Chambardia wahlbergi</i> Zambia	78	81	86	79	62		92	93	82
7	OR525253 <i>Chambardia wahlbergi</i> South Africa	42	87	86	83	65	40		92	82
8	MK764010 <i>Chambardia rubens</i> Egypt	75	81	85	88	61	33	38		82
9	OG KX713462 <i>Etheria elliptica</i>	125	95	96	89	85	89	89	90	

4.3.4 Distribution of bivalves found based on ecological parameters

In Chapter 2, the distribution of all the sample sites were portrayed on maps containing the following data: Level 1 ecoregions, mean annual rainfall, and elevation. Figures 4.14 –4.16 portray where iridinids were found based on these parameters.

Chambardia wahlbergi was found at a high and mid-elevation site (Figure 4.14), in the Highveld and the Lowveld ecoregion (Figure 4.15), and in the region of South Africa where there is a mean annual rainfall of 401 – 600 mm (Figure (4.16).

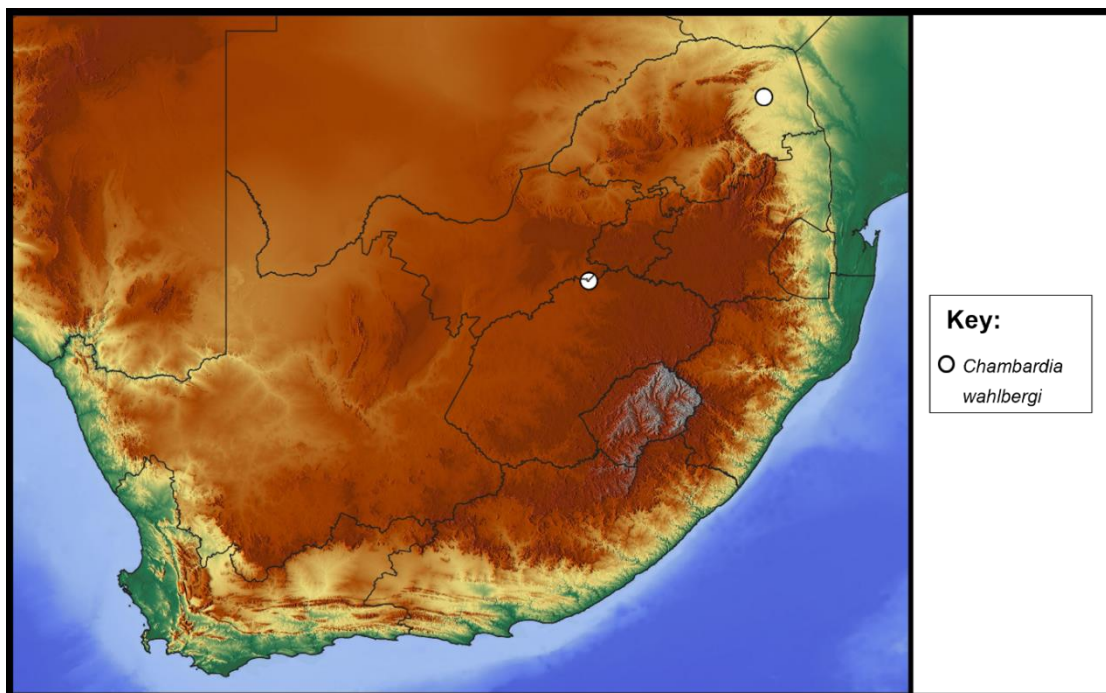


Figure 4.14 South African map indicating at which altitudes the *Chambardia wahlbergi* (Krauss, 1848) were found during this study.

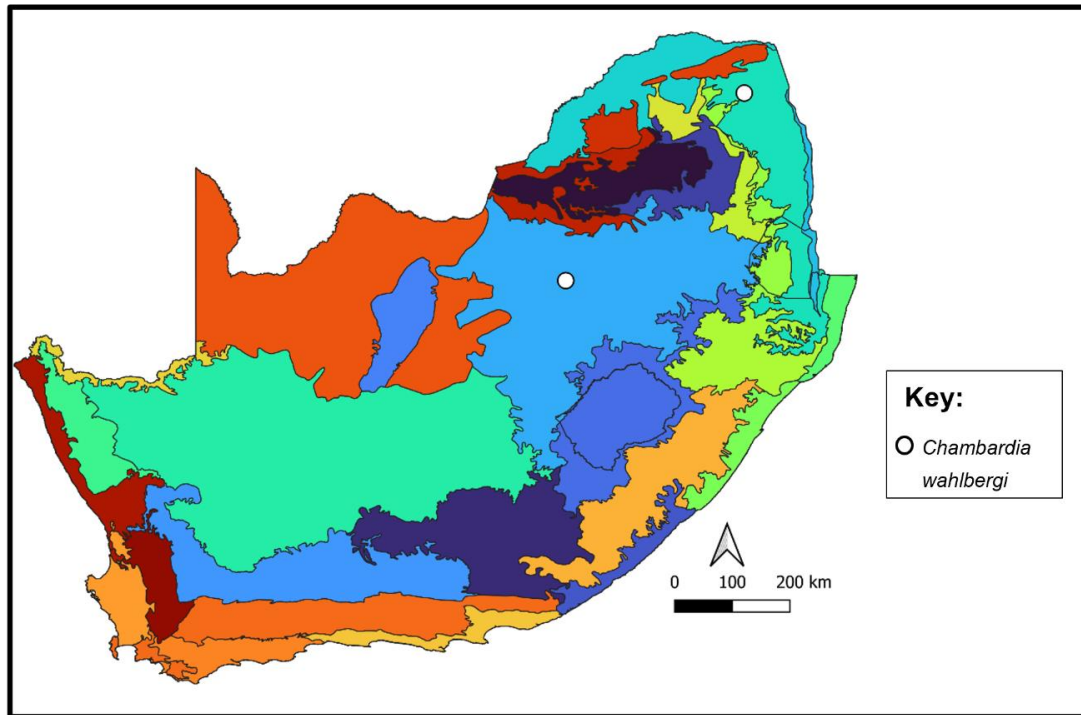


Figure 4.15 South African map indicating the ecoregions *Chambardia wahlbergi* (Krauss, 1848) was found during this project.

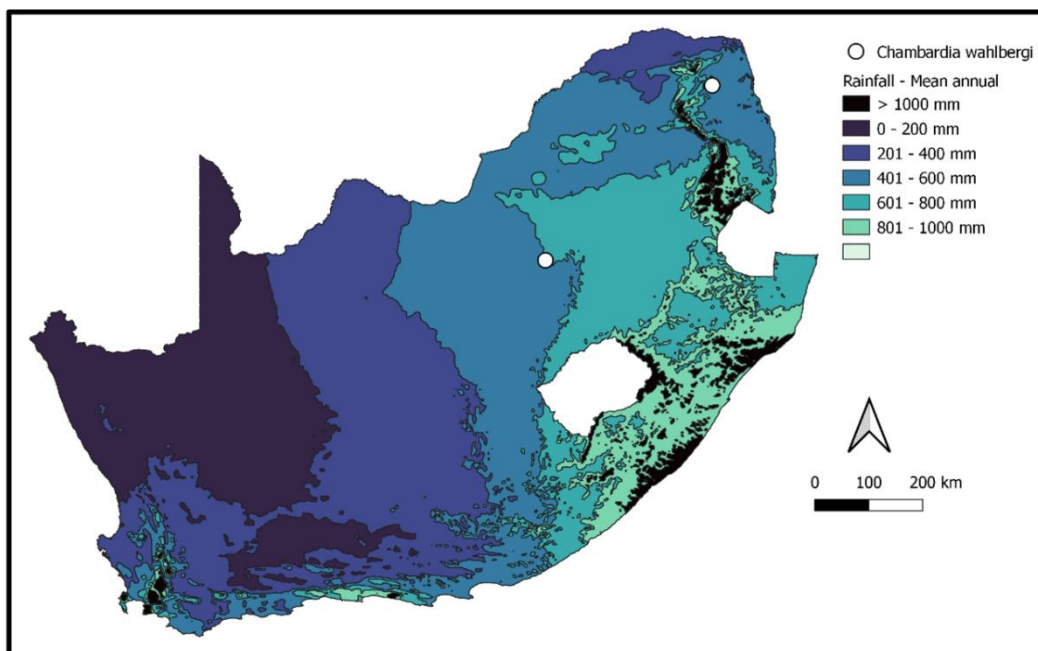


Figure 4.16 South African map of the mean annual rainfall indicating where the *Chambardia wahlbergi* (Krauss, 1848) were found in this project.

4.4 Discussion

Seven iridiniid bivalve specimens were found in the Klein Letaba River and one specimen was found in the Vaal River. In some other sites (Hartbeespoort Dam), only shells were found, but no live specimens. It is unknown why these bivalves' numbers keep decreasing, as mentioned in De Kock et al. (2016), but it was evident in this study as they were only found in two out of the 78 sites sampled. It could be due to persistent droughts (De Kock et al. 2017), as most shells were found on the dry banks of waterbodies, usually buried halfway in the sediment. It is also noteworthy that the Klein Letaba site was very close to a sand mining operation (Mhlongo et al., 2018), although this didn't seem to affect the mollusc diversity as many other mollusc species, likely more resilient, were found.

The 16S phylogenetic tree (Figure 4.12) and the base pair differences/percentage similarity (Table 4.7) indicate the relationship between the novel sequences and the GenBank sequences, confirming that the novel sequences belong to the genus *Chambardia*. Although they did group within the same clade as *Chambardia wahlbergi*, the 16S gene is considered conservative and therefore it cannot be interpreted in isolation and only the genus can be confirmed. The three genera that occur in southern Africa (*Aspatharia*, *Chambardia*, and *Mutela*) are all represented in Figure 4.11 to illustrate the relationship between them and to see where the novel 16S sequences fall. *Aspatharia pfeifferiana* is a closer relative to the *Chambardia* clade than it is to the *Mutela* clade, indicating that *Chambardia* and *Aspatharia* are more closely related to one another than *Chambardia* and *Mutela*. This can also be seen in the base pair differences/percentage similarities table (Table 4.7) where there are 87 bp differences (82% similar) between *Chambardia* and *Aspatharia*, but 111 base pair differences (79 % similar) between *Chambardia* and *Mutela*. Concerning the novel sequences, they had 66 base pair differences (87% similar) with the *Chambardia wahlbergi* sequence from GenBank. This is further supported that the novel sequences belong to the genus *Chambardia*. There is one bp difference between the novel sequence KL7, and the other two (KL1 and KL6) (see Table 4.7) which could indicate inter-species variation. There is not much information about the *Chambardia* morphology in the literature, but the iridiniids in this study matched the description of Appleton (2002) and Mandahl-Barth (1988). The images in Figure x of the bivalves found in Klein Letaba correlates with that of the GBIF images of *C. wahlbergi*. This is further supported by GBIF also having documented occurrences of this species in that area.

When looking at the COI phylogenetic tree (Figure 4.12), it can be deduced that the novel sequence (VR Vaal River) in bold is very closely related to *Chambardia wahlbergi*, with the maximum likelihood and Bayesian inference values (1/100) indicating high support for the

clade. However, the branch containing the novel sequence is more extended than the one from GenBank, suggesting that there are differences and that these two sequences are not identical. This can be clarified when looking at Table 4.8 indicating the base pair differences and percentage similarity between the COI sequences included in this chapter. Here it can be seen that there are 42 base pair differences (92% similar) between the novel sequence and the sequence it claded with (OR25253 *C. wahlbergi*). It is noteworthy that there are 40 base pair differences (92% similar) between the latter sequence from GenBank and another sequence of the same species (KX713448 *C. wahlbergi*). The base pair differences between the novel sequence and the sequence it clades with could be attributed to inter-species variation. Salvi et al. (2014) stated that the interspecies differentiation using the COI gene in large bivalves can reach a threshold of up to 12,45%, further supporting this. It is also noted that the KX713448 *C. wahlbergi* from Zambia only has 33 base pair differences with MK764010 *Chambardia rubens* from Egypt and are 93% similar. So, contrary to the previous statement of inter-species variation, this indicates that there could be less than 40 base pair differences between different species, or *C. rubens* was misidentified and is actually *C. wahlbergi*. In this instance, more sequences or photographs of the *C. rubens* specimens are needed to confirm this. This contradictory information highlights the importance of accurate identifications and linking these identifications to good sequences in this family.

Chambardia wahlbergi is classified as 'least concern' according to the IUCN (Seddon et al., 2011), but De Kock and Wolmarans (2010; 2012) indicated concern based on the fact they did not find this species in previously known localities, and if they were found, it was in fewer numbers compared to previous data. Instead, they were found in a new locality (Vaal River) which was proof that this species had the ability to disperse to higher altitude locations, instead of just east-flowing, warmer areas (Connolly, 1939; Jubb, 1976). Alternatively, the possibility exists that they have always occurred there but were not found in previous studies. During this present study, one iridinid was also found in the Vaal River which correlates with the results of De Kock et al. (2016) where they mention that this species was found in the Vaal River in 2007. *Chambardia wahlbergi* found in this study were found at one high-altitude site in the Highveld and one mid-elevation site in the Lowveld, with multiple shells being found in the Schoonspruit near Klerksdorp in the North West (De Kock et al., 2016). Multiple closed shells were also found during the course of this study at localities such as the Hartbeespoort dam in the North West which is at a higher altitude. Although adult freshwater mussels lack any kind of dispersal, larval stages are well adapted to disperse as they attach to fish in a parasitic life stage (Jubb, 1976). It is not surprising that they were found in the Vaal River and the Schoonspruit, as their tributaries intersect. However, the Mooi River catchment, which is also

a tributary of the Vaal River, has no reported occurrences of this species (Wolmarans et al., 2015).

According to De Kock and Wolmarans (2016), the sizes of the collected *C. wahlbergi* were between 6.7 to 14.9 cm. The range in this study is slightly smaller, ranging from 4.4 to 12.5 cm. However, this is still a good representation as the sample size in this study is likely much smaller than what De Kock et al. (2016) found. They also mention that it can be assumed from their growth lines that the biggest specimen found was approximately 10 years old. From this, we can deduce that the largest specimen found in this study could be around 7 to 8 years old. This is further supported by the fact that this shell from the Vaal River is quite heavily weathered.

It is logical that only *Chambardia* was found in this study when considering the previous coordinates where species of this family were found. For example, there have been no records in GBIF and the NWU Snail Collection of *Aspatharia pfeifferiana* or *Mutela zambesiensis* occurring in South Africa and thus the absence of these bivalves is not unsurprising. With that being said it is noteworthy that the known distribution of *C. wahlbergi* and *C. petersi* are very similar and the possibility exists that there have been misidentifications over the years since there is no substantial morphological evidence that sets the two species apart. Additionally, there were only sequences available for *C. wahlbergi* and not for *C. petersi*. This further supports that these might be the same species and needs further studies for clarification.

4.5 Conclusion

The outcome of this chapter was three novel 16S sequences belonging to the genus *Chambardia*. These specimens morphologically resembled *C. wahlbergi*. Additionally, there was one novel COI sequence that aligned with *C. wahlbergi* and was morphologically characterised as this species. No specimens of *C. petersi* were collected in this study based on both the morphology and phylogenetic analyses, however, it has been reported by GBIF and the NWU Snail Collection. The possibility exists that there has been misidentification of *C. wahlbergi* and *C. petersi* over the years, as they have related distribution records and are morphologically similar, and that they represent one species instead of two. The population numbers for this family were also noted to have decreased over the years, according to literature and the results of this study. In conclusion, the aim for this chapter was achieved as the family Iridinidae was revised and an updated distribution of the southern Africa species in

this family was completed. It is also clear that this family needs further revision since the present study has highlighted some issues that need to be addressed.

CHAPTER 5 AN OVERVIEW OF FRESHWATER BIVALVES IN THE FAMILY UNIONIDAE IN SOUTH AFRICA

5.1 Introduction

Within the class Bivalvia, the Unionidae Rafinesque, 1820 are a varied and important family from an ecological standpoint. With more than 700 species worldwide, this family is essential to freshwater environments because of its filter-feeding practices that improve water quality and aid in the cycling of nutrients (Vaughn and Hakenkamp, 2001; Strayer, 2008). Unionids can be found in a variety of freshwater environments, such as rivers, streams, and lakes (Graf and Cummings, 2010).

The mussel shells are typically olive-brown with ambiguous streaks on the exterior, while the interior is described as “brilliant”. Occasionally, the bivalves exhibit a zig-zag pattern on the posterior dorsal side, and they possess noticeable hinge teeth. Pearls have been found in specimens of this genus (Appleton, 2002). Baird (1840) also mentions that while their form may vary, they consistently have two cardinal teeth and anterior lateral teeth. Rhoads and Pannella (1970) suggest that differences in shell morphology contribute to a higher survival rate during flooding, while Haas (1969b) argues that these variations play a key role in ensuring the successful fertilisation of future unionids. Some of the morphological characteristics have remained consistent over time, with Baird (1840) noting that their umbones are typically damaged due to friction from the sediment in which the bivalve resides and are positioned anterior to the median vertical line (Appleton, 2002).

The family Unionidae has a distinct life cycle that is distinguished by the parasitic glochidia larval stage that clings to the fins or gills of fish hosts. Their reproductive mode is viviparous, similar to that of iridiniids, with eggs developing in chambers formed by the demibranchs, and their larvae are parasitic on fish (Appleton, 2002). Reproduction is entirely random, as the male does not select a specific female to fertilise, and the parasitic larval stage must find a suitable fish host (Mandahl-Barth, 1988; Lopes-Lima et al., 2021). Although this intricate reproductive strategy guarantees the genetic variety and spread of mussel populations, it also leaves them susceptible to changes in the environment and loss of habitat (Howard & Cuffey, 2006). Many species of freshwater mussels are vulnerable or endangered owing to habitat degradation, pollution, and invasive species, making the decrease of these mussels a critical conservation issue (Ricciardi and Rasmussen, 1999).

The significance of Unionidae in preserving biodiversity and ecological balance in freshwater environments has been emphasised by recent studies (Lopes-Lima et al., 2020; Ishii et al., 2024; de Moulpied et al., 2024). The filtration function of mussels enhances the cleanliness of the water and promotes the growth of aquatic plants, both of which benefit other animals (Vaughn et al., 2008). Moreover, the reduction in mussel populations is a sign of more general ecological problems that impact freshwater ecosystems (Lydeard et al., 2004). This decrease in population size could be due to the invasive *Corbicula* clam (Sczarmach et al., 2024) where it was found that unionids in Europe avoided sand that was modified by *Corbicula* and burrowed less in sand with living *Corbicula*.

An example of the important role they play was shown by Aldridge (1998) where it was demonstrated that unionid mussels contribute over 90% of the energy content of the fauna inhabiting the riverbed. The most likely results in better water quality as unionids play an important role in filtering water and removing particulate matter from the water column. Considering this, it is worrying that their numbers are declining not only in South Africa but worldwide (Aldridge, 1998). Furthermore, Randklev et al. (2019) explained that mussels can form communities known as mussel patches, which thrive under conditions where the water is deep enough to keep the sediment stable in high flow, while also protecting from temperature fluctuations when the water level decreases.

Unionids are among the most threatened freshwater taxa, but unfortunately, their conservation is delayed because of the lack of taxonomic structure and ecological data (Lopes-Lima et al., 2017). The family was first classified based on the marsupium, anatomy, larvae type and umbo sculpture by Lea (1836, 1838, 1852, 1870) and Simpson (1900, 1914). It was later discovered that the shell characteristics were too unreliable as there were multiple convergences in shell morphology (Ortmann, 1910, 1911, 1912, 1919; Prashad, 1931). Haas (1969a, b) and Modell (1942, 1949, 1964) compiled classification systems based on shell morphology and anatomical characters, although their variable conchological characteristics caused incoherent identifications. Despite this, the classification system by Haas (1969a) is widely recognised as a more dependable source to identify species.

It was originally thought that unionids were primarily found in freshwaters (Baird, 1840). However, they are now known to inhabit waterbodies with a salinity of up to 3 psu (Mandahl-Barth, 1988; De Kock and Wolmarans, 2010). In 1840, they were found in large abundances (Baird, 1840), which is similar to what is mentioned in De Kock and Wolmarans (2010), however, their numbers are decreasing (De Kock and Wolmarans, 2010). They were also not previously known to occur in Africa, however, GBIF records and recent studies by Lopes-Lima

et al. (2016, 2021, 2024) indicate that the species is now quite widely distributed across the continent.

According to Appleton (2002), there should be four species present in southern Africa, namely: *Unio caffer* Krauss, 1848; *Coelatura framesi* (Connolly, 1925); *Coelatura kunenensis* (Mousson, 1888); and *Coelatura mossambicensis* (E. von Martens, 1860). Recently, *Coelatura framesi* was synonymised with *Coelatura mossambicensis* (Table 5.1), leaving only three known species in this region.

Thus, this chapter aimed to revise the unionid species (Table 5.1) that occur in South Africa and hopefully contribute additional morphological and phylogenetic information in order to start filling in the required knowledge gaps that will lead to the conservation of these species.

Table 5.1 All documented South African unionid species (Mollusca: Bivalvia) (n = 3), as well as their synonyms, presence and abundance of sequences and their category as per the IUCN red list.

Species name and their authority	Synonyms	Sequences	IUCN red list category
<i>Unio caffer</i> Krauss, 1848	• <i>Cafferia caffra</i> (Krauss, 1848) • <i>Cafferia caffra obesa</i> Connolly, 1925 • <i>Unio africanus</i> I. Lea, 1856 • <i>Unio charon</i> Preston, 1910 • <i>Unio diminutis</i> I. Lea, 1859 • <i>Unio diminutus</i> I. Lea, 1859 • <i>Unio mashonae</i> Preston, 1910 • <i>Unio silongweensis</i> Preston, 1912 • <i>Unio vaalensis</i> Chaper, 1885 • <i>Unio verreauianus</i> I. Lea, 1856 • <i>Unio verreuxianus</i> I. Lea, 1867 • <i>Unio vicinus</i> Preston, 1910 • <i>Unio zeledori</i> Dunker, 1866	• 35 COI sequences on GenBank	Least concern
<i>Coelatura kunenensis</i> (Mousson, 1888)	• <i>Nodularia (Caelatura) ellenbergeri</i> Germain, 1920 • <i>Unio kunenensis</i> Mousson, 1888 • <i>Unio zambesiensis</i> Preston, 1905	• 31 COI sequences on GenBank • Two 28S sequences on GenBank	Least concern
<i>Coelatura mossambicensis</i> (E. von Martens, 1860)	• <i>Coelatura framesi</i> (Connolly, 1925) • <i>Coelatura mossambicensis framesi</i> (Connolly, 1925) • <i>Indonaia framesi</i> Connolly, 1925 • <i>Indonaia mozambicensis</i> (E. von Martens, 1860)	• Three COI sequences on GenBank	Least concern

Species name and their authority	Synonyms	Sequences	IUCN red list category
	• <i>Unio borellii</i> Ancey, 1894 • <i>Unio framesi</i> (Connolly, 1925) • <i>Unio lechaptosi</i> Ancey, 1894 • <i>Unio lechaptosi</i> var. <i>minor</i> E. von Martens, 1897 • <i>Unio liederii</i> E. von Martens, 1897 • <i>Unio mossambicensis</i> E. von Martens, 1860 • <i>Unio shirensis</i> Preston, 1910		

5.2 Materials and methods

5.2.1 Collection

All 78 sites in Chapter 2 were targeted for the collection of unionids. These bivalves were collected by digging through the sediment of freshwater bodies with a snail sampler (Figure 3.1b) similar to the manner iridiniids were sampled in section 4.2.1. All specimens collected were placed into 96% ethanol for molecular analyses.

5.2.2 Morphological analysis

The main key that was used for the identification of these unionids was Appleton (2002). The collected bivalves were identified up to species level. The characteristics considered were the shape of the shell, umbo orientation, colour of the inner and outer shell, and the presence of teeth (Figure 1.5). As these specimens were too big to capture images on a dissection microscope, a regular phone camera (Samsung Galaxy A54) was used. A vernier calliper was used to measure the bivalve's width and length (Table 5.3). For length, the calliper was placed at the utmost ends from posterior to anterior, and for width, the measurement started at the top of the umbo and went straight down to where the shell ended (Table 5.3).

5.2.3 Molecular methods

The dissection method was similar to that mentioned in Chapter 4 under 4.2.2 for the iridiniids, where a piece of the foot was dissected off and placed in molecular-grade ethanol until further analysis. The extraction method was performed using Chelex 10% following the manufacturer's protocol as outlined in Chapter 3 under section 3.2.3. The targeted gene regions were COI, 16S, and 28S (see primers in Table 5.2 for each gene region). The volumes of each of the PCR reagents for the 16S and 28S genes were as follows: water = 7.5 μ L,

forward primer = 1 µL, reverse primer = 1 µL, Kapa Hifi HotStart ReadyMix = 12.5 µL. The volumes for the PCR reagents for COI were as follows: water = 7 µL water, forward primer = 1 µL, reverse primer = 1 µL, NEB OneTaq 2x MasterMix with standard buffer = 10 µL.

Table 5.2 Information of primers used for molecular analyses of unionids.

Genetic marker	Primer	Sequence	Reference	PCR conditions (below)
28S	1274F	5'-GACCCGTCTTGAAACACG GA-3'	Markmann and Tautz (2005)	Figure 5.1
	1276R	5'-CTAGTTGCTTCGGCAGGTGAG-3'	Møller et al. (2008)	
COI	LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	Folmer et al. (1994)	Figure 5.1
	HCO2198	5'-TAAACT TCA GGG TGA CCAAAAAT CA-3'		
16S (a)	EU-COI-F	GACGAGAAGACCCYATGGAGC	New for the present study	Figure 5.2
	EU-COI-R	CTGATCCAACATCGAGGTCGTAAACCC		
16S (b)	16Sar	5'-CGCCTGTTTAACAAAAACAT-3'	Hata et al. (2017)	Figure 5.3
	16Sbr	5'-CCGGTCTGAACTCAGATCATGT-3'		

Each of the different genes that were targeted followed different PCR protocols as seen in Table 5.2 and illustrated below.

For 28S and COI genes (Figure 5.1), the following conditions were followed: initial denaturation starts at 95°C for 1 minute. 7 cycles follow with a denaturation of 95°C for 30s and an annealing temperature lasting for 30s starting at 52°C and decreasing with each cycle with 1°C, to end at 46°C. An extension of 72°C for 30s form part of these 7 cycles. Then, 33 cycles of 95°C for 30s, 45°C for 30s, and 72°C for 30s. The final extension was at 72°C for 3min and it was held at 4°C till further analysis. Bößneck et al. (2016) was used as a guideline for this method, but it was tweaked a bit to suit the bivalves' characteristics (Figure 5.1).

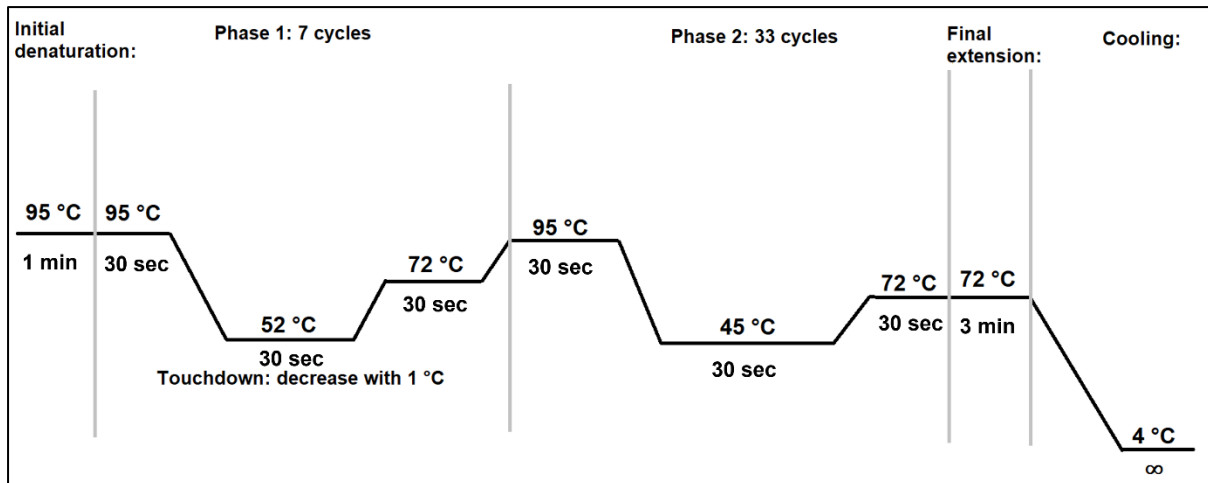


Figure 5.1 An illustration of the touch-down PCR used for the 28S and COI genes for unionids.

The second protocol Figure 5.2 was used for the 16S (a) gene and had an initial denaturation phase of 5 min at 94 °C. Thereafter, 35 cycles of 30 sec at 94 °C, 30 sec at 50 °C, and 1 min at 68 °C were run. A final extension of 10 min at 68 °C was completed whereafter the samples were kept at 4 °C until further analysis (new protocol designed for this study) (see Figure 5.2).

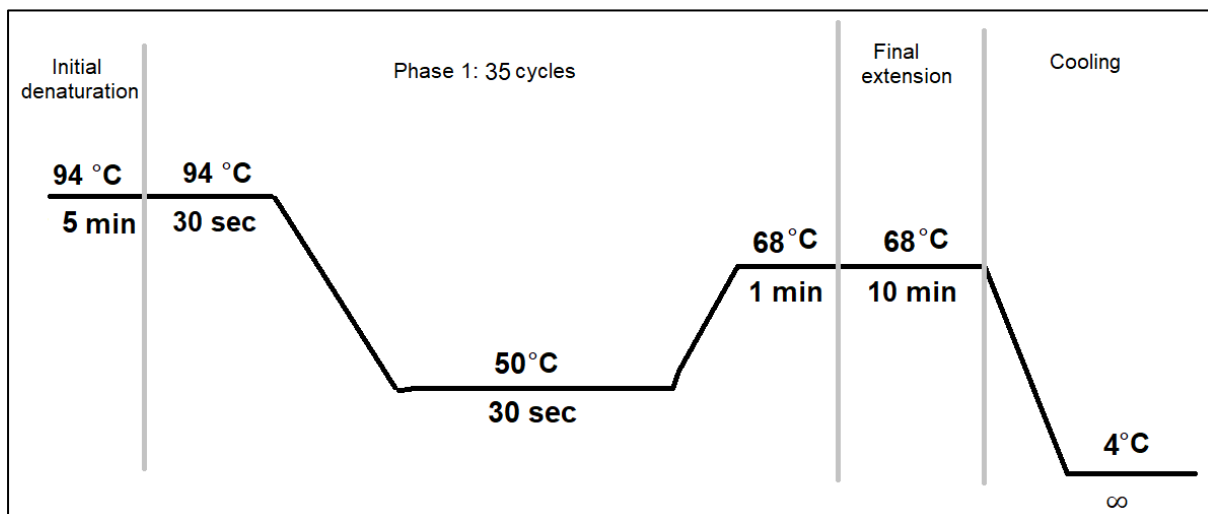


Figure 5.2 An illustration of the PCR protocol used for the COI gene for unionids.

This protocol was used for the 16S (b) gene and had an initial denaturation of 94 °C held for 10 min, followed by 30 cycles of 94 °C for 30 s, 48 °C for 1 min, and 72 °C for 1 min. The final extension was at 72 °C for 10 min, and it was held at 12 °C till further analysis (Hata et al., 2017) (see Figure 5.3).

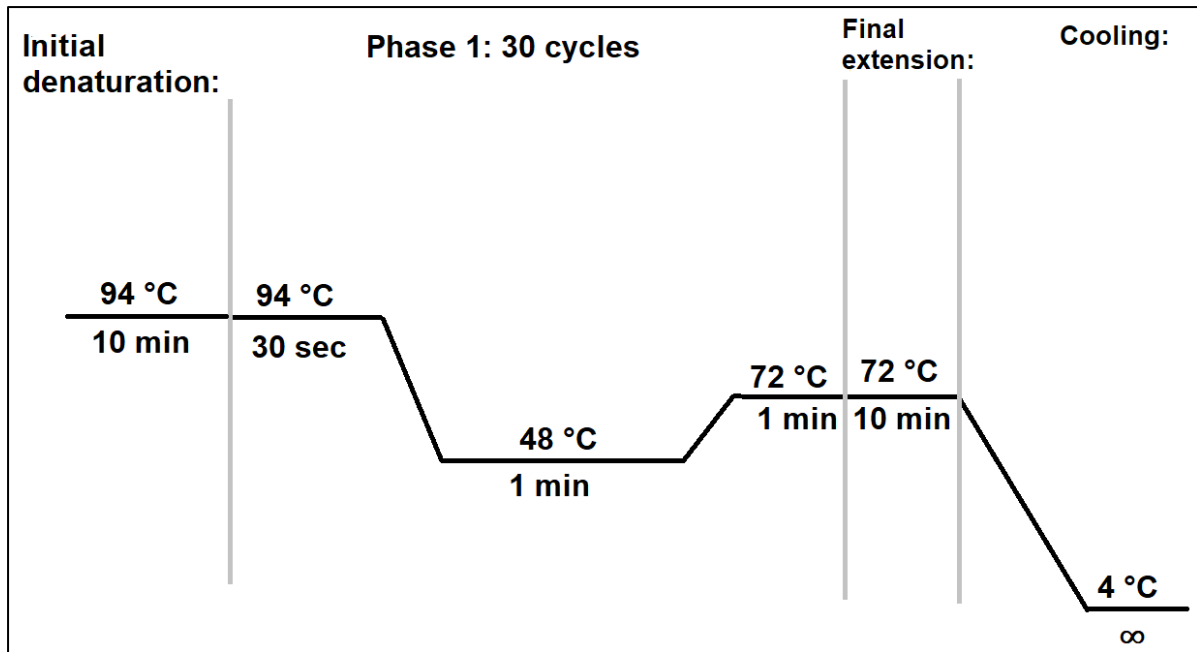


Figure 5.3 An illustration of the PCR protocol used for the 16S gene for unionids.

The PCR products were visualised with gel electrophoresis and successful samples were sequenced by Inqaba. The final sequences underwent the same process as discussed in Chapter 3 under 3.2.3 where the sequences were assembled in Geneious to form alignments with the sequences from GenBank (Table 5.3) and run through different software to obtain the tree files from CIPRES and edited in FigTree.

All relevant sequences included in the study were extracted on or before 31 October 2024 from GenBank. Sequences were selected if they had a country of origin, were published, and if their accepted species name was included i.e., sequences with *cf.* or *sp.* were excluded. Furthermore, normally only one species of each country was included; however, in some cases additional species from a country were included if it illustrated a higher degree of intraspecies variation, i.e. base pair differences. Additionally, unpublished sequences were added if it was the only sequence that was available for that species.

5.2.4 Map creation and coordinate plotting

The maps for this section were made using the QGIS software (Version 3.22) plotting GPS coordinates from GBIF, the NWU Snail Collection, and the current study. The same methods as in Chapter 3 under section 3.2.4 were followed.

Table 5.3 Sequences of the 16S gene region obtained from GenBank along with their sequence name, updated name and authority, accession number, reference for sequence, and country of origin. Outgroup highlighted in grey.

#Sequence name	#Updated name and authority	Accession number	Reference for sequence	Country of origin
<i>Unio tumidiformis</i>	<i>Unio tumidiformis</i> Castro, 1885	EF571344	Reis et al. (2013)	Portugal
<i>Unio bruguierianus</i>	<i>Unio bruguierianus</i> Bourguignat, 1853	KX400175	Araujo et al. (2017)	Greece
<i>Unio ravoisieri</i>	<i>Unio ravoisieri</i> Deshayes, 1848	KX400523	Araujo et al. (2017)	Tunisia
<i>Unio delphinus</i>	<i>Hyriopsis bialata</i> Simpson, 1900	EF571364	Reis et al. (2013)	Portugal
<i>Unio delphinus</i>	<i>Hyriopsis bialata</i> Simpson, 1900	EF571386	Reis et al. (2013)	Portugal
<i>Unio elongatulus</i>	<i>Unio elongatulus</i> Pfeiffer, 1825	KX400362	Araujo et al. (2017)	Italy
<i>Unio foucauldianus</i>	<i>Unio foucauldianus</i> Pallary, 1936	KX400388	Araujo et al. (2017)	Morocco
<i>Unio mancus</i>	<i>Unio mancus</i> Lamarck, 1819	EF635012	Reis et al., (2013)	Spain
<i>Unio mancus</i>	<i>Unio mancus</i> Lamarck, 1819	KX400479	Araujo et al. (2017)	France
<i>Unio mancus</i>	<i>Unio mancus</i> Lamarck, 1819	MF414416	Froufe et al. (2017)	Spain
<i>Unio mancus</i>	<i>Unio mancus</i> Lamarck, 1819	KX400425	Araujo et al. (2017)	Spain
<i>Unio pictorum</i>	<i>Unio pictorum</i> (Linnaeus, 1758)	KX400504	Araujo et al. (2017)	Greece
<i>Unio pictorum</i>	<i>Unio pictorum</i> (Linnaeus, 1758)	OQ564391	Unpublished	Croatia
<i>Unio pictorum</i>	<i>Unio pictorum</i> (Linnaeus, 1758)	MH544952	Abdrakhmanov et al. (2019)	Russia
<i>Unio eucirrus</i>	<i>Unio eucirrus</i> Bourguignat, 1857	MZ518888	Lopes-Lima et al. (2021)	Turkey
<i>Unio pictorum</i>	<i>Unio pictorum</i> (Linnaeus, 1758)	MZ519001	Lopes-Lima et al. (2021)	Turkey
<i>Unio tigridis</i>	<i>Unio tigridis</i> Bourguignat, 1852	KX400524	Araujo et al. (2017)	Israel
<i>Unio terminalis</i>	<i>Unio terminalis</i> Bourguignat, 1852	NC_085470	Unpublished	Israel

#Sequence name	#Updated name and authority	Accession number	Reference for sequence	Country of origin
<i>Unio terminalis</i>	<i>Unio terminalis</i> Bourguignat, 1852	MZ518847	Lopes-Lima et al. (2021)	Israel
<i>Unio delicatus</i>	<i>Unio delicatus</i> Lea, 1863	MZ519024	Lopes-Lima et al. (2021)	Turkey
<i>Unio pictorum</i>	<i>Unio pictorum</i> (Linnaeus, 1758)	MH544962	Abdrakhmanov et al. (2019)	Russia
<i>Unio tumidus</i>	<i>Unio tumidus</i> Philipsson, 1788	MH544963	Abdrakhmanov et al. (2019)	Russia
<i>Unio durieui</i>	<i>Unio durieui</i> Deshayes, 1847	KX400326	Araujo et al. (2017)	Tunisia
<i>Unio gibbus</i>	<i>Unio gibbus</i> Spengler, 1793	KX400390	Araujo et al. (2017)	Tunisia
<i>Corbicula largillierti</i> (outgroup)	<i>Corbicula largillierti</i> (Philippi, 1844)	AB522665	Unpublished	China

#Last updated 31 October 2024

5.3 Results

5.3.1 Morphological results

There are two genera in the family Unionidae from southern Africa: *Coelatura* and *Unio*. Within this area, there are only three known species from these genera, and they will be revised below.

Taxonomic results - *Coelatura*

Phylum Mollusca

Class Bivalvia

Order Unionida

Family Unionidae Rafinesque, 1820

Subfamily Parreysiinae

Tribe Coelaturini

Genus *Coelatura* Conrad, 1853

Notes: Originally, the bivalves in this genus were noted to have the following characteristics: thin elongated shell, corrugated or sub-nodular umbo, laminar, elongated cardinal teeth parallel to the anterior hinge margin, and slightly deflected pallial impressions (Conrad, 1853).

5.3.1.1 *Coelatura kunenensis* (Mousson, 1888)

Original name: *Unio kunenensis* Mousson, 1888.

Type locality: Namibia.

Distribution: Kunene, Okavango, upper Zambesi and Kafue River systems, Ekuma floodplain and Etosha Pan in Namibia (Appleton, 2002). According to GBIF (Figure 5.4), this species occurs only in southern Africa, specifically Botswana, Namibia, Zambia, and one occurrence in the KwaZulu-Natal province of South Africa. It was not found in this study.

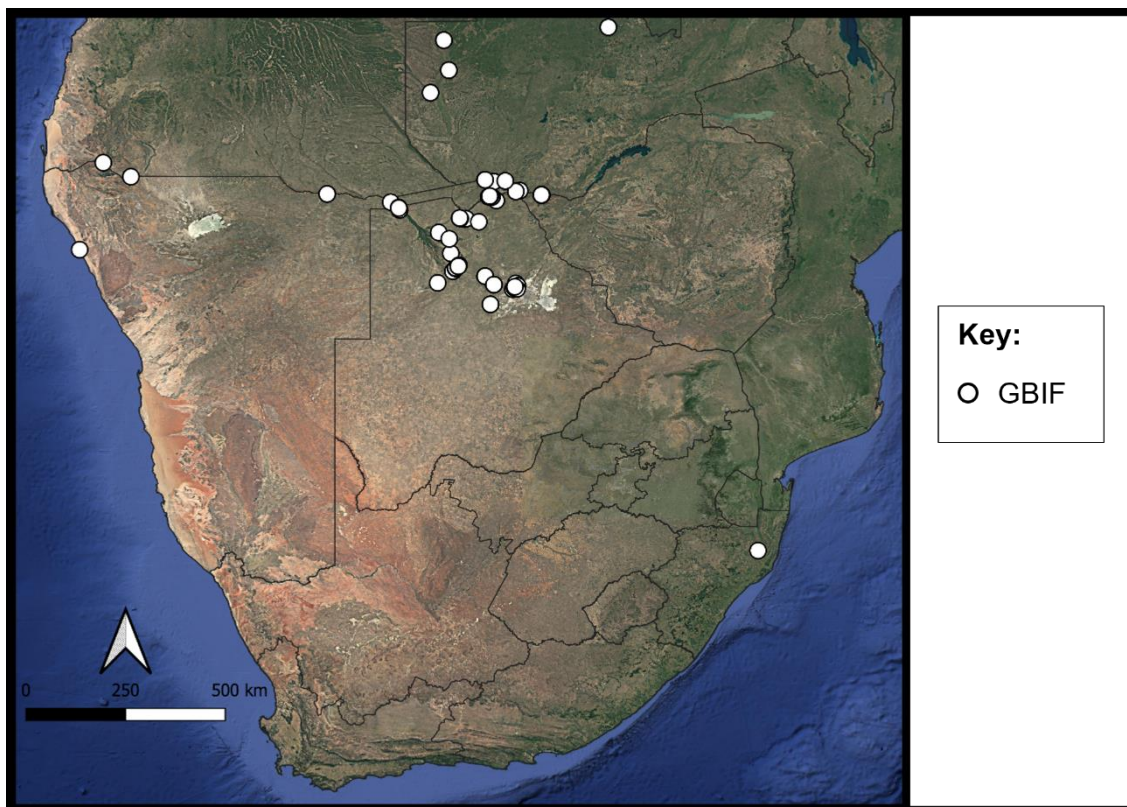


Figure 5.4 Southern African distribution of *Coelatura kunenensis* (Mousson, 1888) based on data points from GBIF (white dots).

Size: An approximate size according to Appleton (2002) is 49 x 33 x 23 mm.

GBIF: 144 occurrences.

Available sequences: There are 31 COI and two 28S sequences on GenBank.

Photos: When searching for *Coelatura kunenensis*, 20 photos were found on GBIF.

Descriptive notes: This is a variable species and can vary in size and colour (from a dark brown to a paler colour) (Figure 5.5). The glochidia (larval stage) do not have hooks.

Remarks: According to the IUCN Red List, this species is of least concern and its population trend is unknown (Kristensen et al., 2010).

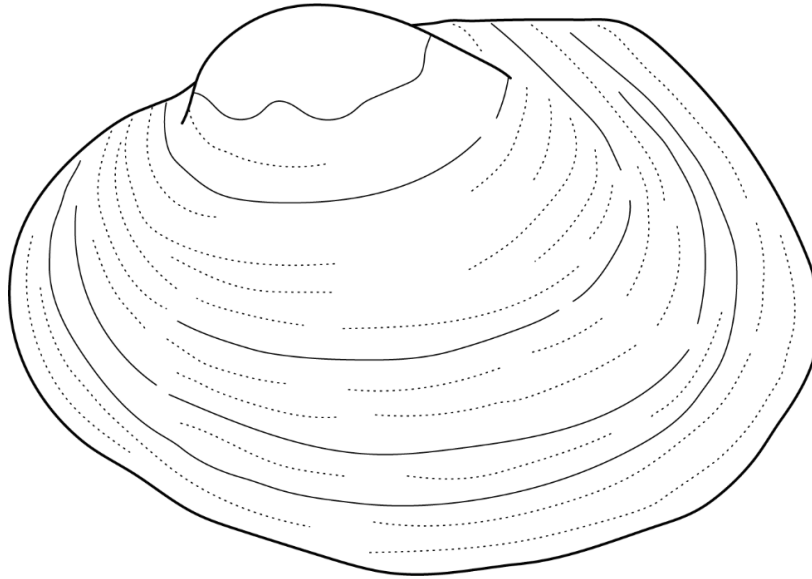


Figure 5.5 A redrawn image of *Coelatura kunenensis* (Mousson, 1888), based on the illustration by Mandahl-Barth (1988).

5.3.1.2 *Coelatura mossambicensis* (E. von Martens, 1860)

Original name: *Unio mossambicensis* E. von Martens, 1860

Type locality: Mozambique.

Distribution: Known from Lake Kariba and the lower Zambezi River to central Mozambique, southern Malawi and eastwards-flowing rivers in Zimbabwe (Appleton, 2002). *Coelatura mossambicensis* can also be found in the eastwards-flowing rivers of Mpumalanga and Limpopo and the Phongolo River floodplain in north-eastern KwaZulu-Natal. According to GBIF, this species only occurs in Southern Africa, in Botswana, Zimbabwe, Zambia, Malawi, Tanzania, and one report in the Mpumalanga province of South Africa (Figure 5.6). It was not found during this study.

Size: According to Appleton (2002), they can grow up to 50 x 30 x 20 mm. This specimen was not examined as part of this study.

GBIF: 36 occurrences.

Photos: While searching for *Coelatura mossambicensis*, three images were found on GBIF.

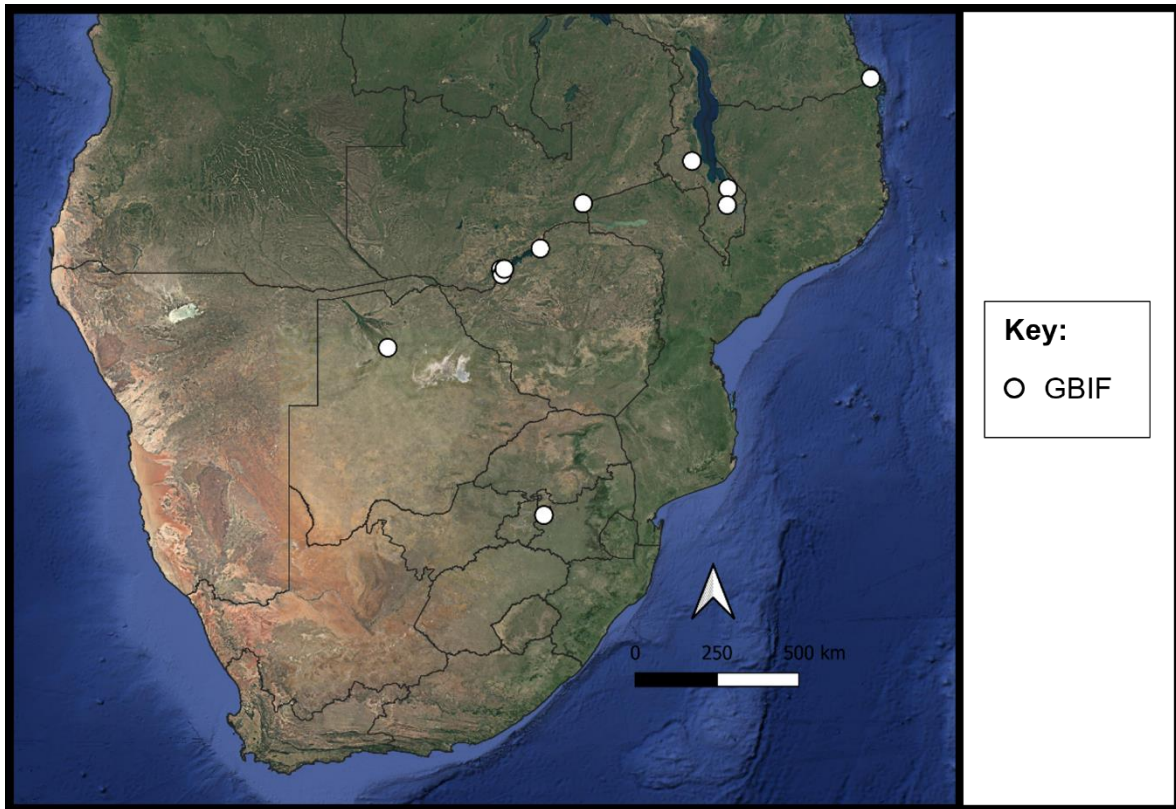


Figure 5.6 Southern African distribution of *Coelatura mossambicensis* (E. von Martens, 1860) based on data points from GBIF (white dots).

Descriptive notes: Their glochidia are without hooks. It is more compressed than the previous species (Figure 5.7).

Remarks: According to the IUCN, this species is of least concern and their population trend is unknown (Van Damme et al., 2018).

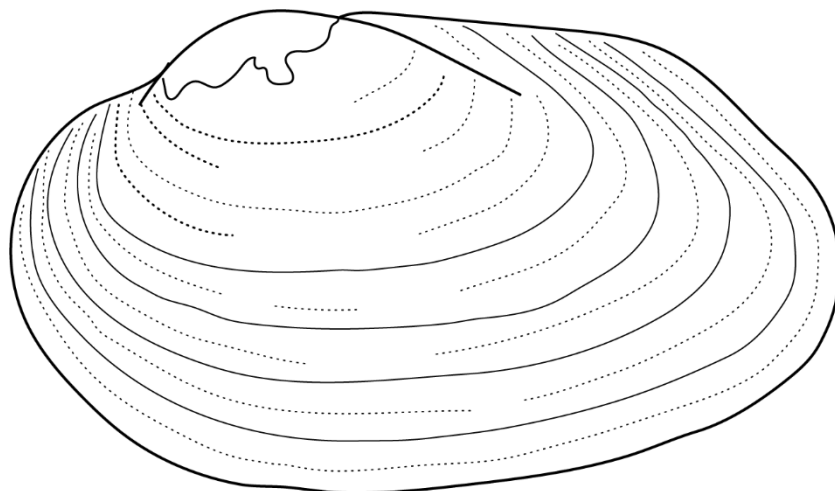


Figure 5.7 A redrawn image of *Coelatura mossambicensis* (E. von Martens, 1860), based on the illustration by Mandahl-Barth (1988).

Taxonomic results – *Unio*

Phylum Mollusca

Class Bivalvia

Order Unionida

Family Unionidae Rafinesque, 1820

Subfamily Unioninae

Tribe Unionini

Genus *Unio* Philipsson, 1788

Notes: The body is laterally flattened. Cardinal teeth rather thin, not forming a distinct angle to the hinge plate. Lateral teeth normally developed.

5.3.1.3 *Unio caffer* (Krauss, 1848)

Original name: *Unio caffer* Krauss, 1848.

Type locality: South Africa (Kwazulu-Natal) (Appleton, 2002).

Distribution: Based on Appleton (2002), they are endemic and widely distributed to southern Africa. They used to be known from rivers in the Berg, Breede, and Kruis rivers, but they have become increasingly rare in south-western Cape. According to GBIF, they have been found in Namibia, Botswana, Zimbabwe, Zambia, Malawi, and across South Africa congregating more towards the Eastern Cape. Based on the NWU Snail Collection, it has been found more toward the Northern provinces of South Africa. Based on the current study, it was found in Mpumalanga in the X-spruit (Figure 5.8).

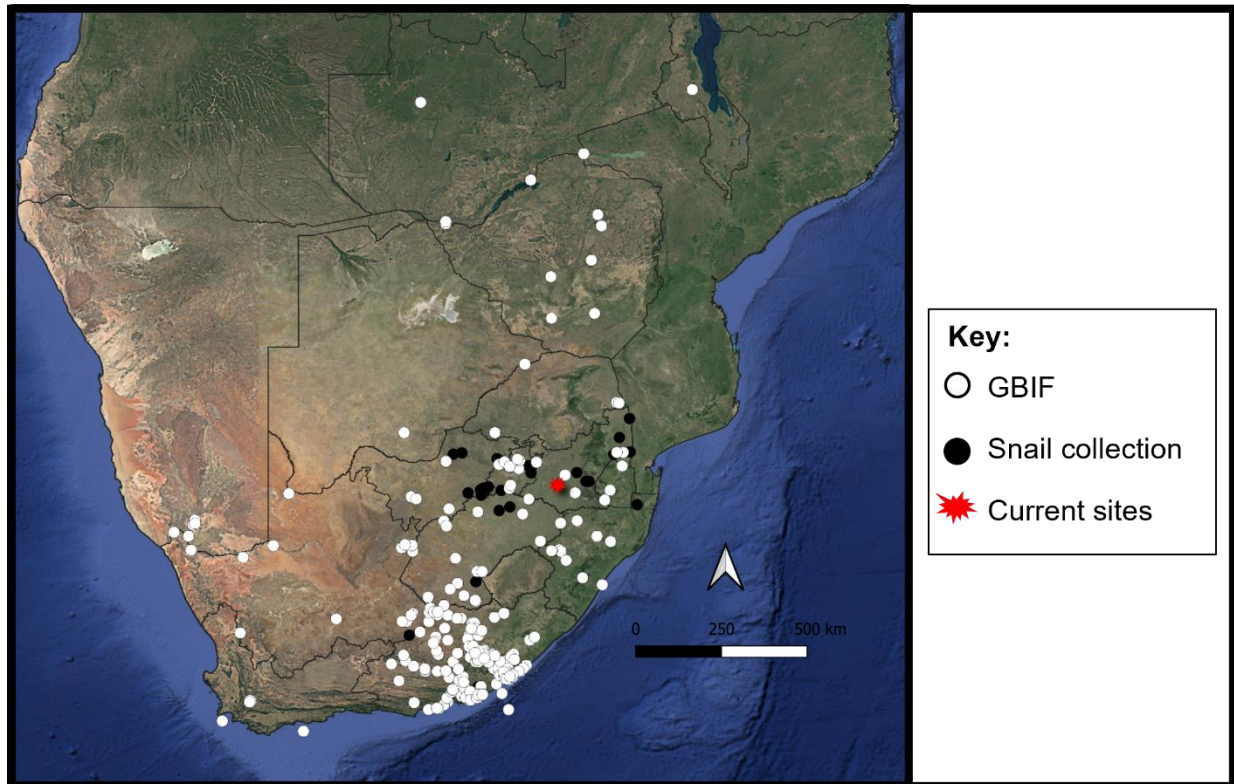


Figure 5.8 Southern African distribution of *Unio caffer* (Krauss, 1848) based on data points from GBIF (white dots) and georeferenced data points from the NWU Snail Collection (black dots) and coordinates from the current study (red star).

Size: According to Appleton (2002), they can grow up to 100 x 45 mm.

GBIF: 562 occurrences.

Photos: When searching for *Unio caffer*, 23 images were found on GBIF.

Descriptive notes: Their posterior end can be angular and varied in their outline. A smooth cardinal tooth can be found on the hinge, together with one anterior and two posterior lateral teeth. Differing from the preceding species, their glochidia have hooks (Appleton, 2002) (see Figure 5.9).

Remarks: Usually, they can be found in seasonal and perennial rivers but are also known to inhabit dams and small lakes (Appleton, 2002). They have been known to be tolerant of brackish water of up to salinities of 3 psu. Pearls have also been found in this species. According to the IUCN Red List, this species is of least concern and their population trend is unknown (Kristensen et al., 2017).

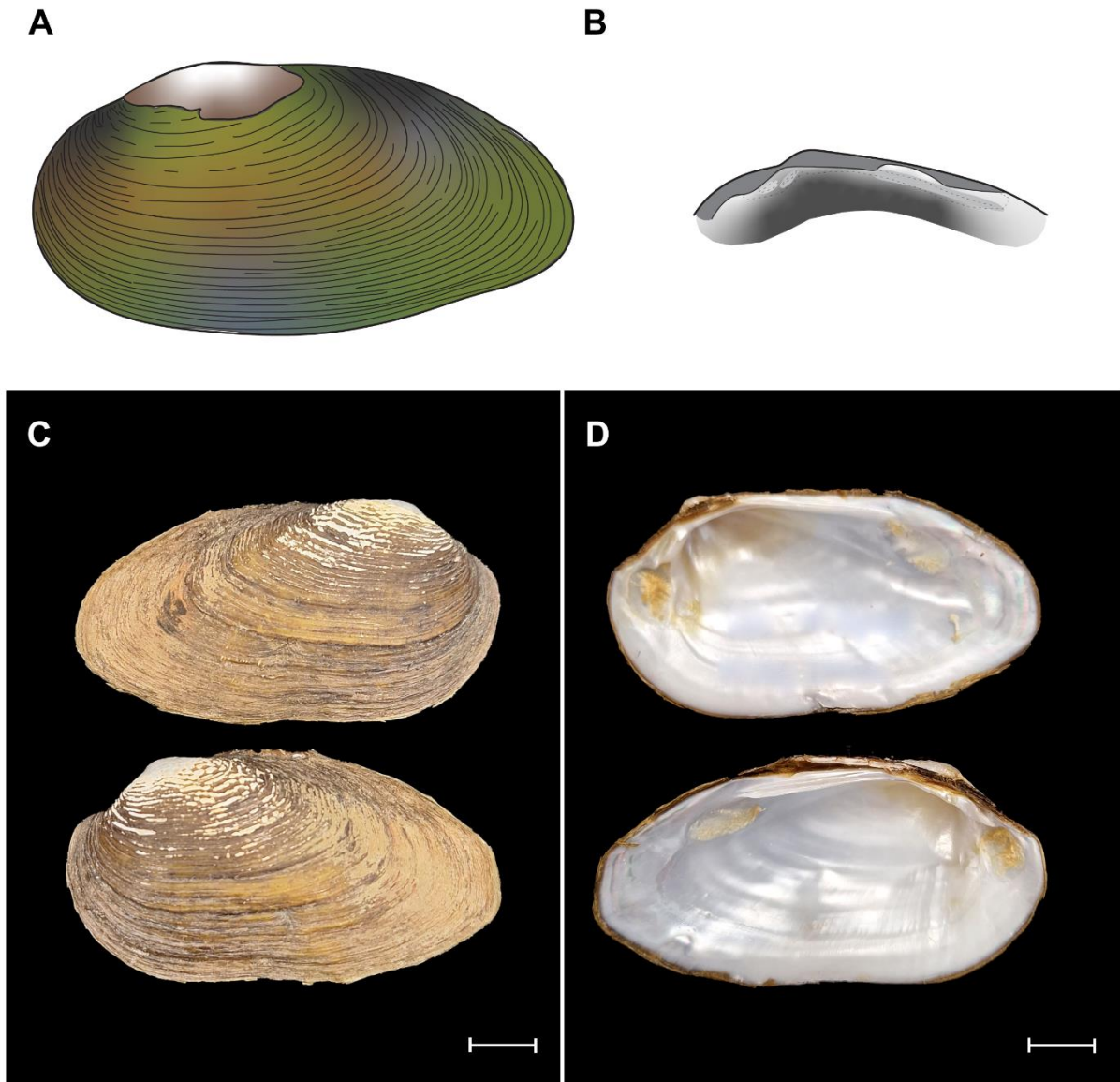


Figure 5.9 Redrawn images of *Unio caffer* (Krauss, 1848) as well as a representative of the bivalves found in this study.

5.3.2 Morphometrics

The width and length of the two bivalves found were measured to compare the unionids to those in literature (Table 5.4). These measurements were used as they were mostly used in literature too.

Table 5.4 The width and length measured in cm of each unionid bivalve collected in this study.

Species code	Length (cm)	Width (cm)
U1	5.5	2.7
U2	6.5	3.3

5.3.3 Phylogenetic results

Two novel 16S sequences were generated for this chapter. All relevant sequences were extracted from GenBank (Table 5.3) to form an alignment with a length of 233 base pairs. The GenBank sequences were generally longer than the novel sequences.

The COI and 28S genes were unsuccessful in amplifying DNA in the PCR process, thus data concerning these genes are not available.

5.3.3.1 Phylogenetics and species diversity

A 16S phylogenetic tree containing the two novel sequences and 25 sequences from GenBank was compiled for this chapter (Figure 5.10). The base pair difference and percentage similarity table can also be seen to provide further support for the reasoning behind why the sequences grouped as they did (Table 5.5).

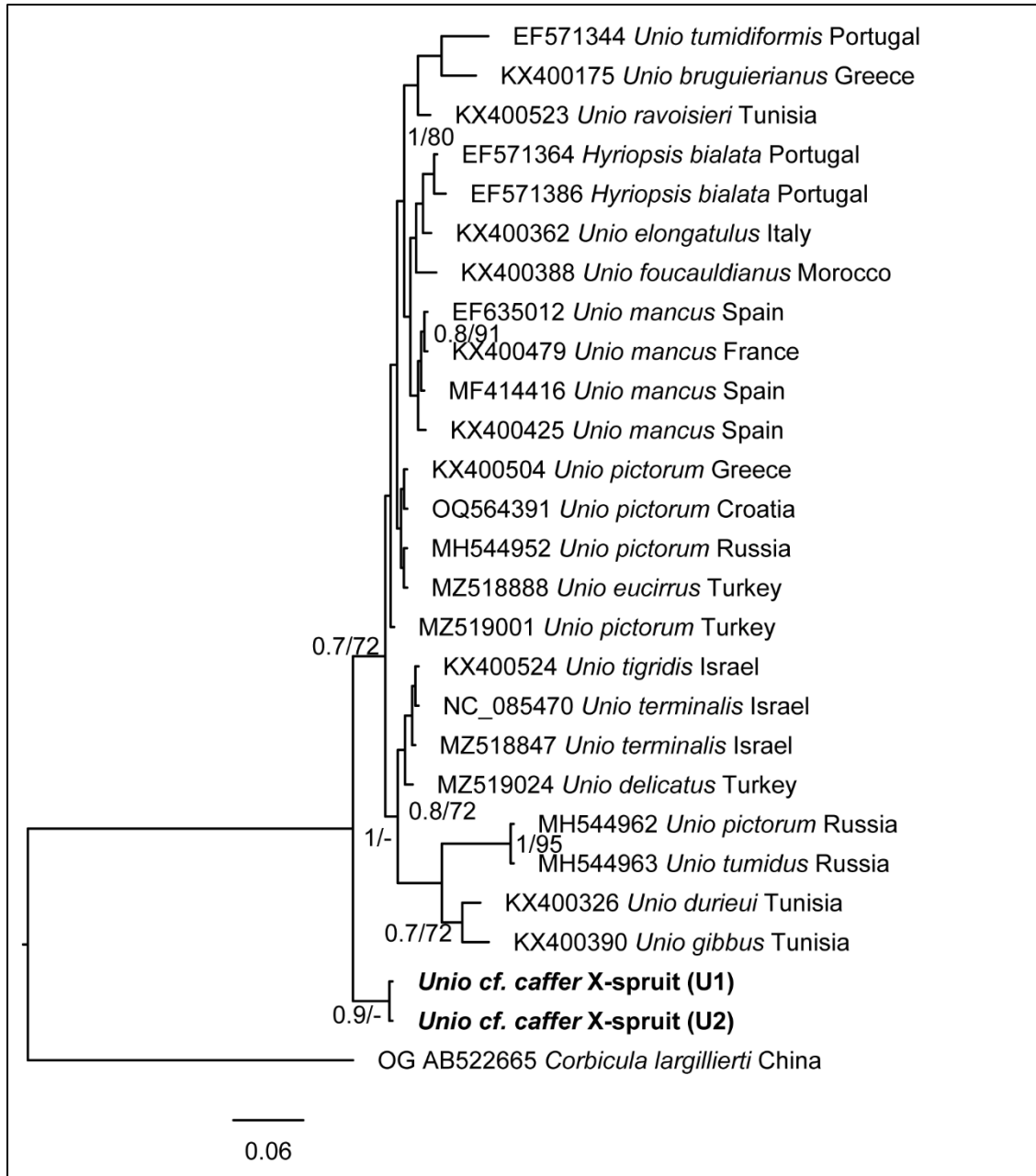


Figure 5.10 A 16S phylogenetic tree containing two novel sequences (bolded), sequences from GenBank, and the Bayesian inference (BI) and maximum likelihood (ML) values.

The novel sequences formed a distinct clade, separate from the sequences from GenBank. The high Bayesian Inference value suggests that the bivalves found in this study are the same (0.9), but Table 5.5 provides clarity on that when considering the base pair differences and percentage similarity. It can also be observed that the GenBank sequences grouped based on their respective species instead of their country of origin.

Table 5.5 Base pair differences (below the diagonal) and percentage similarity (above the diagonal) values for representatives of the 16S gene novel sequences and GenBank sequences for unionids.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1 U1 X-spruit		100	89	90	94	93	95	95	96	95	94	96	96	97	97	97	94	96	96	96	96	93	86	87	89	87	56
2 U2 X-spruit	2		89	90	94	93	95	95	96	95	94	96	96	97	97	97	94	96	96	96	96	93	86	87	89	87	56
3 EF571344 <i>U. tumidiformis</i> Portugal	25	25		94	93	92	93	93	93	93	94	93	93	93	93	92	93	92	92	93	92	92	88	88	91	89	55
4 KX400175 <i>U. bruguierianus</i> Greece	23	23	14		93	93	94	94	94	94	93	94	94	94	94	93	94	92	92	93	93	92	89	89	90	91	57
5 EF571364 <i>U. delphinus</i> Portugal	14	14	16	17		99	98	98	98	98	99	98	98	98	98	98	97	97	97	97	96	97	89	89	91	90	56
6 EF571386 <i>U. delphinus</i> Portugal	17	17	18	17	2		97	97	97	97	98	97	97	97	97	97	96	96	96	96	96	97	90	89	90	90	56
7 EF635012 <i>U. mancus</i> Spain	13	13	16	15	4	6		100	100	100	99	99	99	99	99	99	98	98	98	98	97	97	90	89	92	90	56
8 KX400479 <i>U. mancus</i> France	13	13	16	15	4	6	0		100	100	99	99	99	99	99	99	98	98	98	98	97	97	90	89	92	90	56
9 KX400425 <i>U. mancus</i> Spain	9	9	15	14	5	7	1	1		100	98	99	99	99	99	98	98	98	98	98	97	97	89	89	91	90	56
10 MF414416 <i>U. mancus</i> Spain	13	13	16	15	4	6	0	0	1		99	99	99	99	99	99	98	98	98	98	97	97	90	89	92	90	56
11 KX400362 <i>U. elongatulus</i> Italy	14	14	15	17	3	5	3	3	4	3		99	99	99	99	98	97	98	98	98	97	97	89	89	91	90	56
12 KX400504 <i>U. pictorum</i> Greece	10	10	16	14	4	6	2	2	3	2	3		100	100	100	100	98	98	98	98	98	97	90	90	93	91	56
13 MH544952 <i>U. pictorum</i> Russia	10	10	16	14	4	6	2	2	3	2	3	0		100	100	100	98	98	98	98	98	97	90	90	93	91	56
14 MZ518888 <i>U. eucirrus</i> Turkey	8	8	16	14	4	6	2	2	3	2	3	0	0		100	100	98	98	98	98	98	97	90	90	92	91	55
15 OQ564391 <i>U. pictorum</i> Croatia	8	8	16	14	4	6	2	2	3	2	3	0	0	0		100	98	98	98	98	98	97	90	90	92	91	55
16 MZ519001 <i>U. pictorum</i> Turkey	7	7	17	15	5	7	3	3	4	3	4	1	1	1	1		98	99	99	99	99	97	90	90	93	91	55
17 KX400523 <i>U. ravoisieri</i> Tunisia	14	14	17	13	7	9	4	4	5	4	6	5	5	5	5	4		98	98	98	97	96	89	89	92	90	56
18 KX400524 <i>U. tigridis</i> Israel	9	9	17	17	6	8	4	4	4	4	5	4	4	4	4	3	5		100	100	99	96	90	90	92	91	56
19 NC_085470 <i>U. terminalis</i> Israel	9	9	17	17	6	8	4	4	4	4	5	4	4	4	4	3	5	0		100	99	96	90	90	92	91	56
20 MZ518847 <i>U. terminalis</i> Israel	9	9	17	17	6	8	4	4	4	4	5	4	4	4	4	3	5	0	0		99	96	90	90	92	91	56
21 MZ519024 <i>U. delicatus</i> Turkey	9	9	17	15	8	10	6	6	6	6	7	4	4	4	4	3	7	2	2	2		96	91	91	92	91	56
22 KX400388 <i>U. foucauldianus</i> Morocco	17	17	18	19	6	8	6	6	7	6	7	6	6	6	6	7	9	8	8	8	10		88	88	91	90	55
23 MH544962 <i>U. pictorum</i> Russia	32	32	28	25	24	24	24	24	24	24	25	22	22	22	22	23	25	22	22	22	21	27		100	92	92	55
24 MH544963 <i>U. tumidus</i> Russia	31	31	28	25	24	24	24	24	24	24	25	22	22	22	22	23	25	22	22	22	21	27	0		92	92	55
25 KX400326 <i>U. durieui</i> Tunisia	25	25	20	22	20	22	19	19	19	19	20	17	17	17	17	16	18	17	17	17	17	20	19	19		96	56
26 KX400390 <i>U. gibbus</i> Tunisia	30	30	26	21	23	23	22	22	22	22	23	20	20	20	20	21	23	20	20	20	20	23	19	19	9		58
27 OG AB522665 <i>C. largillierii</i>	101	101	104	100	101	101	101	101	99	101	102	101	101	100	100	100	102	98	98	98	98	103	103	102	101	97	

5.3.4 Distribution of bivalves found based on ecological parameters

In Chapter 2, the distribution of all the sample sites was portrayed on maps containing the following ecological parameters: Level 1 ecoregions, mean annual rainfall, and elevation. Figures 5.11–5.13 portray where *Unio caffer* was found based on these parameters in South Africa.

Unio caffer was found at a higher elevation (Figure.11), in the Highveld ecoregion (Figure 5.12), which coincided with the region that receives 601 – 800 mm per year (Figure 5.13).

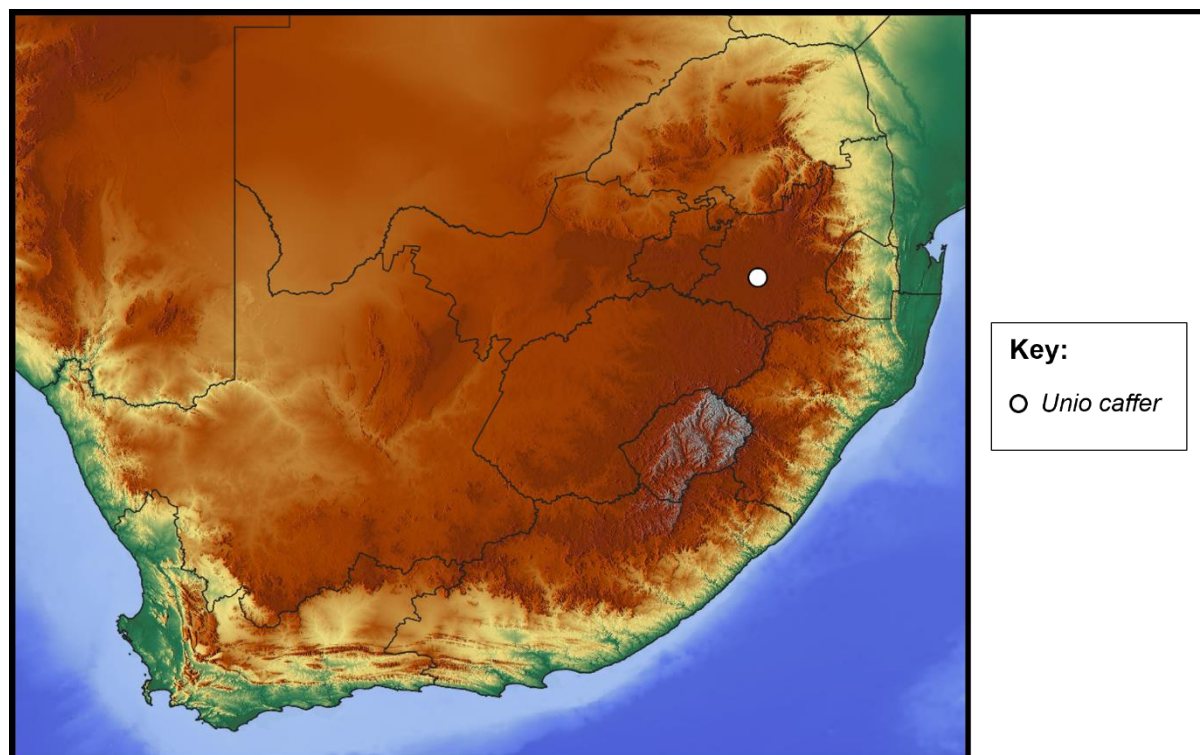


Figure 5.11 South African map indicating at which altitudes *Unio caffer* (Krauss, 1848) was found during this study.

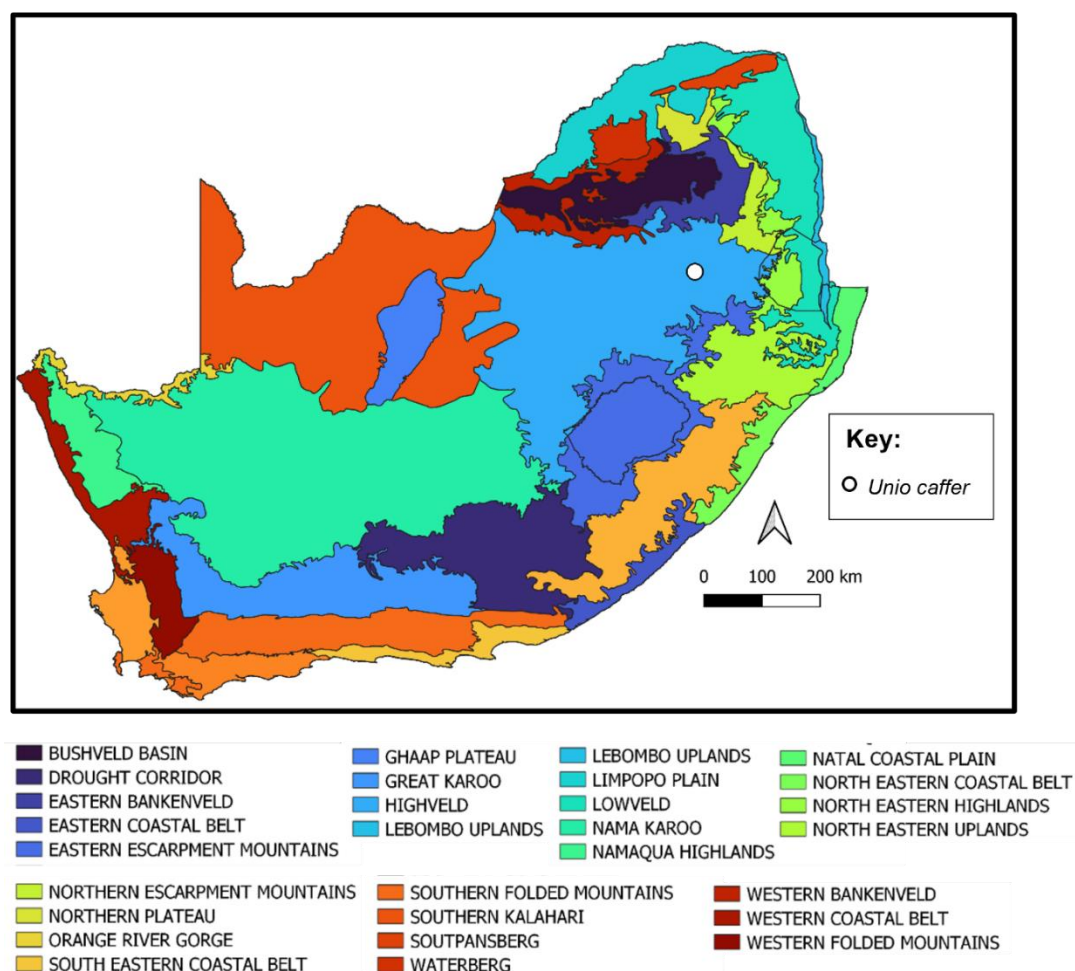


Figure 5.12 South African map indicating in what level 1 ecoregions *Unio caffer* (Krauss, 1848) was found in this project.

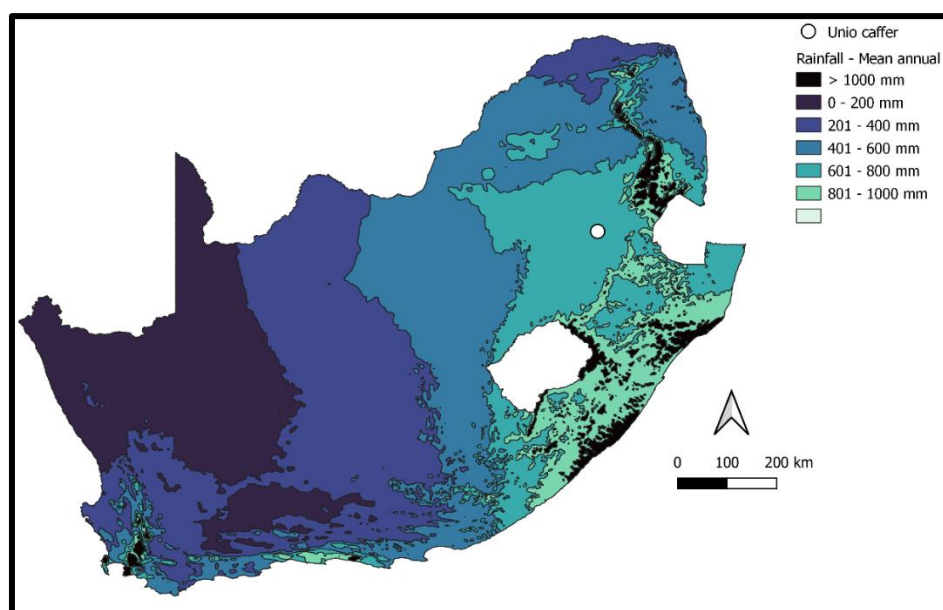


Figure 5.13 South African map of the mean annual rainfall indicating where *Unio caffer* (Krauss, 1848) was found in this project.

5.4 Discussion

There are currently 620 species in 142 genera in the family Unionidae worldwide (Lopes-Lima et al., 2016), but only three are known from southern Africa, with one species being the most common: *Unio caffer*. This number varies quite often as genera and species names change as can be seen in Table 5.3. This family is taxonomically challenging, which makes conservation planning difficult (Lopes-Lima et al., 2016). However, the family has gained more attention recently, contributing to the configuration of the species.

There is not a lot of information available for the *Coelatura* genus, however, each of the species discussed in this chapter was reported from South Africa at least once according to GBIF. The NWU Snail Collection has no information on this genus, suggesting that it was not present at any of the study sites and locations sampled. The possibility of this being a misidentification is unlikely as there are clear morphological differences between the *Coelatura* genus and the *Unio* genus, with *Unio* more elongate than *Coelatura*. This genus was also not found during this study, raising concerns that it may now be threatened or extinct.

In Figure 5.10, in the second clade from the top, there are two sequences of the species *Hyriopsis bialata* Simpson, 1900 (no. 5 and 6 in Table 5.5). These were included as this species was previously known as *Unio delphinus* and changed to its current name in 2021. *Unio delphinus* Gruner, 1841 and *Unio delphinus* Lea, 1840 are both considered invalid names, with *Unio delphinus* Spengler, 1793 as the valid species and authority. The first two names have been synonymised with *Hyriopsis bialata* as they are junior homonyms for the latter name. *Hyriopsis bialata* has 3 and 5 base pair differences respectively compared to *Unio elongatulus* which originates from the base of the *Hyriopsis* clade. This suggests that they are very closely related, and it might mean that *Unio elongatulus* belongs to the same genus as *Hyriopsis*. However, when considering other species for example, comparing *Unio trigradis* (no. 18) to *Unio delicatus* (no. 21), there are only 2 base pair differences, and the sequences are 99% similar. This could indicate that species from this family have a low threshold for the number of base pair differences required to distinguish between species, or it could suggest that these species cluster based on geographic location rather than genetic sequences, as the sequences originate from Turkey and Israel, respectively. On the contrary, *Unio pictorum* (no.12) from Greece, and *U. pictorum* (no. 13) from Russia, have 0 base pair differences and their sequences are 100% similar. This example suggests that the sequences group together based on the identity of the species instead of their country of origin. The same can be said for *U. mancus* from France and Spain (no. 8 and 9), and *U. pictorum* from Turkey (no. 16) compared to *U. pictorum* from Greece and Russia (no.12 and 13). *Unio eucirrus* fell in the same clade as *U. pictorum* (Figure 5.10) and their base pair

differences and percentage similarity suggest that they are the same species, or very closely related. This is further supported by Lopes-Lima et al. (2021) as they mention that *U. eucirrus* and *U. pictorum* are sister taxa of each other. *Unio tigridis*, *U. terminalis*, and *U. delicatus* are all closely related when considering Figure 5.7 as they fall within the same clade, further supported by the low base pair differences and high percentage similarity. Seeing as it is three different species, a possible explanation for this close relatedness could be that they originate close to each other (Israel and Turkey). *Unio delicatus* has high genetic diversity (Lopes-Lima et al., 2021), which could also explain why it grouped with the preceding species. This author further supports the results from this study by mentioning that *U. terminalis* and *U. delicatus* cluster together, as can be seen in Figure 5.10q of this study. There are two *U. pictorum* sequences included from Russia, the first one grouping with *U. pictorum*, and the second one falling in a clade with *U. tumidus*. The second *U. pictorum* is possibly incorrectly identified as it did not fall in the clade with the rest of the *U. pictorum* in the tree, and it has the same amount of base pair differences as *U. tumidus* compared to the other *U. pictorum*. Additionally, it has 0 bp differences compared to *U. tumidus*, and the sequences are 100% similar. Thus, it can be deduced that this sequence is actually *U. tumidus*.

The novel sequences (U1 and U2) formed their own clade suggesting that they are a species different from the GenBank sequences. *Unio caffer* is widely spread throughout South Africa based on the records from GBIF and the NWU Snail Collection. It is also the only species in this family that was found during this study. It brings into question whether this genus is the only one present in South Africa. Morphologically, the bivalves found in this study correlated to the description of *U. caffer*, however, this could not be confirmed molecularly as there are no 16S sequences available for this species. Lopes-Lima et al. (2021) highlights the importance of further studies in phylogenetics for the family Unionidae as morphological characters are not sufficient to distinguish between species. However, in the case of the South African species, their morphology differs enough to be able to say that the bivalves found were *Unio caffer*. Further support of this is the fact that the distribution of this species based on the GBIF records and records from the NWU Snail Collection correlates to the site where the bivalves in this study were found.

5.5 Conclusion

Two 16S sequences were generated from this study, but because of a lack of South African 16S sequences, the bivalves did not match with a GenBank sequence molecularly. However, it did form its own clade which suggests that it is another species in this genus. Based on morphology,

the bivalves were identified as *Unio caffer* and these are the first sequences available for this species. The aim of the chapter was achieved, because it was evident that the occurrences of this species have decreased. The family was revised, and an updated distribution is provided for the South African species. To conclude, this family still has some knowledge gaps, but the outcomes of this study contributed to the distribution information and provided valuable information in terms of species richness and diversity.

CHAPTER 6 CONCLUSION

6.1 General conclusion

Nineteen bivalves that have been reported from South Africa were revised, and five were found during this study: *Euglesa costulosa*, *Euglesa viridaria*, *Eupera ferruginea*, *Chambardia wahlbergi*, and *Unio caffer*. This was the first comprehensive molecular study done for sphaeriids in South Africa. Bivalves have become a prominent focus in recent research, with a plethora of researchers focusing on revising the taxonomy (Behrens et al., 2021; Böhm et al., 2021; Clewing et al., 2022; Bepalaya et al., 2024; Bolotov et al., 2024) and providing basic knowledge to enable more complex studies in the future. The current study achieved a similar outcome by addressing certain knowledge gaps, such as revising family classifications and updating distribution records. In total, 35 COI and 47 16S sequences were generated. However, it also highlighted several gaps in our understanding of South African bivalves, including the vague and inconsistent morphological characteristics currently available and the limited representation of South African sequences on GenBank and BOLD. Nevertheless, if the current research trend continues, these gaps are likely to be filled in future, enabling South African bivalves to receive the conservation attention and importance in freshwater resource management they require.

One of the aims of the study was to contribute to the knowledge of South African freshwater molluscs through molecular data. Molecular data was provided in the form of COI and 16S sequences, representing several species. For the sphaeriids, four species were identified: *Euglesa costulosa*, *Euglesa viridaria*, *Eupera ferruginea*, and possibly *Euglesa langleyana*. In the case of iridiniids, *Chambardia wahlbergi* was the species identified. Similarly, the unionid specimens found in the study were both identified as *Unio caffer*.

The distribution of the sphaeriids remained similar to previous records, with these molluscs being found at 15 out of 78 sites, though their density has decreased. For iridiniids, a decrease in the number of sites was noted, with specimens found at only two sites, which aligned with earlier reports of their decreasing abundance. In the case of unionids, their distribution was even more restricted, with occurrences documented at only one site, suggesting a potential reduction in their range.

Morphological analysis revealed that there were differences within the species groups of sphaeriids, indicating both interspecific and intraspecific variations. The iridiniids and unionids studied exhibited distinct morphological characteristics that allowed identification: the iridiniids were all identified as *Chambardia wahlbergi*, while the unionids were both *Unio caffer*.

Based on the findings presented across all chapters, it is suggested that the number of species within these families is lower than originally estimated. In each chapter, fewer species were identified compared to those reported by GBIF and the NWU Snail Collection. The original species descriptions were based solely on morphological characteristics, which likely contributed to the identification of a larger number of species. However, molecular methods revealed that some species match phylogenetically despite exhibiting morphological differences. This indicates that while morphology may suggest a higher species count, phylogenetic analysis supports the presence of fewer species, with intraspecific variation being more common.

Although this project contributed to the current understanding of South African bivalves, several research gaps remain, particularly regarding the distribution, morphology, and molecular compositions. While comprehensive distribution information exists for certain species, it is insufficient for others. The georeferencing data generated through this study has also enhanced distribution knowledge by making previously inaccessible information available. However, despite these contributions, some species still lack adequate distribution records. Additionally, molecular data for most species in these families remain sparse in South Africa, with only three relevant representative sequences from South African species available for use in this study.

There is a possibility of intraspecific variation within Sphaeriidae, Iridinidae, and Unionidae, indicating variation within these taxa. Kuiper (1964), Mandahl-Barth (1988), and Appleton (2002) described numerous species in each of these families. However, a comprehensive study by De Kock and Wolmarans (2010) identified only a few species, particularly within Iridinidae and Unionidae. While an initially diverse range of Sphaeriidae was documented, recent sampling efforts, including those in the present study, revealed a reduced diversity when analysed using molecular methods.

Intraspecific variation is well-known as a challenge for species identification based on morphology (Aldridge, 1999), and this was particularly evident in this study. This issue was most pronounced in the Sphaeriidae, where multiple species were identified. Although morphological variations within species were noticeable, analysis at the COI level revealed only zero to one base pair difference, highlighting the limited molecular variation within species. However, distinct differences were observed between species (interspecific variation) both morphologically and molecularly. These findings suggest that the threshold for base pair differences required to distinguish between species in Sphaeriidae is exceptionally low.

This project has enabled a better understanding of the distribution, diversity and abundances of selected bivalve families occurring in South Africa. It has provided information that has answered

(or partly answered) the existing research questions but it has unveiled even more research questions for the future. The research on these selected bivalves is vital to ensure the protection of these species as part of South Africa's extensive biodiversity and to ensure their conservation in the future.

6.2 Recommendations

The results contributed to the distribution, molecular data and morphological data of South African bivalves; however, a few recommendations are made below to fill some research gaps and conserve the species.

- As this study was focused on the northern provinces of South Africa, it would be beneficial to sample from the other regions in South Africa too. The ideal situation would be to revisit each of the type localities of the different species in each family and sample extensively to find out if the specific species, or at least the genus, is still present. If something is found, it can be compared to the holotype to compare morphological characteristics and that should be enough to assign a species name after generating a sequence. From this, an updated key of morphological characteristics can be compiled to contain consistent terminology and any interspecific or intraspecific variation that might be observed. Only after all the species in South Africa have undergone this process, will we truly know what the state of the three families are.
- In relation to molecular methods, the following can be recommended: design primers for specific genera to yield a long enough sequence to compare to others, and if multiple genes are available compile at least one whole genome sequence for each species that occurs in South Africa. Compiling a phylogenetic tree for these species will provide a solid baseline for future taxonomic studies to build upon.
- The use of new DNA methods to extract ancient DNA to validate new or existing species could be very beneficial (Guangjie et al., 2024). This approach could be particularly useful if the species can no longer be located in nature or to confirm current species identifications. For instance, in the NWU Snail Collection, a small tissue sample could be used to extract DNA for comparison with currently observed species. This would also allow for the comparison of morphological characteristics and the determination of both interspecific and intraspecific variations.
- Citizen science in the study of sphaeriid bivalves could be promoted more, as images of relevant species are often shared on platforms like iNaturalist, which can contribute valuable data for research. Iridinids and unionids also provide an opportunity for citizen science, as their shells are commonly found along riverbeds. These findings can be

reported to GBIF, encouraging public participation in scientific data collection. This is a good way to regulate the species occurrence and distribution.

- It is recommended that fish surveys be conducted alongside sampling efforts for unionids and iridiniids, as the parasitic larval life stages of these bivalves are highly dependent on the species, abundance, and behaviour of host fish (Aldridge, 1999). Since the specific host fish species in South Africa remain uncertain, tracking fish species in areas where unionids and iridiniids have been reported, such as those identified by GBIF and the NWU Snail Collection, could help narrow down potential host fish. This approach could not only aid in future studies but also provide insights into the decline in unionid and iridiniid populations.
- Engage collaborative partners such as the NRF-SAIAB to support sustained monitoring of these important molluscs and provide more accurate data to inform global conservation agencies such as GBIF and IUCN.

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