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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Upper Cretaceous Staphylinidae from Orapa Diamond Mine in Botswana

by

Sandiso Mnguni

(370596)

Research Project

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Doctor of Philosophy

in

Palaeontology

in the Faculty of Science, University of the Witwatersrand, Johannesburg, South Africa

Supervisor: Dr Ian James McKay

Co-Supervisor: Dr Shaw Badenhorst

March 2022

Declaration

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at any other University.



_____

Signature of candidate

Sandiso Mnguni

Signed this 30th day of March 2022 at the Evolutionary Studies Institute (ESI), School of Geosciences of the University of the Witwatersrand, Johannesburg, South Africa.

Abstract

Upper Cretaceous Staphylinidae from Orapa Diamond Mine in Botswana

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A fauna of Mid-Cretaceous Staphylinidae (Coleoptera) recovered from a Turonian crater-lake in Orapa Diamond Mine in Botswana is described. These are assigned to the following subfamilies: Steninae, Tachyporinae and Paederinae, respectively. Three new species have been erected; these are: *Afristenus orapensis* species nov., *Cretatachinus orapensis* species nov., and *Afrinophilina orapa* species nov., respectively. Furthermore, the paederine, *Afrinophilina orapa*, is the earliest occurrence of a member of the Pinophilina, and the stanine, *Afristenus orapensis*, is a new genus for the family. These are, with the exception of one species from Santana/Crato Formation, a Lower Cretaceous deposit in Brazil, the first Cretaceous fossil staphylinids from the southern hemisphere.

The location, geology and geography, and history of the deposit are provided. The dating of the kimberlites, sediments, and rate of sedimentation are also briefly discussed. Previous publications from the deposit, and the associated palaeoenvironment reconstruction are also provided. As such, an age of the sediments, and the palaeoenvironment are reconstructed using staphylinids, other fossil fauna, and taphonomical studied using the sediments.

The depositional environment of the fossils is discussed, since a taphonomical analysis of the deposit was attempted. The preliminary results suggest that staphylinids inhabited the perimeter of the lake, and were trapped in a nearshore, shallow water oxidising environment. For some unknown reason, most of the plant material is preserved in presumably green, deeper water, un-oxidising environment. This preliminary analysis needs expansion with a larger sample size.

All three fossil insects belonging to the Steninae, Tachyporinae and Paederinae support the previous palaeoenvironmental reconstruction at Orapa as crater-lake situated in a moist, well-vegetated environment. Since all three subfamilies in this study contain numerous species and cannot be assigned to extant genera, it was not possible to draw more confident conclusions than this.

The climate of the deposit in the Cretaceous has been reconstructed as being seasonal and interchanging between tropical and temperate extremes. Based on knowledge of other fossil finds, the staphylinids described here would be predicted to occur in the Cretaceous. The morphological conservatism of the described specimens, together with that of similarly-aged staphylinids, and many other insects, suggests a far more conservative rate of evolution than for vertebrates, which have undergone enormous change in the same period of time. These findings suggest a punctuated equilibrium pattern of evolution, as previously reported in McKay (1990) and Waters (1990).

Keywords: Turonian, Steninae, Tachyporinae, Paederinae, Kimberlite, Palaeoenvironment, Morphological Conservatism and Punctuated Equilibrium.

Dedication

Firstly, I dedicate this achievement to Mr Thamsanqa ‘Thami’ Peteni, my late father.

I equally dedicate this achievement to Mr Cambridge ‘Sthathu’ Nqwili, my late grand-father.

I also dedicate this achievement to Mrs Buyiswa Janet ‘Madongwe’ Mnguni, my late great-grand-mother.

Lastly, I dedicate this achievement to Dr Ian James McKay, my late PhD main supervisor, who has recently passed away, while addressing comments from external examiners he nominated.

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CHAPTER ONE

INTRODUCTION

Palaeoentomology has been an understudied field for many years, despite the huge diversity of insects. The field represents an interface between Palaeontology (i.e., the study of fossils) and Entomology (i.e., the study of insects) (Penney and Jepson 2014). Henceforth, it is a field that allows researchers to investigate insects on Earth (Rasnitsyn and Quicke 2002; Nel et al. 2010). Palaeontologists and entomologists thought that fossils were rare, unreliable, and generally not well-preserved to allow for any taxonomic and systematic studies, which would lead towards palaeoenvironmental reconstructions (Nel et al. 2010). Insects are keystone species in both terrestrial and aquatic ecosystems as pollinators, recyclers, detritivores, predators, scavengers, and food source for other animals (Penney and Jepson 2014). Therefore, fossil insects are used as models to understand the evolution of insects, from the Devonian to the Quaternary age.

1.1 Introduction of Staphylinidae

The Staphylinidae (rove beetles, or staphylinids) is one of the 36 Coleoptera families erected by Latreille in 1802. At the time, Latreille only gave 14 example species. Today, this hyper-diverse family has over 62 453 described species, making it the largest in the animal kingdom (Newton 2016 unpublished data, cited in Lü et al. 2020). This group exists within the largest animal order (i.e., Coleoptera), largest animal class (i.e., Insecta), largest animal phylum (i.e., Arthropoda), and largest kingdom (i.e., Animalia) (Parker 2017).

Ślipiński et al. (2011) summarised the number of described animal species as follows: the Kingdom Animalia has 1 552 319 species; the Phylum Arthropoda has 1 124 050 species, the Class Insecta has 1 020 007 species; while the Order Coleoptera has 387 100 species. According to estimates by Stork (2018), there are still approximately 1 million species of undescribed beetles, 5 million species of undescribed insects, and 6 million species of undescribed arthropods. Therefore, staphylinids constitute 4% of all animals, while they also constitute 16% of all beetles found on Earth.

Traits such as a tough chitinous exoskeleton, wing diversification (from wingless to winged), an emergence of wing folding (i.e., elytra and halteres), and metamorphosis diversification (i.e., complete/holometabolous and incomplete/hemimetabolous), are responsible for much of the success of insects, including staphylinids (Grimaldi and Engel 2005).

In particular, Parker (2017) has suggested that staphylinids are successful because they are mostly small in size (ranging between 0.5-50 mm in length; while many are between 2-8 mm in length), and usually have short elytra (a hardened wing case) that leaves their heavily sclerotised flexible abdomen exposed. Their small size, and heavily sclerotised flexible abdomen enables efficient movement through litter, and allows them to move through tight spaces (Parker 2017). These traits may explain why staphylinids are found in such an enormous variety of microhabitats (Irmler et al. 2018).

Staphylinids have a worldwide distribution, and can be found in nearly all ecosystems of the world (Irmler et al. 2018). Despite their diversity, they share a similar body plan, although it tends to vary slightly, or dramatically, within and between taxonomic groups (Parker 2017). Nevertheless, it is possible that having an effective common body plan explains their success.

Several species of staphylinids serve as natural enemies, and are used as effective biological control agents of crop pests (Parker 2017; and Irmler et al. 2018). Due to their predominance in many habitats, researchers expect them to play a key role in contributing to ecosystem services (Parker 2017), which suggests that they may be used to assess the health and functioning of biodiversity in ecosystems (Thayer 2005). The high species richness, diversity, and abundance of staphylinids in many microhabitats, and their ability to colonise new areas, while successfully withstanding environmental disturbances, suggests they may be useful as indicators of ecosystem health (Parker 2017). Therefore, they may be used for monitoring purposes.

For example, in applied sciences such as forestry and conservation, they have a potential to be extensively used as bio-indicators of environmental conditions (Thayer 2005). Their chemical secretions may also be useful for making biomedical compounds, including anti-cancer and anti-microbial treatments (Parker 2017).

Astonishingly, their general morphology has largely remained uniform over time, a phenomenon called bradytely (i.e., slow rate of morphological change, or “arrested evolution”). Various

explanations for this bradytely have been proposed, including slow extinction rates, and the steady presence of mesic habitats over a geological time (Clarke and Chatzimanolis 2009).

Despite their common body plan, staphylinids exhibit numerous morphological adaptations. For example, those found in soil surfaces have an extensive elongated shape and flexible body for efficient movement (Irmler et al. 2018). However, those found in deeper soil layers have reduced eyes, legs, and antennae (Irmler et al. 2018). Staphylinids found in natural holes such as burrows and nests of mammals evolved longer legs and antennae (Irmler et al. 2018). Furthermore, staphylinids found in vegetation evolved broad adhesive tarsi, while those found under bark of deadwood have become dorsoventrally flattened for efficient movement (Irmler et al. 2018).

Staphylinids are mostly known to be generalist predators, although some specialise in feeding on insects, or mites (Thayer 2005; and Parker 2017). Some staphylinids do not entirely ingest their prey; instead, they chew, exude midgut digestive fluids, and suck in the partly-digested liquid, before discarding the hard parts (Thayer 2005). Other staphylinids are fungus, humus, pollen, and plant feeders (Thayer 2005; Parker 2017; and Irmler et al. 2018). Extensive research has so far shown that predation (18 subfamilies), mycophagy (16 subfamilies), and saprophagy (14 subfamilies) are the most widespread types of feeding habits of staphylinids (Thayer 2005).

Some staphylinids live in close association on a mutually-exclusive relationship with social insects such as hymenopterans (i.e., ants, bees and wasps) and termites (Thayer 2005; Parker 2017; and Irmler et al. 2018). They either feed directly on the host, or on parasites of the host. Some are fed by the host, or feed on remnants of the host's food, or waste product (Thayer 2005; and Parker 2017). Other staphylinids live in nests of mammals and birds (Thayer 2005; and Parker 2017).

No fossil staphylinids are known from the Triassic (252-201 Ma), although time-calibrated phylogenies suggest that they originated in the Triassic/Jurassic (252-145 Ma), diversifying in the Jurassic/Cretaceous (145-66 Ma) (McKenna et al. 2015; Toussaint et al. 2016; Zhang et al., 2018; Lü et al. 2020; and Cai et al. 2021). Furthermore, the first fossil staphylinids appeared in the Middle to Late Jurassic of Europe and Asia (McKenna et al. 2015; Toussaint et al. 2016; and Cai et al. 2021).

Phylogenetic studies have suggested that three of the four informal groups of subfamilies currently recognised were present in the Middle to Late Jurassic (McKenna et al. 2015; Toussaint et al. 2016;

and Zhang et al. 2018). The four informal and non-monophyletic groups of staphylinids, which are assigned based on larval and adult morphologies are omaliine, tachyporine, oxyteline and staphylinine groups (Lawrence and Newton 1982, 1995; and McKenna, et al. 2014).

Thayer (2005) described the general features of the four informal and non-monophyletic groups: (1) the omaliine group are characterised by having a pair of ocelli, although this is lacking in some taxa, (2) the tachyporine group predominantly has predaceous subfamilies within it, (3) the oxyteline group is associated with saprophagy or mycophagy, and (4) the staphylinine group includes subfamilies that are predaceous, some feeding *via* extraoral digestion and possessing of defensive glands.

A more comprehensive, well-resolved, and highly supported phylogeny of the Staphylinidae is still required. Many efforts have been made to reconstruct the phylogeny of the Staphylinidae (e.g., Lawrence et al. 2011; Zhang and Zhou 2013; Bocak et al. 2014; McKenna et al. 2015, 2019; Toussaint et al. 2016; Yuan et al. 2016; Zhang et al. 2018; Baca et al. 2021, and Cai et al. 2021). Most recently, especially during the last decade, there has been a steady rise in the number of descriptions of fossil staphylinids that have been recovered from deposits across the globe (Peris and Rust 2020). Moreover, most of the fossil staphylinids have been recovered from amber deposits (Grimaldi and Engel 2005; and Rasnitsyn and Quicke 2007). As such, recently, many studies have used a combination of evidence from molecular, morphological, and fossil databases (e.g., Żyła et al. 2021), to create total evidence phylogenies.

The fossil record of staphylinids can still be improved with more accurate and readily available descriptions, especially of rock fossils, which are currently poorly studied, and represent different palaeoenvironments from amber fossils (Grimaldi and Engel 2005). Henceforth, this study described a fauna of the Upper Cretaceous staphylinids that were recovered from Orapa Diamond Mine (ODM), which is situated in north-eastern Botswana. They are the first described fossil staphylinids from Africa, and only the second in the southern hemisphere. In addition, this study also attempts to reconstruct an ancient ecosystem of the deposit during the Cretaceous.

Firstly, the deposit where the staphylinids were recovered is described with reference to the locality, geology and geography, age of the deposit, fossil fauna, and taphonomy. Secondly, the classification, phylogeny, evolution of the staphylinids, and their fossil record are reviewed. Thirdly, the materials and methods, terminology, and techniques that were used to identify and

describe the fossils are detailed. Thereafter, a selection of well-preserved fossil staphylinids is described. Lastly, to reconstruct an ancient ecosystem of the deposit; the fossil fauna is discussed in accordance with the age of the deposit, palaeoclimate, palaeoecology, palaeoenvironment, and the general considerations of the staphylinid evolution and palaeogeography.

Research Aim, Hypothesis, and Objectives

1.2 Aim

This project aimed to study evolution using taxonomy and taphonomy of fossil Staphylinidae from Orapa Diamond Mine in Botswana, to reconstruct the palaeoenvironment and palaeoecology of the area during the Cretaceous.

1.3 Hypothesis

A study of evolution using taxonomy and taphonomy of the fossil Staphylinidae from Orapa Diamond Mine in Botswana will contribute towards the palaeoecological and palaeoenvironmental reconstruction of the area during the Cretaceous.

1.4 Objectives

1. To study the taxonomy of fossil Staphylinidae from the Orapa Diamond Mine.

2. To evaluate the taphonomic processes that biased the fossil beetle assemblage.

3. To describe well-preserved subfamilies of Staphylinidae to the lowest possible taxonomic level.

4. To reconstruct the palaeoecology and palaeoenvironment of the Orapa Diamond Mine during the Cretaceous age.

CHAPTER TWO

LOCALITY, GEOGRAPHY AND GEOLOGY OF THE ORAPA DIAMOND MINE

2.1 Introduction of Orapa Diamond Mine

The Orapa Diamond Mine (ODM) is situated in north-eastern Botswana (Figure 2.1), approximately 240 km due west of Francistown (21°18.465'S; 25°22.177'E) (Rayner and McKay 1986). It is unique and of heritage importance, as it is the only Cretaceous deposit in Africa that is rich in compressions and impressions of fossil plants and insects. This is important, considering that the Cretaceous is key in the history of insect evolution (Nel 2015; and Mayhew 2018).

The ODM is situated over a pair of adjoining diamondiferous kimberlitic pipes. It is the largest conventional open-pit diamond mine producer by carats in the world (Brook 2019), with a length of 1 600 m and a width of 1 000 m (Rayner and McKay 1986). The Orapa kimberlite (known as AK/1 in Rayner and McKay 1986; McKay 1990; Rayner et al. 1991, 1997; Gernon et al. 2009 a, b; and Brook 2019) is a rare example of a kimberlite pipe that still has its crater lake facies preserved, termed epiclastic kimberlite (Gernon et al. 2009 a). Other examples of epiclastic kimberlites are known from neighboring countries in Africa such as Tanzania, Zambia, Angola, Democratic Republic of the Congo, Mali and South Africa (McKay 1990; and Gernon et al. 2009 b).

About 93.1 Ma (Upper Cretaceous, Turonian), a pair of kimberlites from the same kimberlitic source pipe erupted; first, the North pipe, and then the South pipe (Gernon et al. 2009 a, b). It is the latter pipe that formed a large crater at the surface (Gernon et al. 2009 a, b). In modern times, mining operations have removed the sediments overlying the Orapa pipe, and this has produced a large oval pit that is 80 m deep on average (Figure 2.2).

The ODM is one of 60 kimberlitic pipes and dykes in the area (Gernon et al. 2009 a, b). The geographical area is flat with a few low hills and ridges, and an average elevation of 960 m above sea level (Rayner 1993), with a semi-arid climate, experiencing an average annual rainfall of 500 mm (McKay 1990). Mopane bush is the dominant vegetation in the area (Rayner 1993). Temperatures range from 0°C in winter to 42°C in summer, but fluctuate between 21-32°C for

most days (McKay 1990). Previous research suggests that the locality was 34% further south at the time of the formation, which would place it at a similar latitude as modern-day Cape Town (McKay 1990, 1991; and Rayner et al. 1991). The topography was flat, and it was from this flat topography that a volcano protruded with a lake in the centre (McKay 1990).

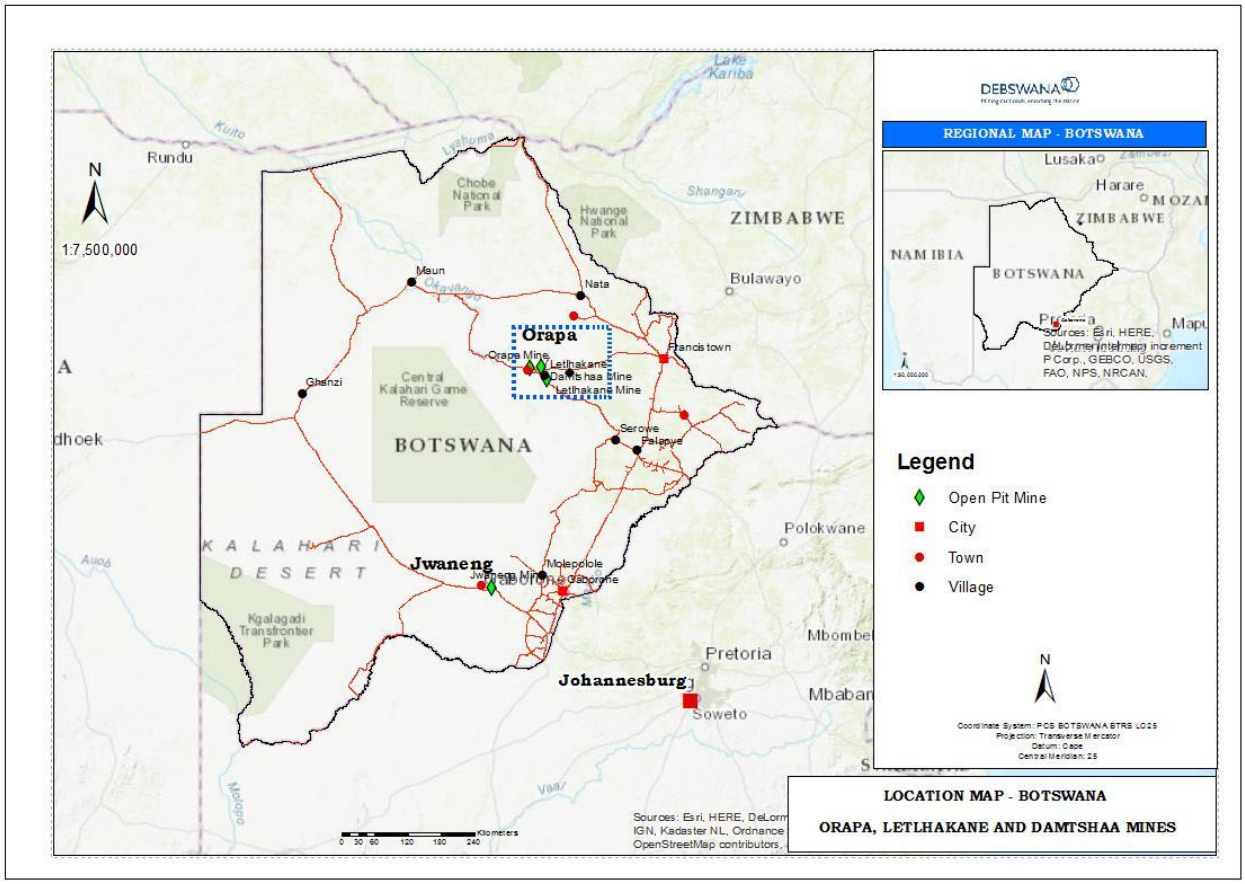


Figure 2.1 Orapa Diamond Mine in Botswana locality map. Taken from Mashabila (2019, page 9).

2.2 History of Orapa Diamond Mine

The first diamond discoveries in Botswana were reported in 1938, but mining, excavation and exploration started in 1959 (De Wit 2018). The Orapa kimberlite was discovered in 1967 (De Wit 2018). In Botswana, the first recognised diamond find was made by prospecting geologists from Consolidated African Selection Trust (CAST) in 1955, and this discovery also gave rise to the first diamond exploration by De Beers in the same year (Brook 2019).

The first diamondiferous kimberlite in the country was discovered on the 1st of March in 1967, six months after Botswana gained its independence from British colonial rule (Brook 2019). Thereafter, the mine came into production four years later, sometime in 1971 (De Wit 2018). Since then, 397 kimberlites have been discovered in the country (De Wit 2018). The Orapa pipe has a roughly oval outline and consists of two kimberlite pipes; a north and south pipe, giving it a bilobate shape (McKay and Rayner 1986; McKay 1990; and Gernon et al. 2009 a, b).

The ODM kimberlite eruption was caused by deformed Archaean basement rocks overlain by volcanic and sedimentary rocks of the Permian-Jurassic Karoo Supergroup (Gernon et al. 2009 a, b). The eruption of the kimberlites formed a large volcanic crater at the surface (Gernon et al. 2009 a, b), in which a lake soon formed (Rayner and McKay 1986; and Rayner et al. 1991, 1997). It is estimated that the crater would have had a maximum depth of 140 m and a cone of 114 m (Hawthorne 1975; and Dobbs 1978), whereas the sedimentary infill of the crater was between 60-90 m thick (Hawthorne 1975; and Dobbs 1978).

Early infilling of the crater would have been caused primarily by gravity-driven catastrophic processes, which resulted in the slumping and erosion of the cone, which consisted of kimberlitic material, and chunks of surrounding country rock, which the kimberlite collected on its journey to the surface (McKay 1990; and Gernon et al. 2009 a, b). Rock falls and debris avalanches, heavy rainfall from the hot-humid Cretaceous climate all played a part in triggering sheet-flood deposits, and debris flows from the steep crater walls (Gernon et al. 2009 a).

Interestingly, part of this early infill was a pyroclastic flow from a nearby erupting kimberlite volcano (Gernon et al. 2009 a, b). As the crater stabilised and the lake formed, debris flows and fine-grained lacustrine deposition predominated (Hawthorne 1975; Dobbs 1978; and McKay 1990). In addition to the above, there are also small portions of fluvial deposits, which were probably formed by a small stream (Dobbs 1978; and McKay 1990).

Debris flows occur when masses of unconsolidated sediments on steep slopes become saturated by rainstorms, or melting (Blatt et al. 1980; and McKay 1990). Seismic events, such as earthquakes or earth tremors can also cause debris flows (Blatt et al. 1980; and McKay 1990). Debris flows are postulated to be common in environments such as talus slopes in mountain environments, and volcanic cones (Blatt et al. 1980; and McKay 1990). Kimberlites are prone to absorbing large

amounts of water, forming extremely unstable and slippery mud; thus, they would have formed an ideal environment for debris flow deposition (McKay, personal communication).

At ODM, debris flows were found on the inside of the crater, flowing from the periphery towards the centre (McKay 1990). The debris flows consisted of large chunks, or rock separated and transported by a fine-grained matrix such as mud. At ODM, finer grained granular flows precede and succeed debris flows (Figure 2.2), which were either extensions of the debris flows, or were formed by a similar catastrophic mechanism of finer grained material on the crater wall (Dobbs 1978; and McKay 1990).



Figure 2.2 The top view of the Orapa Diamond Mine in Botswana taken in June 2018 with the fossiliferous sediments removed (Photo by I.J. McKay).

Movement of debris and granular flows into the lake resulted in the deposition of coarser grained sediments amongst the fine-grained sediments, which were formed by the slow settling of finer grained sediments in the water column (McKay 1990). Debris and granular flows also stirred up mud, which also slowly settled, resulting in the formation of thick layers of unlaminated mudstone (McKay 1990). The result of these process was a thick layer of lacustrine sediments, consisting of

interbedded fine-grained stones, with coarser grained sediments. These lacustrine sediments are today of different colors. There are green sediments, consisting of relatively unoxidised kimberlite, which tends to be blue-green in color (Figure 2.3); brown-red sediments (Figure 2.4), which appear to have been oxidised; and pale pink (Figure 2.5), or cream sediments (Figure 2.6), which were perhaps altered by post-depositional action of ground water (McKay 1990).



Figure 2.3 Green sediments (BP/2/18544) from Orapa Diamond Mine in Botswana. The block is housed at the Evolutionary Studies Institute (ESI), University of the Witwatersrand.

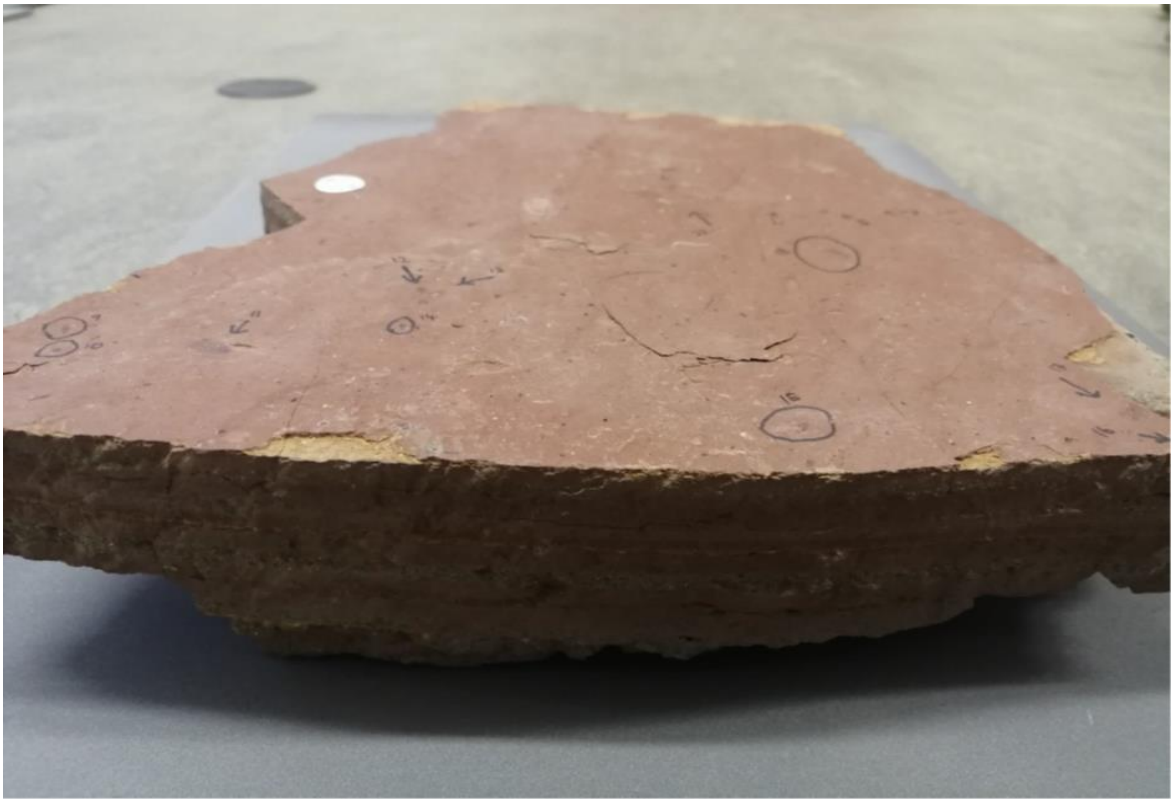


Figure 2.4 Brown-red sediments (BP/2/28058) from Orapa Diamond Mine in Botswana. The block is housed at the Evolutionary Studies Institute (ESI), University of the Witwatersrand.

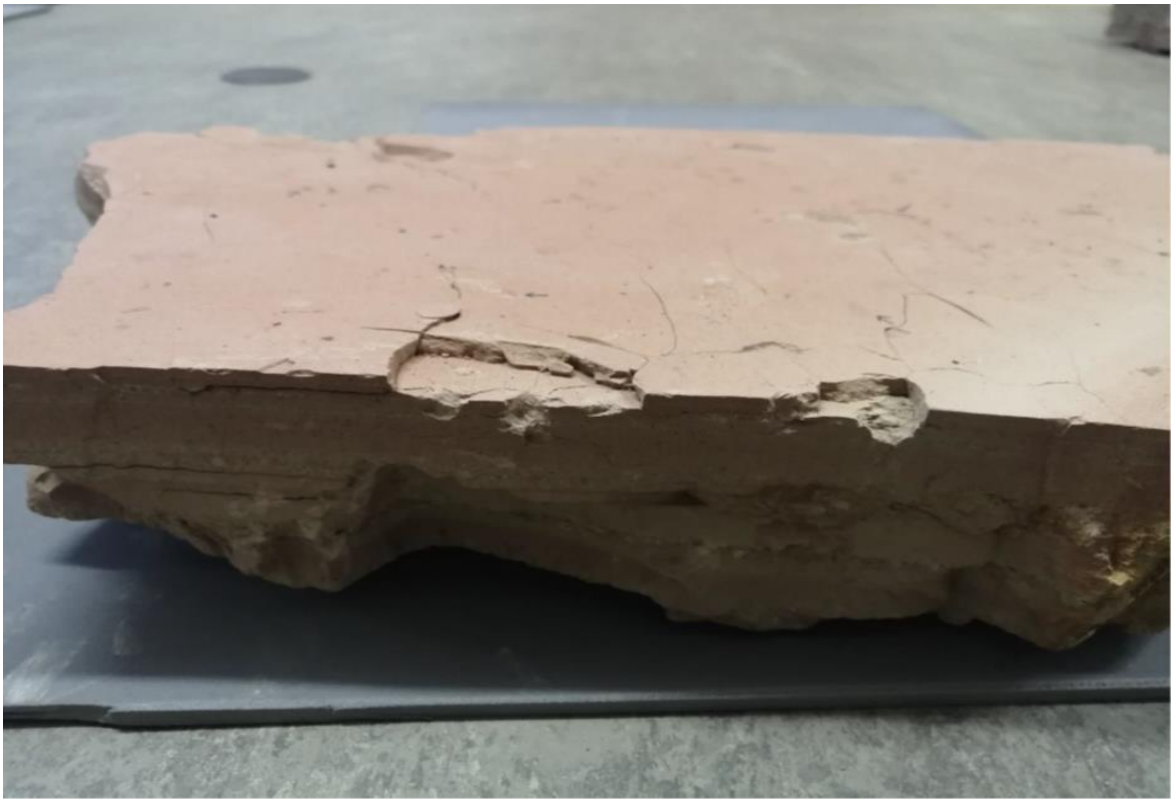


Figure 2.5 Pink sediments (BP/2/26533) from Orapa Diamond Mine in Botswana. The block is housed at the Evolutionary Studies Institute (ESI), University of the Witwatersrand.



Figure 2.6 Cream sediments (BP/2/17890a) from Orapa Diamond Mine in Botswana. The block is housed at the Evolutionary Studies Institute (ESI), University of the Witwatersrand.

The green unoxidised sediments were interpreted as being formed in deep-water, while the brown-red oxidised sediments were interpreted as being deposited in shallow, oxygen-rich conditions (McKay 1990). The brown-red sediments would have been deposited on the shore of the ancient lake at ODM. Fossil insects and plants on the green and brown-red sediments are preserved as coalified compressions; possibly with the remnants of the cuticle, while those in the pink and cream sediments are preserved as impressions, supporting the notion that they were subjected to a post depositional action of the ground water. These differently-colored fossiliferous lacustrine sediments contain interesting varve-like structures, mud or desiccation cracks, and calcite layers which are described in more detail below.

2.3 Varve-like structures

Varves form in aquatic depositional environments. They result in clastic, biological, and chemical sedimentary processes caused by seasonal variation (Wohlfarth 1996). Varves usually form under unoxidised conditions. At ODM, they are thin layers, light in color, which produce pairs of laminations usually under 0.5 mm thick . They have light and dark layers; the light layer was formed by coarse silt and fine sand that would have been deposited in spring and summer, while the dark layer was formed by clay and fine silt that that would have accumulated during the winter season (Greenwalt et al. 2014). Laminations can also be formed by microbial, bacterial, and algal blooms (Greenwalt et al. 2014). According to Smith (1986), laminations in some of the mudstones overlying the Stompboor kimberlite may have been formed in seasonal conditions. Light layers may have formed in summer, when plant growth and evaporation would have resulted in calcite being deposited on the lake bottom. Similar seasonal laminations are known from Quaternary lakes (McKay 1990).

2.4 Un-laminated mudstones

The un-laminated mudstones vary in color, and are the same as those that overlie the mudflows (McKay 1990). Some are brown-red in color, and on a few occasions, even have many small pits that look like impressions left by raindrops (McKay 1990). These un-laminated mudstones preserve clusters of insect remains in high concentrations, many with their wings stretched out (McKay 1990). According to McKay (1990), the red color of the mudstones and raindrop impressions suggest that they were deposited in shallow, near shore, oxidising conditions.

The sedimentary sequence in lacustrine sediments in general includes interbedded mudflows, sandstones, and massive mudstone (McKay 1990). The laminated and un-laminated mudstones preserve a great number of fossil fauna and flora (McKay 1990).

2.5 Calcite layers

The thick calcite layers consist of fibrous coarse calcite crystals that were deposited during dry periods when the lake level dropped (Smith 1986). Calcite layers and mud-cracks are sometimes found alongside deep water sediments, which suggests that there were times when the lake dried up completely (McKay 1990). Calcite layers were possibly deposited after the lacustrine sediments had lithified. This prediction is suggested by the calcite layers that run diagonally over the bedding

planes (McKay 1990). These calcite layers may have crystallised from ground water in cracks and joints (McKay 1990).

2.6 Raindrop impressions

As already mentioned above, some of the un-laminated mudstones are covered with tiny pits that look like the impressions raindrops leave behind in soft mud. The raindrop impressions may be rich in evaporite crystals (McKay 1990).

2.7 Mud-cracks

When mud dries, mud-cracks may form. These are also known as desiccation cracks, and they are sometimes found in the sediments from Orapa (Dobbs 1978; and McKay 1990). It can be difficult to tell the difference between the mud-crack which formed 90 million years ago and those that are seldom formed in modern muddy conditions. They are often found to be associated with evaporite layers of calcite (Dobbs 1978; and McKay 1990). They are used in geology to interpret the original orientation of a rock (to detect which side is up). Mud-cracks are often indicative of predominately sunny or shady environments (Kindle 1917). Fast drying (prominent in sunny environments), yield widely-spaced, irregular mud-cracks (Kindle 1917; and Weinberger 1999). Slow-drying (prominent in shady environments), results in closer-spaced, regular mud-cracks (Kindle 1917).

Unfortunately, these fine-grained lacustrine sediments have been removed by mining operations, and have been dumped in a small spoil heap at the mine. More effort is needed to preserve the heritage of the mine.

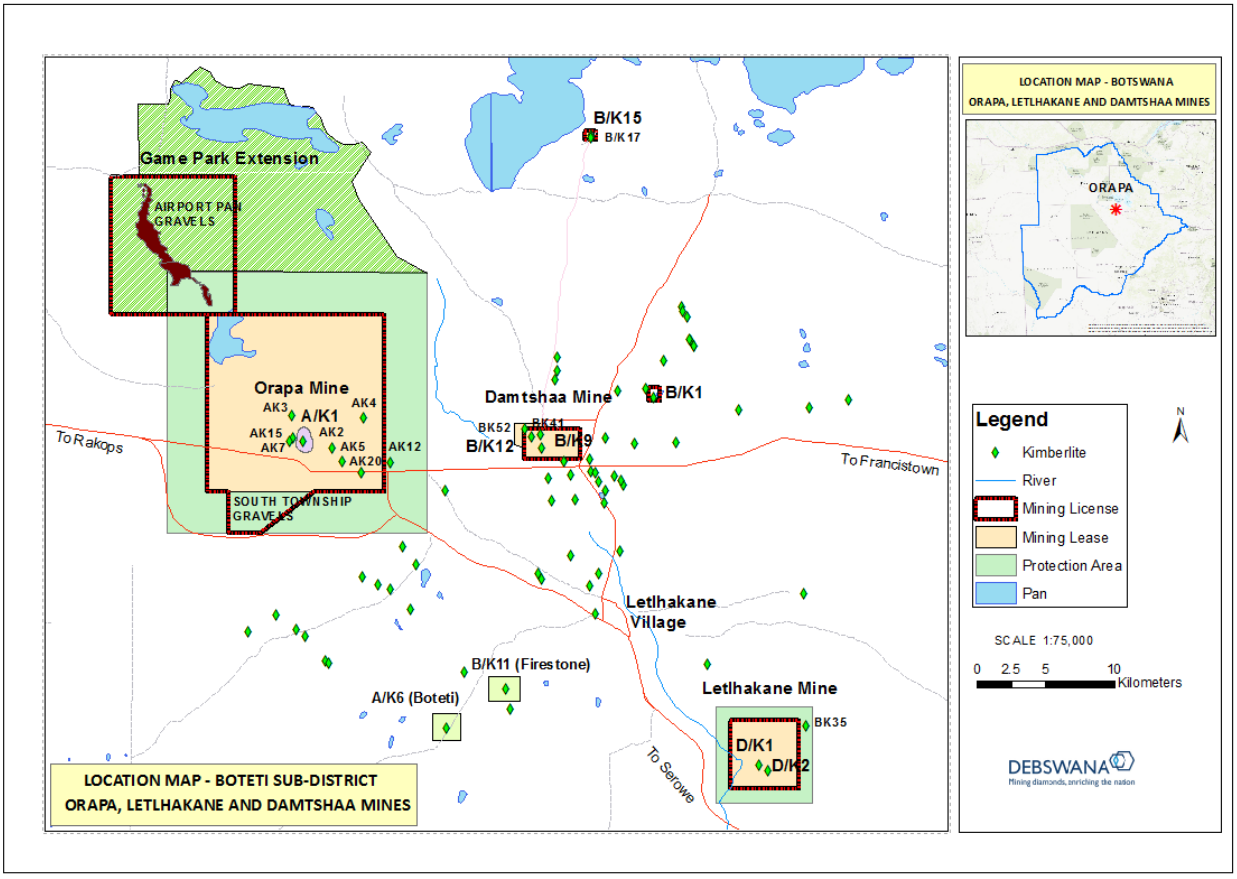


Figure 2.7 Botswana's kimberlite fields and craton geology. Taken from Mashabila (2019, page 13).

CHAPTER THREE

DATING METHODS USED AT ORAPA DIAMOND MINE

3.1 Introduction

The date of the ODM kimberlite has been estimated using the age of the kimberlite, the dating of fossils within the lacustrine sediments, and the estimates of rapid deposition of sediments in the crater.

3.2 Dating of the kimberlite

The age of the kimberlite at the ODM was estimated using two methods based on the decay of extracts from radioactive low-uranium zircons ^{238}U into ^{206}Pb (Davis 1977; and Haggerty et al. 1983). These were: low-contamination hydrothermal U/Pb dating, and fission track dating (Haggerty et al. 1983) (Figure 3.1). According to Davis (1977), the ODM kimberlite dates to 93.1 Ma, while according to Haggerty et al. (1983), it dates to 87.4 ± 5.7 to 92.4 ± 6.1 Ma. It is worth noting that, according to Rayner (1987), the methods indicate the time of eruption of the kimberlite.

If we take the error range in Haggerty et al. (1983) into account, the kimberlite at the ODM is aged between 81.7 and 98.5 Ma, with a midpoint of 90.1 Ma. This midpoint is very close to the estimated age of 93.1 Ma in Davis (1977). The kimberlite at the ODM is, therefore, probably Turonian, but possibly Cenomanian (on the upper side of the error range), or Coniacian (on the lower side of the error range) in age. In general, kimberlite pipes found within the Kaapvaal Craton date between 95.4 and 81.7 Ma (Davis 1977).

There are also other kimberlite pipes (Table 3.1) that have been used as a proxy.

Table 3.1: Estimated ages of kimberlite pipes of sites in Botswana, Lesotho and South Africa. Taken from Davis (1977, page 82).

Kimberlite pipe (deposit)	**Estimated age (Ma)**
Bultfontein, South Africa (duplicate)	81.7

Bultfontein, South Africa	83.8
Kampfersdam, South Africa	86.9
Mothae, Lesotho	87.1
Wesselton, South Africa	90.3
Monastery, South Africa	90.4
Bultfontein, South Africa	91.2
De Beers, South Africa	92.0
Roberts Victor, South Africa	92.2
Orapa, Botswana	93.1
Leicester, South Africa	93.6
Finsch, South Africa	94.1
Kimberley Pool, South Africa	95.4

3.3 Dating of the sediments

The sediments were dated using plant macrofossils, and fossil pollen (palynomorphs) (Bamford 1990; and McKay 1990). Bamford (1990) reported on an angiosperm species assigned to the genus *Sapindopsis*, which has only been recorded from the Albian to Cenomanian (105-93 Ma) age.

Palynology analysis by Scholtz (1985, cited by Bamford 1990; McKay 1990; Rayner 1993; and Rayner et al. 1991, 1997), also reported on *Ephredripites*, a tricolpate and *Classopollis cf jardinei* types, which indicate a Mid-to-Late Cretaceous age, with a fairly dry climate [i.e. not older than Albian (100 Ma), or younger than mid-Senonian (75 Ma)] (Figure 3.1). Furthermore, Rayner et al. (1991) have argued that the low abundance of pollen may favor the older, rather than younger age.

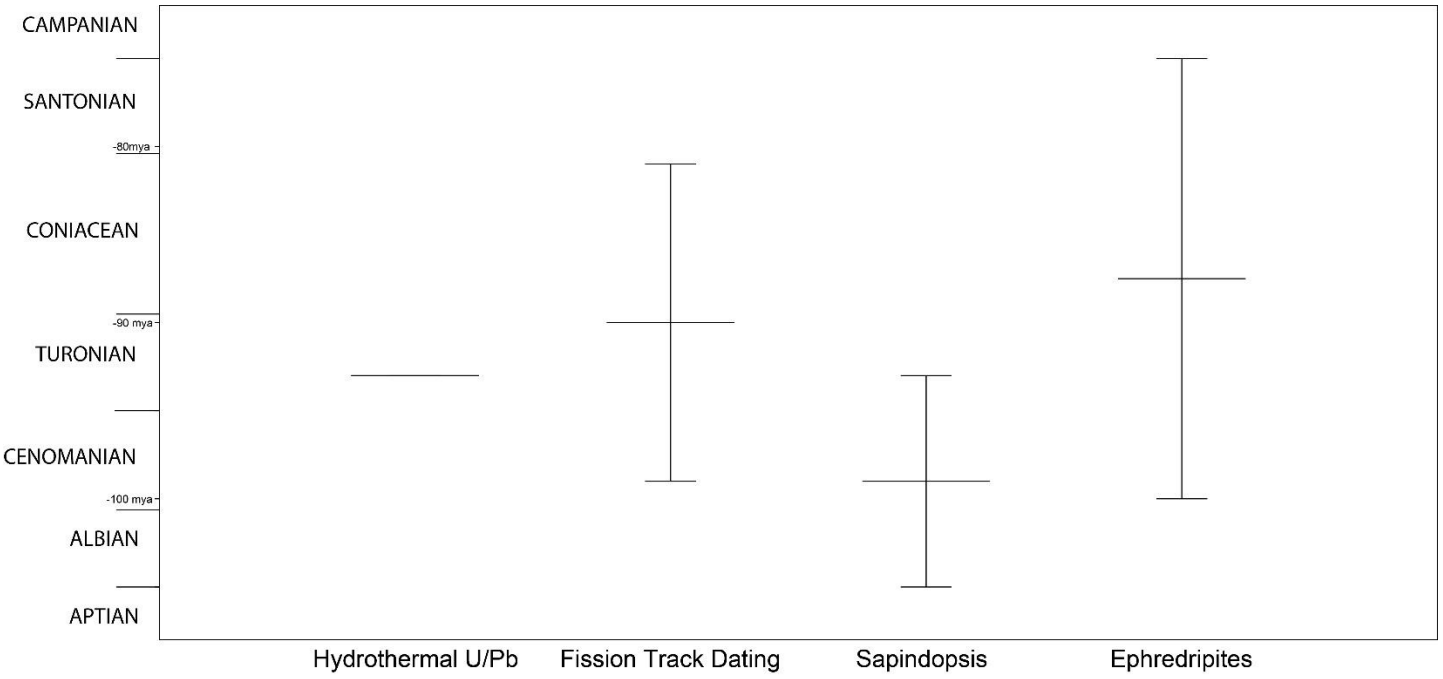
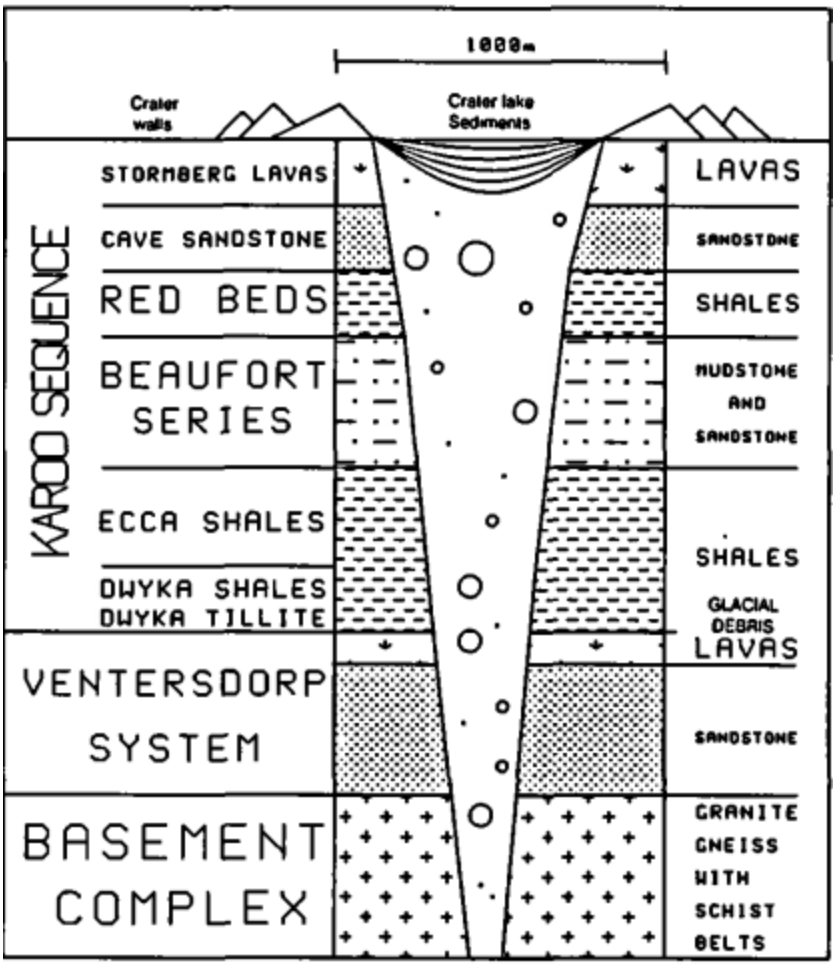


Figure 3.1 Figure showing the methods used to date the ODM in Botswana. Adapted from Rayner et al. 1991.

3.4 Rate of sedimentation

Kimberlitic sediments are known to be very unstable in nature. Mud and debris flows are almost instantaneous. The rate of sedimentation would have likely been rapid, provided that the velocity of the mud and debris flow were high (McKay 1990). Dobbs (1978), based on observations of sedimentation rates in similar alluvial fan environments, estimates the rate of sedimentation in the ODM crater to have been between 311 000 to 1 842 1015 million years. In addition, Smith (1986) estimates, based on annual laminations, or varves at Stompboor kimberlite pipe give a rate of sedimentation of approximately 220 000 years for 76 m of sediments, and 440 000 years for 140 m of sediments that were deposited at ODM. This, therefore, suggests that the age difference between the formation of the kimberlite, and the sediment infilling was probably not significant (Figure 3.2). However, since there was probably a period of erosion, there were periods when there was no deposition at all. As such, to date, there is no independent check on the estimates.


1000m
Crater walls
Crater lake Sediments
KAROO SEQUENCE
STORMBERG LAVAS
CAVE SANDSTONE
RED BEDS
BEAUFORT SERIES
ECCA SHALES
DWYKA SHALES
DWYKA TILLITE
VENTERSDORP SYSTEM
BASEMENT COMPLEX
LAVAS
SANDSTONE
SHALES
MUDSTONE AND SANDSTONE
SHALES
GLACIAL DEBRIS
LAVAS
SANDSTONE
GRANITE GNEISS WITH SCHIST BELTS

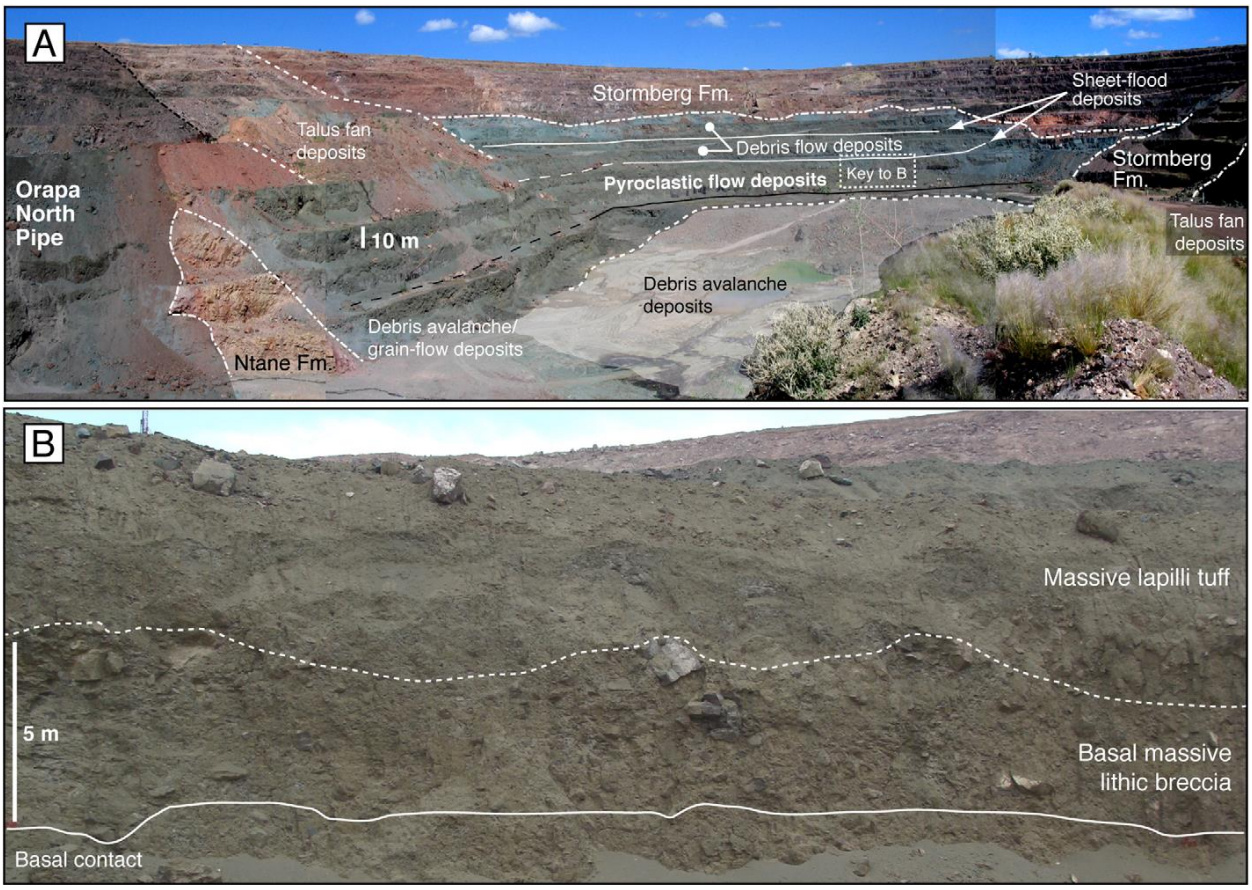


Figure 3.2 Figure showing the kimberlite pyroclastic flow. Taken from Rayner et al. (1991, page 148) and Gernon et al. (2009, page 568).

CHAPTER FOUR

PREVIOUS PALAEOENTOMOLOGICAL RESEARCH AND PALAEOENVIRONMENT AT ORAPA DIAMOND MINE

4.1 Introduction

Following on from an initial collection of fossil insects described by Dobbs (1978), a group of scientists from the then Bernhard Price Institute (BPI) of Palaeontology at the University of the Witwatersrand, Johannesburg, South Africa were invited to visit ODM in 1983, and collected material uncovered by mining operations. This initial visit was followed by visits in 1986, 1987, and 1988 (McKay 1990), and a more recent trip by Dr I.J. McKay and Mr. S. Mnguni in June 2018. The result of all these trips was approximately 6 800 lacustrine mudstone blocks housed in the Herbarium of the ESI, and 220 lacustrine mudstone blocks housed in the Gabarone Museum, Botswana. In addition, there are over 100 lacustrine mudstone blocks collected in the most recent trip that are still in need of cataloging.

4.2 Review of the Orapa Diamond Mine collection

According to McKay (1990), the collection from the ODM comprises of 51% insects, 44.6% plants and 4.4% unidentifiable specimens, based on examining the entire collection. Brothers and Rasnitsyn (2003) provide a detailed description of the generally well-preserved insect taxa they found on blocks that include Hymenoptera. They recorded 55% insects, 40% plants and 5% unidentifiable specimens. A more detailed breakdown of their analysis is given in Table 4.1. Generally, the collection lacks termites and bees, while spiders occur in low numbers. Also, no aquatic or terrestrial vertebrate skeletons have been discovered at ODM (McKay 1990), possibly owing to the acidic conditions in the lake that were not conducive for their preservation.

The assemblage is largely dominated by terrestrial insects (McKay 1990; and Brothers and Rasnitsyn 2003). The major insect orders such as Coleoptera, Hymenoptera, Diptera, Hemiptera and Blattodae are well represented (McKay and Rayner 1986; Rayner and McKay 1986; and Rayner et al. 1991, 1997). There are also minor insect orders such as Orthoptera, Thysanoptera,

Dermaptera and Neuroptera (McKay and Rayner 1986; Rayner and McKay 1986; and Rayner et al. 1991, 1997; and Brothers and Rasnitsyn 2003). More importantly, particularly for this project, the most common insect order is the Coleoptera. This is partly because beetles are the most speciose insect group on Earth, and they tend to preserve better than other insect groups because of their richness and diversity, robust exoskeleton and elytra (Grimaldi et al. 2005).

Table 4.1: The assemblage of the Orapa insect fauna by order based on selecting 68 blocks with Hymenoptera. Taken from Brothers and Rasnitsyn (2003).

Taxa	**Number of specimens**	**Percentage**
Beetles (Coleoptera)	183	28.6
Leaf hoppers, etc., (Hemiptera)	115	17.9
Cockroaches (Blattodea)	112	17.5
Flies, mosquitoes, etc., (Diptera)	81	12.6
Thrips (Thysanoptera)	45	7.0
Wasps, bees, ants, etc., (Hymenoptera)	40	6.2
Crickets, locusts, etc., (Orthoptera)	38	5.9
True bugs (Hemiptera)	17	2.6
Earwigs (Dermaptera)	3	0.5
Moths (Lepidoptera)	3	0.5
Bark-lice (Psocoptera)	2	0.3
Dragonflies, damselflies, etc., (Odonata)	1	0.2
Spiders (Arachnida)	1	0.2
Total	**641**	**100**

Bamford (1990) investigated the fossil flora of ODM. Using trees, Bamford (1990) described 27 genera and 28 new species of leaves, three genera and three new species of flowers, together with 13 genera and 14 new species of seeds and fruits. Plants present in the collection from ODM include angiosperm leaves (45%), angiosperm seeds (17%), angiosperm stems (15%), angiosperm flowers (14%), and Pteridophytes (9%). There is also a small amount of charcoal found in the collection (Bamford 1990), suggesting that there was a fire at the time.

Additionally, pollen grains were studied by Scholtz (1985), and were identified as Araucariaceae or Cupressaceae types. There were *Ephredripites* sp. and *Classopollis* cf. *jardinei* that indicated a Mid to Late Cretaceous age. With these findings, Bamford (1990) postulated that the environment at ODM was tropical to subtropical, with seasonal rainfall, while the micro-environment around the crater-lake probably had successional scrub vegetation around the unstable sides.

McKay (1990) used carabid beetles to postulate that the environment at Orapa was tropical to temperate (i.e., seasonal with cool winters), warm and very moist, with substantial seasonal rainfall. The insect fauna is extremely variable, but many of the taxa described belong to diverse groups that either provide no palaeoenvironmental information, or are not considered to be good palaeoenvironmental indicators (McKay 1990; and Rayner et al. 1991, 1997). However, recording phytophagous, saprophagous and mycophagous insects suggests a steady vegetation cover (McKay and Rayner 1986). In addition, the presence of flies from the family Bibionidae indicate seasonality with high humidity, forestry microenvironment condition that would have been adjacent to water (Rayner et al. 1991).

McKay (1990) proposed that the general lack of aquatic insects in the crater-lake suggests that the water was unfavorable for faunal and floral material, and that the few adult aquatic insects possibly flew in from nearby habitats. These include the Zygoptera, and possible aquatic beetles from the family Coptoclavidae. It is probable that the crater-lake may have been toxic with excessive salinity, and this is supported by numerous evaporite layers within the sediments (McKay and Rayner 1986).

The poisoning of the crater-lake is supported by the lack of remains of other aquatic animals, including aquatic insect larvae (McKay 1990). In addition, the general lack of dragonflies, caddisflies, mayflies, and stoneflies, which are used to assess the ecosystem health of aquatic environments, is another line of evidence indicating that the ODM crater-lake was probably poisoned, although there are several Zygoptera that have been recorded in the collection.

It is possible that a more thorough analysis of the sediments has the potential to contribute to a better understanding of the water quality at ODM during the Cretaceous age, by revealing indicator organisms such as ostracods and diatoms (McKay, personal communication). Therefore, a geochemical and geophysical analysis is warranted. This offers an opportunity for collaboration in the future. However, the presence of possible aquatic Diptera and Coleoptera larvae observed in

the ODM collection when conducting the taphonomical analysis of this study challenges the hypothesis that the water was consistently toxic.

4.3 Published research from Orapa Diamond Mine

A summary of all the research completed on the fossil fauna and flora from the Orapa Diamond Mine in Botswana is given in Table 4.2.

Table 4.2: Table showing all the research completed on the fossil fauna and flora from the Orapa Diamond Mine in Botswana.

Publication	**Source**
Cretaceous fossil insects from Orapa, Botswana.	McKay and Rayner 1986
The treasure chest of the Orapa Diamond Mine.	Rayner and McKay 1986
March flies from an African Cretaceous springtime.	Rayner 1987
A new hybotine dipteran from the Cretaceous of Botswana.	Waters 1989a
A Cretaceous dance fly (Diptera: Empididae) from Botswana.	Waters 1989b
A new aphid from the Cretaceous of Botswana.	Rayner and Waters 1989
The angiosperm palaeoflora from the Orapa pipe, Botswana.	Bamford 1990
Cretaceous Carabidae (Coleoptera) from Orapa, Botswana.	McKay 1990
A Cretaceous crane-fly (Diptera: Tipulidae): 93 million years of stasis.	Rayner and Waters 1990
Cretaceous Diptera from Orapa, Botswana.	Waters 1990
Cretaceous Promecognathinae (Coleoptera: Carabidae): a new genus, phylogenetic reconstruction and zoogeography.	McKay 1991

The mid-Cretaceous palaeoenvironment of central Southern Africa (Orapa, Botswana).	Rayner et al. 1991
The first Mesozoic Vespidae (Hymenoptera) from the Southern Hemisphere, Botswana.	Brothers 1992
The fossils from the Orapa Diamond Mine: A Review.	Rayner 1993
Cretaceous weevils from southern Africa, with description of a new genus and species and phylogenetic and zoogeographical comments (Coleoptera: Curculionoidea).	Kuschel et al. 1994
Cretaceous weevils from southern Africa, with description of a new genus and species and phylogenetic and zoogeographical comments (Coleoptera: Curculionoidea).	Rayner et al. 1994
Cretaceous fossils from the Orapa Diamond Mine.	Rayner et al. 1997
Diversity of Hymenoptera and other insects in the Late Cretaceous (Turonian) deposits at Orapa, Botswana: a preliminary review.	Brothers and Rasnitsyn 2003
The first Late Cretaceous ants (Hymenoptera: Formicidae) from southern Africa, with comments on the origin of the Myrmicinae.	Dlussky et al. 2004
Two new hymenopteran fossils from the mid-Cretaceous of southern Africa (Hymenoptera: Jurapriidae: Evaniidae).	Rasnitysn and Brothers 2007
A new genus and species of Euparagiinae from the Late Cretaceous of southern Africa (Hymenoptera: Vespidae).	Brothers and Rasnitsyn 2008
New genera and species of Maimetshidae (Hymenoptera: Stephanoidea s.l.) from the Turonian of Botswana, with comments on the status of the family.	Rasnitsyn and Brothers 2009

Two new labenopimpline ichneumonids (Hymenoptera: Ichneumonidae) from the Upper Cretaceous of southern Africa.	Kopylov et al. 2010
The first scarabaeid beetle (Coleoptera, Scarabaeidae, Melolonthinae) described from the Mesozoic (Late-Cretaceous) of Africa.	Woolley 2016

Summary of the palaeoenvironment of Orapa Diamond Mine:

Previous research on Coleoptera and other invertebrates at ODM identified a number of taxa. Phytophagous and predaceous groups (e.g., gryllids, heteropterans, carabids, scarabids, elateroids, staphylinids, curculionids, and dipterans) suggests that there was continuous vegetation cover in the crater (McKay and Rayner 1986; Rayner and McKay 1986; Waters 1989; Bamford 1990; McKay 1990; Rayner and Waters 1990; McKay 1991; Brothers 1992; Rayner et al. 1991, 1994, 1997; Kuschel et al. 1994). Many of the insects are preserved whole, suggesting lack of scavengers (McKay and Rayner 1986; and Rayner and McKay 1986). The crater-lake may have been toxic (i.e., there is no evidence of bioturbation) (McKay and Rayner 1986; Rayner and McKay 1986).

Insects indicate that the climate was strongly seasonal, with a warm, high humidity, wet summer, and a dry, cold winter, while some have suggested that it was seasonal, temperate, with spring and summer rains. For example, fossil insects such as gryllids, orthopterans, dipterans, heteropterans, carabids, curculionids, blattids, hymenopterans; and staphylinids imply a strongly seasonal, with a warm, wet summer, and a dry, cold winter (Rayner and McKay 1986). Bibionids indicate a humid and cool climate (Rayner 1987). An aphid indicates a seasonal and temperate climate, with spring and summer rains (Rayner and Waters 1989). Lastly, carabids imply that the climate was tropical to temperate (i.e., seasonal with cool winters), warm and very moist, with substantial seasonal rainfall (McKay 1990).

Plants indicate that the climate was tropical to subtropical, with seasonal rainfall, while the micro-environment around the crater-lake probably had successional scrub vegetation around the unstable sides, although the crater was probably forested, densely or heavily vegetated, or well-

wooded, with rocks and leaf litter near the lake, at the time of deposition (Bamford 1990; Waters 1990; Rayner et al. 1991, 1994, 1997; and Kuschel et al. 1994).

Therefore, both insects and plants indicate that the climate at Orapa was tropical, subtropical to temperate (i.e., seasonal with cool winters), warm and very moist, with substantial seasonal rainfall, while the surrounding area was largely forested.

There is very little known about the palaeoenvironment, paleoclimate and geology of Africa during the Cretaceous age. Generally, on a global scale, it is widely accepted that the climate during the Cretaceous age was arid, warm, humid with seasonal rain and wind patterns (de Souza Carvalho et al. 2010). As such, the climate at the time has been compared to greenhouse environmental conditions. Moreover, more analysis of the fossil fauna and flora from ODM concurs that there are remarkable faunal and floral interconnections between southern Africa and northeastern Laurasia.

CHAPTER FIVE

TAPHONOMY OF THE ORAPA DIAMOND MINE

5.1 Introduction

Taphonomy (from Greek *taphos*, a tomb) deals with patterns of the fossil record. Essentially, taphonomy investigates the processes which affect how an organism becomes a fossil. It is defined as the "study of the transition of animal remains from the biosphere to the lithosphere" (Smith 2012). Taphonomic processes generally involve two stages. The first stage happens between death and burial (termed biostratinomy), while the second stage occurs between burial and being recovered by a palaeontologist (termed diagenesis) (Lyman 2010). However, there is also pre-death filtering, where factors like ecology, behavior, and morphology affect the likelihood of an insect dying in a locality, with fossilisation potential in the first place (Smith 2012). Once an organism dies, it changes (*via* biological, physical, and chemical processes), before and after burial, and this affects the information reserved in the fossil record (Lyman 2010).

Fossil insects, possibly dating back to the Devonian (Grimaldi and Engel 2005), are preserved in many different palaeoenvironments, including rivers, lakes, lagoons, deltas, forests and seas (Smith 2012). They have been found in sediments deposits (including crater-lake sediments like those at the ODM), amber, peat, tar, calcium carbonate precipitates and chert (Smith 2012). Insects preserved in fossilised tree resin, usually of gymnosperm origin, dating back to the Early Cretaceous, preserve the finest detail, and may be compared directly to extant species, like the description of *Octavius electrospinosus* Clarke and Chatzimanolis, 2009 (Clarke and Chatzimanolis 2009), which was described from the Burmese amber of Myanmar.

However, insects preserved in amber do not sample the true insect diversity, as they tend to be species associated with forests (Smith 2012). Fossils preserved in lacustrine sediments sample a wider variety of habitats, and can also preserve extraordinary detail (Smith 2012; and Liu et al. 2021). The fossils preserved in lacustrine sediments from the ODM Lagerstätten fall into this category, and may often preserve the fine details of specimens (McKay and Rayner 1986; Rayner

and McKay 1986; and Rayner et al. 1991, 1997). There are still numerous questions outstanding regarding the taphonomy of the ODM fossils. For example, do the brown-red and green lacustrine sediments possibly reflect different depths of water and environments? why are there many whole-bodied insects present in the collection? Why are there few aquatic insects that are preserved?

Smith (2012) developed a useful model detailing taphonomic processes that influence insect preservation in lacustrine environments, and these are similar to those that came into play at the ODM. These are partly based on actualistic studies targeting the preservation of insects in modern settings, and laboratory experiments (Martínez-Delclòs and Martinell 1993; and Smith et al. 2006), and partly on extensive studies, targeting the preservation of fossil insects in Cenozoic lakes (Smith 2012). This model (Figure 5.1) is used to guide the discussion that follows.

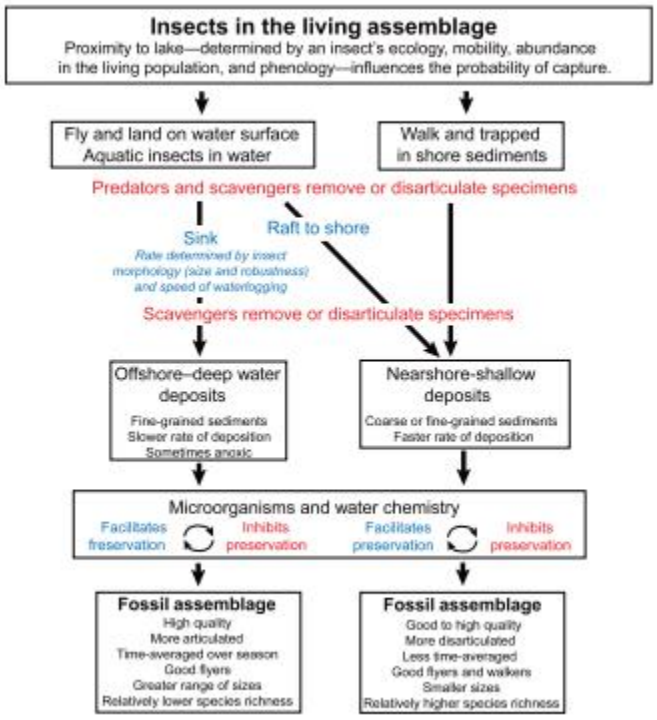


Figure 5.1 A model that depicts the main taphonomic controls on insect preservation in lacustrine settings. Taken from Smith (2012, page 349).

5.2 Ecology of a crater-lake

Smith (2012) described the most important determinants that contribute towards the preservation potential of all insects. Insect ecology is a variable which determines whether an insect will reach a lake in the first place, and this includes factors such as habitat, feeding preference (diet), abundance, phenology (periodic events), and mobility. A good example at the ODM is an abundance of bibionid flies, which were apparently swarming on the lake shore, resulting in them becoming trapped in lake, and ultimately fossilised (Rayner 1987).

5.3 Entry into the lake (landing on water surface or becoming trapped in nearshore sediments)

Lacustrine environments vary from small to large in size, ephemeral to long-lived, and open to closed systems (Smith 2012). They can either be saline or freshwater; with variable nutrient content, physico-chemical properties, and temperature measures (Cohen 2003).

When insects land on the water surface, and are not fed upon, or scavenged, they may either sink to the bottom of the lake (depending on factors such as size, robustness, and waterlogging), or be rafted to the side of the lake, and become trapped on the periphery. Alternatively, they may experience decay, which completely removes them from the record. If they are trapped on the periphery, then that is where they will stay (Martínez-Delclòs and Martinell 1993). That is where they will become preserved, if they are not predated upon, or scavenged (Smith 2012).

Generally, most insects float on the surface of the water. Insects floating on the surface of the water may be included in a mat, and be transported to the bottom with it (Martínez-Delclòs and Martinell 1993). The length of the floating duration of an insect depending on their density, wing size, body size (subsequent tracheal system size), and whether the insect entered the surface of the water alive, or dead (Smith 2012). Small insects tend to outnumber large insects on the surface of the water (Smith 2012), and denser insects break the water surface tension faster than less dense insects (Martínez-Delclòs and Martinell 1993). Insects that enter the surface of the water alive actively inhale the water through their tracheal system, and drown, and this inevitably increases their density, reducing their floatation duration (Martínez-Delclòs and Martinell 1993). Furthermore, larger, and more complex tracheal systems result in faster flooding, rapid density increase, and an even shorter floatation duration (Martínez-Delclòs and Martinell 1993). In addition, it has been

shown that any incident that destabilises the surface tension of the water surface (e.g., heavy rain) causes all insects to sink to the bottom (Martínez-Delclòs and Martinell 1993).

It is possible that insects which spend extended periods of time in water may experience decay, which removes them from the record (McKay 1990; and Smith 2000, 2012). The decay process may get hindered by environmental factors such as hyper-salinity, or low temperatures, which slow down metabolic processes of decomposition bacteria (Martínez-Delclòs and Martinell 1993). Alternatively, when insects experience longer floatation durations, they may become surrounded by microbial biofilms that protect them from most physical damages (Smith 2000, 2012).

Once an insect has broken the surface tension of the water surface, it may sink slowly, and vertically; and the time of descent depends on its density, and water depth (Martínez-Delclòs and Martinell 1993). It is at this stage that insects may be fragmented by natural enemies (i.e., predators, parasites, parasitoids, and scavengers) (Martínez-Delclòs and Martinell 1993). Research has shown that complete and articulated insects of the same taxa tend to typically fall, rest and become buried in similar positions (Martínez-Delclòs and Martinell 1993).

Finally, insects reach the sediment surface and become buried. This is where the depositional environment (e.g., salinity, oxygen levels, sediment composition, and rates of sedimentation), as well as the robustness of an insect come into play (Martínez-Delclòs and Martinell 1993). During this stage, the absence of benthic scavengers and bioturbators results in a completely articulated specimen (Martínez-Delclòs and Martinell 1993). A rapid burial is considered to be necessary for exceptional preservation, but many researchers have recently argued that early mineralisation is the most important factor (Martínez-Delclòs and Martinell 1993; and Smith 2000, 2012).

Insects may be deposited in fine sediments that had slow deposition; or they may be deposited in coarser sediments that had a faster rate of deposition (Smith 2012). All these factors influence the preservation quality of specimens, disarticulation, and time-averaging in fossil assemblages (Smith 2012).

The most common disarticulation spots are between: (1) head and thorax, (2) the head and base of antennae, (3) the antennal segments, (4) the thoracic segments and legs, (5) the articulated parts of the leg, (6) the prothorax and wings and (7) the metathorax and abdomen (Rasnitsyn and Quicke 2007).

Actualistic studies have provided more insights that the disarticulation of appendages varies significantly with the following patterns: (1) antennae disarticulate fast and remain on the surface, (2) legs disarticulate from the coxae or trochanter, (3) wings are the last appendages to detach, even when the other parts of the body have disarticulated, (4) heads almost always disarticulate at a later stage and, (5) thorax is the last part of the body to disarticulate (Carpenter et al. 1992).

5.4 Different sedimentation conditions in offshore vs nearshore deposits (rate of sedimentation, grain size, and anoxic vs oxic conditions)

There are differences between nearshore and offshore environments. Generally, it is thought that sediments in deeper water tend to be finer grained in structure. Insects that were slowly buried in these finer grained sediments, which would have been found in anoxic conditions, may be better preserved than those insects that were rapidly buried in coarser, or fine sediments, which would have been found in shallower, oxidising conditions (Wilson 1988; McKay 1990; and Smith 2012).

5.5 Microorganisms and water chemistry facilitate or inhibit preservation

Biofilms created by diatoms coat and protect insects from agents of decay and decomposition (McKay 1990; Smith et al. 2006; and Smith 2012). Microenvironment and organics in a depositional environment also facilitate the mineralisation of insect soft tissues (Smith 2012). Research has shown that if mineralisation occurs soon after burial, it can lead to three-dimensional preservation (Smith 2012). Thereafter, sinking (which is seldom determined by size and robustness of the insect), and the rate at which an insect becomes waterlogged, further enhances or facilitates the preservation quality (Smith et al. 2006).

5.6 Difference between nearshore and offshore fossil deposits

Fossil assemblages in deep water conditions tend to contain rather strong flyers, better preservation (fine grained sediments, slow deposition, anoxic conditions), variable size range of insects, low species richness, and more articulated specimens (less exposure to predation, scavenging and other destructions) (Figure 5.1). Fossil assemblages in shallow water conditions tend to contain both strong and weak fliers, variable preservation, small size range of insects, high species richness, and less articulated specimens (more exposure to predation, scavenging and other destructions) (Figure 5.1). As a result, it is clear that water depth and distance from the shore are important factors in fossil insect preservation (Smith 2012). The type of lake, its associated sediments, water

chemistry, and sampling region within a lake influences the quality of insect taxa that become preserved (Wilson 1980, 1982, 1988; and Smith 2000).

Wilson (1988) investigated the taphonomy of insects in lacustrine deposits by comparing nearshore and offshore insect assemblages. According to Wilson (1988), lacustrine paleoenvironments can be reconstructed using evidence from sedimentology, geochemistry, stratigraphy, taphonomy, and taxonomy; and that such reconstructions of past environments are feasible through the process of uniformitarianism. For example, if insects belonging to the same taxonomic level all inhabit the same environment, or climatic regime, it is seldom safe to infer that earlier congeneric species had similar habits, and researchers can use that as a matrix to reconstruct the paleoenvironment accordingly (Wilson 1988).

Taxonomic and taphonomic datasets are considered to be very useful. Therefore, in this study in particular, the taphonomic data of insects was preliminary investigated to reconstruct the palaeoecology and palaeoenvironment of the ODM during the Cretaceous. At the ODM, the green fossiliferous sediments are thought to reflect deeper-water-anoxic-conditions, while the brown-red sediments are thought to reflect shallow-water-oxidising-conditions.

Therefore, the insect fauna of the two differing sediment types were compared in terms of body size, articulation or disarticulation, and diversity of insect taxa. The taphonomic assessment was limited to 100 differently-colored, randomly-selected lacustrine mudstone blocks. The results of this work have been subsequently discussed in greater detail in chapter 8 of this study.

CHAPTER SIX

REVIEW OF THE FOSSIL RECORD OF THE STAPHYLINIDAE FROM ORAPA DIAMOND MINE

In this chapter, I give a brief overview of some of the proposed classification and phylogenetic schemes of the Staphylinidae. This is followed up by a review of the molecular clock estimates, detailing the appearance of the staphylinid clades; the summary of the fossil record for this hyper-diverse group; and a more detailed review of selected subfamilies of the Staphylinidae, which have been found at ODM, and are described in chapter 8: Steninae, Tachyporinae, and Paederinae.

A phylogenetic analysis based on the morphological characters of the fossil Staphylinidae from ODM is useful for assigning the specimens into appropriate subfamilies (e.g., Beutel and Leschen 2005; Grebennikov and Newton 2009; Bogri et al. 2020; and Shaw et al. 2020). However, it has its own challenges, including lack of consistency in selecting appropriate subfamilies, models and methodologies. Other challenges include the grouping of selected subfamilies, calibration steps, topologies which impact node ages and divergence estimates. In addition, the lack of agreement about using stem and crown fossils for mixed types of data has been contentious (Ware and Barden 2016). Taking this into account, a phylogenetic analysis based on the morphological characters of the fossil Staphylinidae from ODM forms part of future studies. It is beyond the scope of this project.

6.1 Classification and phylogenetic schemes

Making sense of the enormous diversity of staphylinids has been an ongoing enterprise for many years. The classification of Lawrence and Newton (1982) based on traditional larval and adult morphological characters paved the way. This scheme was modified by Lawrence and Newton (1995, cited by Thayer 2005; Zhang and Zhou 2013; McKenna et al. 2014; and Liu et al. 2021), and consolidated by many other authors, using traditional morphological characters in rigorous phylogenetic settings (e.g., Beutel and Leschen 2005; Clarke and Grebennikov 2009; and Grebennikov and Newton 2009, 2012).

In recent years, more especially in the last decade or so, previous research has been expanded, uncovering and explaining the evolutionary history of the Staphylinidae, and their hyper-diversity. These studies have gone beyond traditional morphological data, and used genetics and fossils of the Staphylinidae (e.g., McKenna et al. 2014, 2015, 2019; Toussaint et al. 2016; Lü et al. 2020; Baca et al. 2021; and Cai et al. 2021). Some studies have used all the different types of data simultaneously, to produce the "so called" total-evidence phylogenetics (e.g., Żyła et al. 2019, 2021). Nonetheless, Lawrence and Newton (1982, 1995) provided a firm foundation for future research. In their scheme, they identified four non-monophyletic major lineages or subgroups, consisting of subfamilies of the Staphylinidae which are described below.

However, before presenting their scheme, it is necessary to explain that the Silphidae were not always included within the Staphylinidae in literature. Their inclusion within the family has been summarised by McKenna et al. (2015), who described how these groups were discovered to be paraphyletic, proposing Silphidae to be included within the Staphylinidae. Essentially, Lawrence and Newton (1982, 1995) recognised that the Silphidae were staphylinids, and Grebennikov and Newton (2009) added the Scydmaenidae, by demoting it into Scydmaeninae. The Silphidae are commonly known as carrion beetles, and comprise approximately 187 described species (A. F. Newton unpublished database, 18-Sep-2016, cited by Lü et al. 2020). The Scydmaeninae are commonly known as ant-like stone beetles, and comprise approximately 5 252 described species (A. F. Newton unpublished database, 18-Sep-2016, cited by Lü et al. 2020).

For a long time, it was hypothesised that the Scydmaenidae was rooted deep within the Staphylinoidea, until Grebennikov and Newton (2009) reported that it does not form an independent family, but is rather a sister clade to the Steninae + Euaesthetinae lineage within the Staphylinidae. Recently, Staphylinidae, Silphidae and Scydmaenidae have been consistently grouped as a monophyletic unit, based on larval and adult morphological characters (Grebennikov and Newton 2009). Lawrence and Newton (1982, 1995) classification scheme, as updated by McKenna et al. (2014), is presented below in Table 6.1.

Table 6.1: The four non-monophyletic major lineages or subgroups of the Staphylinidae. From McKenna et al. (2014), modified from Lawrence and Newton (1982, 1995).

Taxonomic rankings	**Subgroups**	**Subfamilies**
Kingdom: Animalia Phylum: Arthropoda Subphylum: Mandibulata Superclass: Hexapoda Class: Insecta Subclass: Pterygota Superorder: Holometabola Order: Coleoptera Suborder: Polyphaga Infraorder: Staphyliniformia Superfamily: Staphylinoidea Family: Staphylinidae	Omaliine Group	Dasycerinae Reitter, 1887
		Empelinae Newton and Thayer, 1992
		Glypholomatinae Jeannel, 1962
		Micropeplinae Leach, 1815
		Microsilphinae Crowson, 1950
		Neophoninae Fauvel, 1905
		Omaliinae MacLeay, 1825
		Proteininae Erichson, 1839
		Protopselaphinae Newton and Thayer, 1995
		Pselaphinae Latreille, 1802
	Tachyporine Group	Aleocharinae Fleming, 1821
		Habrocerinae Mulsant and Rey, 1876
		Olisthaerinae Thomson, 1858
		Phloeocharinae Erichson, 1839
		Tachyporinae MacLeay, 1825
		Trichophyinae Thomson, 1858
	Oxyteline Group	Apateticinae Fauvel, 1895
		Osoriinae Erichson, 1839
		Oxytelinae Fleming, 1821
		Piestinae Erichson, 1839
		Scaphidiinae Latreille, 1806
		Trigonurinae Reiche, 1866
	Staphylinine Group	Euaesthetinae Thomson, 1859
		Leptotyphlinae Fauvel, 1874
		Megalopsidiinae Leng, 1920

		Oxyporinae Fleming, 1821
		Paederinae Fleming, 1821
		Pseudopsinae Ganglbauer, 1895
		Scydmaeninae Leach, 1815
		Silphinae Latreille, 1806
		Solieriinae Newton and Thayer, 1992
		Staphylininae Latreille, 1802
		Steninae MacLeay, 1825

The four major lineages, or subgroups presented above have consistently been shown to be non-monophyletic (Thayer 2005; Zhang and Zhou 2013; McKenna et al. 2014; and Liu et al. 2021). Using total-evidence approaches that combine morphology with molecular and fossil evidence, progressively, researchers have been able to create better corroborated, and more stable phylogenies. Nonetheless, there is yet to be a general agreement about the classification of the Staphylinidae (Coleoptera: Staphylinoidea). Examples of studies which produce divergent results are Hunt et al. 2007; Zhang and Zhou 2013; McKenna et al. 2014, 2015, 2019; Toussaint et al. 2016; Zhang et al. 2018; Żyła et al. 2019, 2021; Lü et al. 2020; Baca et al. 2021; Cai et al. 2021; Liu et al. 2021; and Song et al. 2021.

Despite disagreements over the phylogenetic framework for the Staphylinidae, it has been possible for authors to start asking, and posing evolutionary questions, particularly those related to fields like palaeoecology, palaeoenvironment, palaeogeography and palaeoclimate (e.g., McKenna et al. 2014; and Lü et al. 2020). For example, McKenna et al. (2014) studied the evolution of microhabitat and feeding habits within the Staphylinidae, while Lü et al (2020) were interested in the relationship between staphylinid diversification and climate change.

Ideally, a study that seeks to address phylogenetic relationships among the subfamilies of the Staphylinidae has to include several representative of taxa from all the recognised 33 subfamilies, and closely related families as outgroup (Irmler et al. 2018). Good examples of this would be studies by McKenna et al. (2015), Toussaint et al. (2016), Lü et al. 2020, Cai et al. 2021, and Song et al. 2021. All studies have focused on a particular group of interest, and have selected representatives taxa, and outgroups, based on the questions that needed to be answered. Thus,

many studies (e.g., Solodovnikov and Schomann 2009; Zhang and Zhou 2013; Liu et al. 2021; and Song et al. 2021) investigating the family-level relationships within the Coleoptera incorporate very few staphylinid subfamilies, and are unable to shed light about subfamily-level relationships. Fikáček et al. (2020) argue that the origin and early diversification of the Staphylinidae (Staphylinoidea: Staphyliniformia: Polyphaga) remains an area of active research.

6.2 Molecular clock estimates of fossils

Using the molecular clocks, based on estimates of number of genetic difference between taxa, it is possible to assign divergence estimates and branching events on phylogenetic trees (Zhang and Zhou 2013; and Toussaint et al. 2016). In addition, by incorporating fossils to the datasets, researchers have been able to estimate the time of divergence of major groups, thereby producing elegant, time-calibrated phylogenetic trees. As the number of studies has progressed, there has been an increase in agreement on relatedness over time. Examples of these studies are Hunt et al. (2007); Zhang and Zhou (2013); McKenna et al. (2014, 2015, 2019); Toussaint et al. (2016); Zhang et al. (2018); Cai et al. (2021); and Liu et al. (2021). Authors have developed molecular clock estimates, and examples are summarised in Table 6.2. The table highlights the notable differences in divergence estimates, one of the challenges in phylogenetics.

Table 6.2: The age estimates of the various taxonomic levels of the Staphyliniformia. Taken from Zhang and Zhou (2013), Lü et al. (2020) and Cai et al. (2021).

Taxonomic rank	**Zhang, Zhou '13**	**Lu et al. '20**	**Cai et al. '21**
Osoriinae (minus Osoriini)	146.86	107.42	-
Osoriinae (Osoriini)	146.86	117.61	-
Dasycerinae	-	121.53	-
Steninae	79.88	122.22	-
Oxyporinae	124.97	125.00	-
Pselaphinae + Protopselaphinae	-	131.36	-
Leptotyphlinae	-	134.10	-
Euaesthetinae	131.06	135.54	-
Phloeocharinae (*Charhyphus* related)	-	136.43	-

Megalopsidiinae	-	137.31	-
Mycetoporini of Tachyporinae	-	139.35	-
Aleocharinae	154.08	139.40	-
Pseudopsinae	-	142.40	-
Neophoninae	-	144.33	-
Paederinae	176.74	147.58	-
Phloeocharinae (*Phloeocharis* related)	-	151.52	-
Micropeplinae	-	151.52	-
Habrocerinae	-	153.21	-
Proteininae	-	153.74	-
Scydmaeninae	-	158.10	-
Staphylininae	176.03	160.41	-
Solieriinae	-	162.94	-
Piestinae	-	162.99	-
Ptiliidae	-	164.56	-
Trichophyinae	-	164.98	-
Trigonurinae	-	164.98	-
Tachyporinae (minus Mycetoporini)	154.08	165.00	-
Scaphidiinae	168.15	165.72	-
Olisthaerinae	-	166.58	-
Oxytelinae	126.16	168.38	-
Leiodidae (minus Coloninae)	-	170.24	-
Omaliinae-complex [Omaliinae, Empelinae, Glypholomatinae, and Microsilphinae]	168.15	170.53	-
Silphidae	-	170.97	-
Agyrtidae	-	174.00	-
Apateticinae	-	174.81	-

Hydrophiloidea (*sensu stricto*)	-	179.00	-
Histeroidea	-	181.15	-
Hydraenidae	-	181.50	-
Scarabaeoidea	-	184.65	-
Coloninae of Leiodidae	-	186.90	-
Staphylinidae (including Silphidae)	243.35	187.86	209-184
Staphylinoidea	-	194.57	258-238
Root	-	208.90	322-306

The majority of the known subfamilies of Staphylinidae originated in the Jurassic age, and it was during this period that three of the four major lineages or subgroups in Lawrence and Newton (1982, 1995) appeared (Clarke and Chatzimanolis 2009; Grebennikov and Newton 2009; Zhang and Zhou 2013; Lü et al. 2020; and Cai et al. 2021). Furthermore, some hyper-diverse subfamilies such as Aleocharinae, Paederinae, Pselaphinae, and Staphylininae (Staphylinini), possibly emerged in the Late Jurassic, or Early Cretaceous ages (Clarke and Chatzimanolis 2009; Grebennikov and Newton 2009; Zhang and Zhou 2013; Lü et al. 2020; and Cai et al. 2021). In addition, events in the Jurassic appear to have set the stage for a substantial diversification within the Staphylinidae during the Cretaceous (Thayer et al. 2012).

Lü et al. (2020) modelled the dynamics of speciation (λ) and extinction (μ) using the Markov Chain Monte Carlo (MCMC) method, which detects shifts in net diversification rates ($r = \lambda-\mu$), give a contrary position. They suggest that the net diversification rate of the Staphylinidae increased exponentially in the Early Jurassic age, peaked in the Middle-Jurassic age, was stationary in the Cretaceous age, and climbed exponentially since the Paleogene age (Lü et al. 2020). To formulate these results, Lü et al. (2020) produced a phylogeny of Staphyliniformia and Scarabaeoidea from 664 terminal taxa using six genes. In addition, they selected 50 staphylinid fossils to calibrate the divergence time of the different clades. In the future, investigating how the staphylinids from ODM fit into the Lü et al. (2020) scheme would be worthwhile.

6.3 Fossil record of the Staphylinidae on Earth

The fossil record of the Staphylinidae on Earth is described with regards to occurrences in the Mesozoic and Cenozoic ages. The main source of this information is Smith and Marcot (2015) database, which I have verified, and subsequently updated, with regards to the included taxa. Using the taxa named in Smith and Marcot (2015) database, I was able to find the deposit and age of the species that have been described, and subsequently place them to a particular subfamily in which they belong, using the literature and online resources like Google Scholar.

No fossil Staphylinidae have been formally described in the following subfamilies: Empelinae, Habrocerinae, Leptotyphlinae, Microsilphinae, and Neophoninae (Chatzimanolis 2018). However, given the extremely long evolutionary history of the Staphylinidae, it is possible that fossils for all the above-mentioned subfamilies will soon be discovered and formally described (Chatzimanolis 2018). Currently, the described fossils for subfamilies Staphylininae, Pselaphinae, Omaliinae, Paederinae, Oxytelinae, Tachyporinae and Aleocharinae is higher compared to other subfamilies. More importantly, many of the species have been described recently (e.g., Schomann and Solodovnikov 2017; Bogri et al. 2020; Shaw et al. 2020; Tihelka et al. 2020; Yamamoto 2021; and Żyła et al. 2019, 2021).

6.3.1 Triassic age

For 10 years, the oldest rove beetle known to was considered to be *Leehermania prorova* Chatzimanolis, et al., 2012 (Chatzimanolis et al. 2012). This species was reportedly found in a Late Triassic deposit in Virginia, dated between 230-220 Ma old. However, this was recently questioned and disputed by Fikáček et al. (2020), who used an extensive morphological matrix of extant Coleoptera, and placed this fossil specimen within the Myxophaga, closest to the Hydroscaphidae. Currently, there is no Staphylinidae recorded from the Triassic age. However, there are two silphids that were recorded from Molteno Formation (Table 6.3) of South Africa. The fossils are described as *Pseudosilphites triassicus* Zeuner 1931 and *Pseudosilphites natalensis* Zeuner 1961. They are poorly preserved, and thus heir assignment to the Silphidae is questionable (Crowson 1960). There is also an undescribed species from Grès à Voltzia of France (Papier et al., 2005).

Table 6.3: The Staphylinidae recorded during the Triassic. Modified from the database of Smith and Marcot (2015).

Subfamily	**Taxa**	**Deposit**
Silphinae	*Pseudosilphites natalensis* Zeuner 1961	Birds's River, Molteno Fm., South Africa
Silphinae	*Pseudosilphites triassicus* Zeuner 1961	Birds's River, Molteno Fm., South Africa
Incertae sedis	*Leehermania prorova* Chatzimanolis et al., 2012	Cow Branch Fm., United States of America
Incertae sedis	Undescribed species Papier et al., 2005	Grès à Voltzia, France

6.3.2 Jurassic age

Thirty three Staphylinidae and Silphidae species have been recorded from the Jurassic (Table 6.4). Nine of these were assigned to the Omaliinae, eight to the Tachyporinae,, three to the Oxytelinae, while Glypholomatinae, Olisthaerinae and Scaphidiinae each had two fossils assigned to them. Many of these were recovered from Karatau-Mikhailovka Galkina, a deposit that is situated near Baydibek, Turkistan Region, in Kazakhstan. Notably, no staphylinids from the staphylinine group of subfamilies have been recorded during this age. This is congruent with divergence estimates (e.g., McKenna et al. 2015). Two of the three fossil staphylinids from ODM described in chapter 8 (i.e., Steninae and Paederinae) of this study are from the staphylinine group of subfamilies, confirming that the lineage is rather much more recent than the tachyporine group of subfamilies.

Table 6.4: The Staphylinidae recorded during the Jurassic. Modified from the database of Smith and Marcot (2015).

Subfamily	**Taxa**	**Deposit**
Apateticinae-Trigonurinae	*Mesapatetica aenigmatica* Cai et al., 2014	Karatau, Kazakhstan
Glypholomatinae	*Juroglypholoma antiquum* Cai et al., 2012	Daohugou Fm., China
Glypholomatinae	*Juroglypholoma talbragarense* Cai et al., 2013	Talbragar Fossil Fish Bed, Australia
Olisthaerinae	*Anicula inferna* Ryvkin, 1985	Novospassky, Russia

Olisthaerinae	*Protolisthaerus jurassicus* Cai et al., 2015	Daohugou Fm., China
Omaliinae	*Archodromus brachypterus* Tikhomirova, 1968	Karatau, Kazakhstan
Omaliinae	*Archodromus comptus* Tikhomirova, 1968	Karatau, Kazakhstan
Omaliinae	*Eophyllodrepa propinqua* Ryvkin, 1985	Novospassky, Russia
Omaliinae	*Globoides microps* Tikhomirova, 1968	Karatau, Kazakhstan
Omaliinae	*Globoides oculatus* Tikhomirova, 1968	Karatau, Kazakhstan
Omaliinae	*Globoides sp.* Zhang, 2002	Neimonggol, China
Omaliinae	*Morsum abdominale* Ryvkin, 1985	Kubekovo, Russia
Omaliinae	*Porrhodromus communis* Tikhomirova, 1968	Karatau, Kazakhstan
Omaliinae	*Sinanthobium daohugouense* Cai and Huang 2013	Daohugou Fm., China
Oxytelinae	*Mesoxytelus mandibularis* Tikhomirova, 1968	Karatau, Kazakhstan
Oxytelinae	*Mesoxytelus parvus* Tikhomirova, 1968	Karatau, Kazakhstan
Piestinae	*Abolescus glabratus* Tikhomirova, 1968	Karatau, Kazakhstan
Scaphidiinae	*Scaphidiopsis aequivoca* Weyenbergh, 1869	Solnhofen, Germany
Scaphidiinae	*Scaphidiopsis hageni* Weyenbergh, 1869	Solnhofen, Germany
Staphylininae	*Tunicopterus sigara* Tikhomirova, 1968	Karatau, Kazakhstan
Staphylininae-Paederinae	*Subcelytrinus antiquus* Tikhomirova, 1968	Karatau, Kazakhstan
Tachyporinae	*Abscondus regularis* Tikhomirova, 1968	Karatau, Kazakhstan
Tachyporinae	*Abscondus similis* Tikhomirova, 1968	Karatau, Kazakhstan
Tachyporinae	*Mesotachinus major* Tikhomirova, 1968	Karatau, Kazakhstan
Tachyporinae	*Mesotachinus medius* Tikhomirova, 1968	Karatau, Kazakhstan
Tachyporinae	*Mesotachinus minor* Tikhomirova, 1968	Karatau, Kazakhstan
Tachyporinae	*Protachinus minor* Cai et al., 2013	Talbragar Fossil Fish Bed, Australia
Tachyporinae	*Protostaphylinus mirus* Lin, 1976	Jiulongshan, China
Tachyporinae	*Tachyporoides villosus* Tikhomirova, 1968	Karatau, Kazakhstan
Trigonurinae	*Abolescus glabratus* Tikhomirova, 1968	Karatau, Kazakhstan
Incertae sedis	*Sinostaphylius xiejiajieensis* Hong, 1992	Jiuda Formation, China
Incertae sedis	*Sulcelytrinus antiquus* Tikhomirova, 1968	Karatau, Kazakhstan
Incertae sedis	*Tunicopterus sigara* Tikhomirova, 1968	Karatau, Kazakhstan

6.3.3 Cretaceous age

According to Smith and Marcot (2015) database, which I have subsequently updated for the taxa described in this study, my results show that there are 156 Staphylinidae and Silphidae that have been recorded during the Cretaceous age (Table 6.5). However, the actual number must be much higher, because Liu et al (2021) lists 33 from the Yixian Formation of China. According to my findings, 29 of Cretaceous taxa were assigned to the Scydmaeninae, 27 to the Staphylininae, 14 to the Oxytelinae, 10 to the Paederinae, nine to the Pselaphinae, eight to the Tachyporinae, six to the Oxyporinae and Dasycerinae, while Aleocharinae, Steninae, Osoriinae, Piestinae and Solieriinae also deserve a notable mention. In the Smith and Marcot (2015) database, some of the species have been misplaced, and belong to other families, such as Carabidae and Buprestidae. These have been excluded in the table, together with a few taxa that could not be found in any online resource.

Table 6.5 shows that the majority (70 descriptions) of the genera and species were recovered from the Burmese amber, a deposit that is situated in Hukawng Valley, in northern Myanmar. In addition, 36 descriptions have been recorded from Yixian Formation, a deposit that is situated near Jinzhou, Liaoning Province, in China. Lastly, eight were recovered from Laiyang Formation, a deposit that is situated near the village of Tuanwang, Laiyang City, Shandong Province, in China.

Table 6.5: The Staphylinidae recorded during the Cretaceous. Modified from the database of Smith and Marcot (2015).

Subfamily	**Taxa**	**Deposit**
Aleocharinae	*Cretodeinopsis aenigmatica* Cai and Huang, 2015	Burmese Amber, Myanmar
Aleocharinae	*Cretotrichopsenius burmiticus* Cai et al., 2017	Burmese Amber, Myanmar
Aleocharinae	*Mesosymbion compactus* Yamamoto et al., 2016	Burmese Amber, Myanmar
Dasycerinae	*Cedasyrus dimorphus* Yin and Cai, 2021	Burmese Amber, Myanmar
Dasycerinae	*Protodasycerus aenigmaticus* Yamamoto, 2016	Burmese Amber, Myanmar
Dasycerinae	*Protodasycerus corpulentus* Yin and Cai, 2021	Burmese Amber, Myanmar
Dasycerinae	*Protodasycerus gigas* Yin and Cai, 2021	Burmese Amber, Myanmar

Dasycerinae	*Protodasycerus tuberculatus* Yamamoto, Newton and Yin, 2021	Burmese Amber, Myanmar
Dasycerinae	*Vetudasycerus burmiticus* Cai et al., 2018	Burmese Amber, Myanmar
Euaesthetinae	*Nordenskioldia (Libanoeuaesthetus) pentatarsus* Lefebvre et al., 2005	Lebanese Amber, Lebanon
Euaesthetinae	*Octavius electrospinosus* Clarke and Chatzimanolis, 2009	Burmese Amber, Myanmar
Megalopsidiinae	*Megalopinus extinctus* Yamamoto and Solodovnikov 2016	Burmese Amber, Myanmar
Micropeplinae	*Micropeplus pengweii* Jiang et al., 2020	Burmese Amber, Myanmar
Micropeplinae	*Protopeplus cretaceous* Cai and Huang, 2014	Burmese Amber, Myanmar
Olisthaerinae-Phloeocharinae	*Megolisthaerus chinensis* Solodovnikov and Yue, 2010	Yixian Fm., China
Omaliinae	*Daiodromus umbrosus* Ryvkin, 1990	Daya Fm., Russia
Omaliinae	*Duocalcar geminum* Peris and Thayer, 2014	Charentese Amber, France
Omaliinae	*Mesodeliphrum rufulum* Ryvkin, 1990	Turga Fm., Russia
Omaliinae	*Paraphloeostiba sonani* Bernhauer, 1943	Burmese Amber, Myanmar
Omaliinae	*Prodaia inflatas* Ryvkin, 1990	Daya Fm., Russia
Osoriinae	*Cretochirus newtoni* Yamamoto, 2019	Burmese Amber, Myanmar
Osoriinae	*Mesallotrochus longiantennatus* Cai and Huang, 2015	Burmese Amber, Myanmar
Osoriinae	*Priochirus thayerae* Yamamoto, 2019	Burmese Amber, Myanmar
Osoriinae	*Priochirus (Eopriochirus) trisclerite* Peng et al., 2022	Burmese Amber, Myanmar
Oxyporinae	*Cretoxyporus extraneus* Cai and Huang, 2014	Yixian Fm., China
Oxyporinae	*Oxyporus cretaceous* Yamamoto, 2017	Burmese Amber, Myanmar
Oxyporinae	*Oxyporus yixianus* Solodovnikov and Yue, 2011	Yixian Fm., China
Oxyporinae-Megalopsidiinae	*Paroxyporus fossilis* Cai, 2021	Yixian Fm., China
Oxyporinae	*Protoxyporus grandis* Cai and Huang, 2014	Yixian Fm., China
Oxytelinae	*Anotylus archaicus* Yue et al., 2012	Yixian Fm., China
Oxytelinae	*Gollandia planata* Makranczy et al., 2018	Burmese Amber, Myanmar

Oxytelinae	*Megalymma gigantea* Tikhomirova, 1980	Manlay, Mongolia
Oxytelinae	*Megalymma rohdendorfi* Tikhomirova, 1980	Manlay, Mongolia
Oxytelinae	*Mesocoprophilus clavatus* Cai and Huang, 2013	Yixian Fm., China
Oxytelinae	*Morda mora* Ryvkin, 1990	Daya Fm., Russia
Oxytelinae	*Prajna tianmiaoae* Lü et al., 2017	Burmese Amber, Myanmar
Oxytelinae	*Protodeleaster glaber* Cai et al., 2013	Yixian Fm., China
Oxytelinae	*Pseudanotylus archaicus* Yue et al., 2012	Yixian Fm., China
Oxytelinae	*Sinoxytelus breviventer* Yue et al., 2010	Yixian Fm., China
Oxytelinae	*Sinoxytelus euglypheus* Yue et al., 2010	Yixian Fm., China
Oxytelinae	*Sinoxytelus longisetosus* Yue et al., 2010	Yixian Fm., China
Oxytelinae	*Sinoxytelus transbaicalicus* Cai et al., 2013	Urey Fm., Russia
Oxytelinae	*Turgaphloeus pubescens* Ryvkin, 1990	Turga Fm., Russia
Paederinae	*Apticax solidus* Schomann and Solodovnikov, 2012	Santana Fm. / Crato Fm., Brazil
Paederinae	*Apticax volans* Schomann and Solodovnikov, 2012	Santana Fm. / Crato Fm., Brazil
Paederinae	*Cretoprocirrus trichotos* Shaw et al., 2020	Burmese Amber, Myanmar
Paederinae	*Diminudon kachinensis* Żyła et al., 2019	Burmese Amber, Myanmar
Paederinae	*Diminudon schomannae* Żyła et al., 2019	Burmese Amber, Myanmar
Paederinae	*Mesostaphylinus antiquus* Solodovnikov and Yue, 2013	Yixian Fm., China
Paederinae	*Mesostaphylinus elongatus* Solodovnikov and Yue, 2013	Yixian Fm., China
Paederinae	*Mesostaphylinus laiyangensis* Zhang, 1988	Laiyang Fm., China
Paederinae	*Mesostaphylinus yixianus* Solodovnikov and Yue, 2013	Yixian Fm., China
Paederinae	*Paederus fuscipes* Lin et al. 2018	Zhengzhou Fm., China
Phloeocharinae	*Phloeocharis agerata* Chatzimanolis et al., 2013	New Jersey Fm., United States of America
Piestinae	*Paleosiagonium adaequatum* Yue et al., 2016	Yixian Fm., China
Piestinae	*Paleosiagonium brevelytratum* Yue et al., 2016	Yixian Fm., China
Piestinae	*Propiestus archaicus* Yamamoto et al., 2019	Burmese Amber, Myanmar
Proteininae	*Vetuproteinus cretaceous* Cai et al., 2016	Burmese Amber, Myanmar

Protopselaphinae	*Protopselaphus newtoni* Liu et al., 2021	Burmese Amber, Myanmar
Protopselaphinae	*Protopselaphus thayerae* Liu et al., 2020	Burmese Amber, Myanmar
Pselaphinae	*Boreotethys arctopteryx* Parker, 2016	Burmese Amber, Myanmar
Pselaphinae	*Boreotethys grimaldii* Parker, 2016	Burmese Amber, Myanmar
Pselaphinae	*Cretasonoma corinformibus* Peris et al., 2014	Albian Spanish Amber, Spain
Pselaphinae	*Cretobrachygluta laurasiensis* Yin et al., 2019	Burmese Amber, Myanmar
Pselaphinae	*Cretobythus excavates* Yin et al., 2018	Burmese Amber, Myanmar
Pselaphinae	*Penarhytus tenebris* Peris et al., 2014	Albian Spanish Amber, Spain
Pselaphinae	*Priscaplectus carinatus* Yin et al., 2019	Burmese Amber, Myanmar
Pselaphinae	*Priscaplectus grandiceps* Yin et al., 2019	Burmese Amber, Myanmar
Pselaphinae	*Protrichonyx rafifrons* Parker, 2016	Burmese Amber, Myanmar
Pseudopsinae	*Cretaceonanobius fossilis* Liu et al., 2022	Burmese Amber, Myanmar
Pseudopsinae	*Cretopseudopsis maweii* Liu et al., 2020	Burmese Amber, Myanmar
Scydmaeninae	*Archemastax divida* Yin et al., 2019	Burmese Amber, Myanmar
Scydmaeninae	*Archeutheia magnifica* Jałoszyński and Peris, 2016	Albian Spanish Amber, Spain
Scydmaeninae	*Cascomastigus monstrabilis* Yin and Cai, 2018	Burmese Amber, Myanmar
Scydmaeninae	*Cenomaniola carinata* Jałoszyński and Yamamoto, 2017	Burmese Amber, Myanmar
Scydmaeninae	*Cenomaniola macrophthalma* Jałoszyński and Yamamoto, 2017	Burmese Amber, Myanmar
Scydmaeninae	*Clidicostigus arachnipes* Jałoszyński et al., 2017	Burmese Amber, Myanmar
Scydmaeninae	*Clidicus cretaceus* Kirejtshuk et al., 2015	Archingeay French Amber, France
Scydmaeninae	*Cretoleptochromus archaicus* Cai and Huang, 2016	Burmese Amber, Myanmar
Scydmaeninae	*Cretoleptochromus burmiticus* Yin et al., 2017	Burmese Amber, Myanmar
Scydmaeninae	*Electroatopos castaneus* Chatzimanolis et al., 2010	Burmese Amber, Myanmar
Scydmaeninae	*Ektatotricha paradoxa* Chatzimanolis et al., 2010	Burmese Amber, Myanmar
Scydmaeninae	*Hapsomela burmitis* Poinar and Brown, 2004	Burmese Amber, Myanmar

Scydmaeninae	*Hapsomela minor* Yin, 2020	Burmese Amber, Myanmar
Scydmaeninae	*Hapsomela tibialis* Yin, 2020	Burmese Amber, Myanmar
Scydmaeninae	*Hyperstenichnus vendeanus* Jałoszyński and Perrichot, 2017	Vendean Amber, France
Scydmaeninae	*Kachinus antennatus* Chatzimanolis et al., 2010	Burmese Amber, Myanmar
Scydmaeninae	*Kachinus magnificus* Peris et al., 2014	Albian Spanish Amber, Spain
Scydmaeninae	*Kuafu borealis* Yin et al., 2018	Burmese Amber, Myanmar
Scydmaeninae	*Loeblitoides separatus* Jałoszyński, 2020	Burmese Amber, Myanmar
Scydmaeninae	*Nuegua elongata* Yin et al., 2018	Burmese Amber, Myanmar
Scydmaeninae	*Palaeoleptochromus schaufussi* O'Keefe et al., 1997	Medicine Hat, Canada
Scydmaeninae	*Pangusyndicus excavates* Yin et al., 2019	Burmese Amber, Myanmar
Scydmaeninae	*Pangusyndicus longirostris* Jałoszyński, 2020	Burmese Amber, Myanmar
Scydmaeninae	*Praphennium carinatum* Jałoszyński, 2018	Burmese Amber, Myanmar
Scydmaeninae	*Scydmaenus linqibini* Yin and Zhou, 2020	Burmese Amber, Myanmar
Scydmaeninae	*Scydmaenus minor* Yin and Cai, 2019	Burmese Amber, Myanmar
Scydmaeninae	*Scydmobisetia dentipes* Jałoszyński and Bai, 2020	Burmese Amber, Myanmar
Scydmaeninae	*Scydmobisetia vetutissima* Jałoszyński and Yamamoto, 2016	Burmese Amber, Myanmar
Scydmaeninae	*Taimyraphes microscopicus* Jałoszyński and Perkovsky, 2019	Santonian Taimyr Amber, Yantardakh
Silphinae	*Cretosaja jinjuensis* Sohn and Sam, 2021	Jinju Fm., China
Silphinae	*Sinosilphia punctata* Grebennikov and Newton, 2012	Jinju Fm., China
Silphinae	*Prosilpha nigrita* Grebennikov and Newton, 2012	Jinju Fm., China
Solieriinae	*Prosolierius crassicornis* Thayer et al., 2012	Burmese Amber, Myanmar
Solieriinae	*Prosolierius mixticornis* Thayer et al., 2012	Burmese Amber, Myanmar
Solieriinae	*Prosolierius parvus* Peris et al., 2014	Albian Spanish Amber, Spain
Solieriinae	*Prosolierius tenuicornis* Thayer et al., 2012	Burmese Amber, Myanmar
Staphylininae	*Cretoquedius distinctus* Solodovnikov and Yue, 2013	Yixian Fm., China

Staphylininae	*Cretoquedius dorsalis* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Cretoquedius infractus* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Cretoquedius oculatus* Ryvkin, 1988	Magadanskaia, Russia
Staphylininae	*Cretoprosopus problematicus* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Durothorax creticus* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Laostaphylinus fuscus* Zhang, 1988	Laiyang Fm., China
Staphylininae	*Laostaphylinus nigritellus* Zhang, 1988	Laiyang Fm., China
Staphylininae	*Megolisthaerus minor* Cai and Huang, 2013	Yixian Fm., China
Staphylininae	*Mesostaphylinus fraternus* Zhang et al., 1992	Laiyang Fm., China
Staphylininae	*Mesostaphylinus laiyangensis* Zhang, 1988	Laiyang Fm., China
Staphylininae	*Paleothius gracilis* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Paleowinus ambiguous* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Paleowinus chinensis* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Paleowinus fossilis* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Paleowinus mirabilis* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Paleowinus rex* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Philonthus kneri* Alroy et al., 2017	Lulworth Fm., Middle Purbeck (Berriasian)
Staphylininae	*Quedius cretaceous* Cai and Huang, 2013	Yixian Fm., China
Staphylininae	*Sinostaphylina nanligezhuangensis* Hong and Wang, 1990	Laiyang Fm., China
Staphylininae	*Thayeralinus fieldi* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Thayeralinus fraternus* Zhang et al., 1992	Laiyang Fm., China

Staphylininae	*Thayeralinus giganteus* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Thayeralinus glandulifer* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Thayeralinus longelytratus* Solodovnikov and Yue, 2013	Yixian Fm., China
Staphylininae	*Vetatrecus adelfiae* Kypke et al., 2019	Burmese Amber, Myanmar
Staphylininae	*Vetatrecus secretum* Kypke et al., 2019	Burmese Amber, Myanmar
Steninae	*Festenus gracilis* Żyła et al., 2017	Burmese Amber, Myanmar
Steninae	*Festenus robustus* Żyła et al., 2017	Burmese Amber, Myanmar
Steninae	*Stenus (Nestus) imputribilis* Ryvkin, 1988	Magadanskaia, Russia
Steninae	*Stenus (Tesnus) inexpectatus* Schlüter, 1978	Bezonnais Amber, France
Tachyporinae	*Cuneocharis elongates* Ryvkin, 1990	Daya Fm., Russia
Tachyporinae	*Glabrimycetoporus amoenus* Yue et al., 2009	Yixian Fm., China
Tachyporinae	*Hesterniasca obesa* Zhang et al., 1992	Laiyang Fm., China
Tachyporinae	*Hesterniasca lata* Cai et al., 2011	Yixian Fm., China
Tachyporinae	*Mesotachyporus puer* Gusarov, 2000	New Jersey Amber, United States of America
Tachyporinae	*Procileoporus burmiticus* Yamamoto, 2016	Burmese Amber, Myanmar
Tachyporinae	*Ryvkinius gracilis* Ryvkin, 1990	Daya Fm., Russia
Tachyporinae	*Undiatina roduc* Ryvkin, 1990	Semyon Fm., Russia
Trichophyinae	*Trichophya minor* Yamamoto and Newton, 2021	Burmese Amber, Myanmar
Trigonurinae	*Kovalevia onokhoica* Ryvkin, 1990	Onozhoi, Russia
Trigonurinae	*Trigunda lata* Ryvkin, 1990	Semyon, Russia
Incertae sedis	*Carirriderma roduc* Martins-Neto, 1990	Santana Fm. / Crato Fm., Brazil
Incertae sedis	*Scaphylinus ammanicus* Kaddumi, 2005	Aarda-Subeihi, Jordan
Incertae sedis	Undescribed species Schlüter, 1978	Bezonnais Amber, France
Incertae sedis	Undescribed species Jell and Duncan, 1986	Queensland, Australia
Incertae sedis	Undescribed species Soriano et al., 2007	Las Hoyas, El Montsec, Spain
Incertae sedis	Undescribed species Alonso et al., 2000	Álava, Spain
Incertae sedis	Undescribed species Perrichot et al., 2007	Charente-Maritime, France

6.3.4 Cenozoic age

According to Smith and Marcot (2015) database, to date, in total, there are approximately 383 Staphylinidae and Silphidae that have been recorded during the Cenozoic age (Table 6.6). The highest contributors were Staphylininae (57, 15%), Pselaphinae (47, 12%), Omaliinae (42, 11%), Scydmaeninae (38, 10%), Paederinae (30, 8%), Oxytelinae (28, 7%), Tachyporinae (28, 7%), , Aleocharinae (24, 6%), and Steninae (17, 5%). From the Palaeocene + Eocene eras, which have recorded a total of 235 descriptions; Pselaphinae (38, 16%), Scydmaeninae (36, 15%), Staphylininae (32, 14%), Omaliinae (30, 13%), Tachyporinae (17, 7%), Aleocharinae (14, 6%), Paederinae (14, 6%), Oxytelinae (14, 6%) and Steninae (11, 5%) were the biggest contributors.

Of the 235 descriptions recorded from the Palaeocene + Eocene eras,. 126 of these were recorded from the Baltic Amber, which is situated near Kaliningrad Oblast in Russia. Fifty six descriptions have been recorded from the Florissant, which is situated near the Florissant Fossil Beds National Monument (Colorado), in the United States of America. Forty four descriptions were recorded from the Oligocene era. Fifty one descriptions were recorded from the Miocene era. Forty four descriptions were also recorded from the Pleistocene + Holocene eras. The remaining were recorded from the Pliocene era.

Table 6.6: The Staphylinidae recorded during the Cenozoic. Modified from the database of Smith and Marcot (2015).

Palaeocene and Eocene

There are 235 staphylinids recorded during the Palaeocene and Eocene eras.

Subfamily	**Taxa**	**Deposit**
Aleocharinae	*Adinopsis groehni* Zerche, 1999	Baltic Amber, Russia
Aleocharinae	*Aleochara baltica* Paśnik and Kubisz, 2002	Baltic Amber, Russia
Aleocharinae	*Aleocharopsis caseyi* Herman, 2001	Florissant Fm., United States of America
Aleocharinae	*Aleocharopsis secunda* Wickham, 1913	Florissant Fm., United States of America
Aleocharinae	*Atheta jantarica* Paśnik, 2005	Baltic Amber, Russia

Aleocharinae	*Atheta florissantensis* Wickham, 1913	Florissant Fm., United States of America
Aleocharinae	*Baltioligota electrica* Paśnik, 2005	Baltic Amber, Russia
Aleocharinae	*Dictyon antiquus* Paśnik and Kubisz, 2002	Baltic Amber, Russia
Aleocharinae	*Electrogymnusa baltica* Wolf-Schwenninger, 2004	Baltic Amber, Russia
Aleocharinae	*Gyrophaena saxicola* Scudder, 1900	Green River Fm., United States of America
Aleocharinae	*Homalota recisa* Scudder, 1900	Green River Fm., United States of America
Aleocharinae	*Leptusa (Protoleptusa) defuncta* Semenov et al., 2001	Rovno Amber, Ukraine
Aleocharinae	*Palaeomesoporus electiricus* Yamamoto and Maruyama, 2017	Baltic Amber, Russia
Aleocharinae	*Phymatura electrica* Paśnik and Kubisz, 2002	Baltic Amber, Russia
Apateticinae	*Miosilpha necrophiloides* Madge, 1980	Florissant Fm., United States of America
Euaesthetinae	*Euaesthetus ‘‘sp. A ‘‘ (prope nitidulus)* Puthz, 2008	Baltic Amber, Russia
Euaesthetinae	*Euaesthetus ‘‘sp. B ‘‘ (prope nitidulus)* Puthz, 2008	Baltic Amber, Russia
Euaesthetinae	*Stenaesthetus sp. (prope quadrisculatus)* Puthz, 2005	Baltic Amber, Russia
Euaesthetinae	*Stenaesthetus sp. (prope quadrisculatus)* Puthz, 2008	Baltic Amber, Russia
Omaliinae	*Anthobium alekseevi* Shavrin, 2020	Baltic Amber, Russia
Omaliinae	*Eusphalerum bukejsi* Shavrin and Kairišs, 2021	Baltic Amber, Russia
Omaliinae	*Eusphalerum kanti* Shavrin and Kairišs, 2021	Baltic Amber, Russia
Omaliinae	*Geodromicus amplissimus* Shavrin, 2019	Yunnan Fm., China
Omaliinae	*Geodromicus balticus* Shavrin, 2019	Baltic Amber, Russia
Omaliinae	*Geodromicus crassipalpis* Shavrin, 2019	Yunnan Fm., China
Omaliinae	*Geodromicus spadiceus* Shavrin, 2019	Yunnan Fm., China

Omaliinae	*Homalium antiquorum* Wickham, 1913	Florissant Fm., United States of America
Omaliinae	*Microptilium geistautsi* Alekseev, 2013	Baltic Amber, Russia
Omaliinae	*Nipponophloeostiba longilobata* Shavrin, 2020	Laos, Thailand
Omaliinae	*Omalium antiquorum* Peris et al., 2014	Florissant Fm., United States of America
Omaliinae	*Omalium khmericum* Shavrin, 2020	Laos, Thailand
Omaliinae	*Paraphloeostiba amooraphila* Shavrin, 2020	Laos, Thailand
Omaliinae	*Paraphloeostiba ampliata* Shavrin, 2020	Yunnan Fm., China
Omaliinae	*Paraphloeostiba apicalis* Shavrin, 2020	Yunnan Fm., China
Omaliinae	*Paraphloeostiba brancuccii* Shavrin, 2020	Laos, Thailand
Omaliinae	*Paraphloeostiba cooteri* Shavrin, 2020	Zhejiang Fm., China
Omaliinae	*Paraphloeostiba electrica* Zanetti et al., 2016	Baltic Amber, Russia
Omaliinae	*Paraphloeostiba formosana* Shavrin and Smetana, 2016	Laos, Thailand
Omaliinae	*Paraphloeostiba gayndahensis* Shavrin, 2020	Yunnan Fm., China
Omaliinae	*Paraphloeostiba morosa* Shavrin, 2019	Baltic Amber, Russia
Omaliinae	*Paraphloeostiba opacicollis* Shavrin, 2020	Laos, Thailand
Omaliinae	*Paraphloeostiba sulcicollis* Shavrin, 2020	Yunnan Fm., China
Omaliinae	*Phloeostiba assingi* Shavrin, 2020	Laos, Thailand
Omaliinae	*Phloeonomus (Phloeonomus) rougemonti* Shavrin, 2020	Yunnan Fm., China
Omaliinae	*Phyllodrepa antiqua* Zanetti et al., 2016	Baltic Amber, Russia
Omaliinae	*Phyllodrepa daedali* Shavrin, 2019	Baltic Amber, Russia
Omaliinae	*Phyllodrepa icari* Shavrin, 2019	Baltic Amber, Russia
Omaliinae	*Pseudolesteva insinuans* Schaufuss, 1890	Baltic Amber, Russia
Omaliinae	*Sucinoptinus bukejsi* Alekseev, 2012	Baltic Amber, Russia
Osoriinae	*Paleosorius cambayensis* Ortega-Blanco et al., 2013	Ypresian Cambay Basin Amber, India
Oxyporinae	*Oxyporus blumenbachii* Gravenhorst, 1806	Baltic Amber, Russia
Oxyporinae	*Oxyporus impressus* Piton, 1940	Menant Fm., France
Oxyporinae	*Oxyporus stiriacus* Scudder, 1890	Florissant Fm., United States of America

Oxytelinae	*Bledius adamus* Scudder, 1878	Green River Fm., United States of America
Oxytelinae	*Bledius faecorum* Scudder, 1900	Green River Fm., United States of America
Oxytelinae	*Bledius morsei* Scudder, 1900	Florissant Fm., United States of America
Oxytelinae	*Bledius osborni* Scudder, 1900	Florissant Fm., United States of America
Oxytelinae	*Bledius primitiarum* Scudder, 1900	Florissant Fm., United States of America
Oxytelinae	*Bledius soli* Scudder, 1900	Florissant Fm., United States of America
Oxytelinae	*Deleaster grandiceps* Scudder, 1900	Florissant Fm., United States of America
Oxytelinae	*Oxytelus levis* Förster, 1891	Brunstatt, Alsace, France
Oxytelinae	*Oxytelus pristinus* Scudder, 1876	Chagrin Valley, United States of America
Oxytelinae	*Oxytelus subapterus* Wickham, 1913	Florissant Fm., United States of America
Oxytelinae	*Oxytelus ominosus* Förster, 1891	Brunstatt, Alsace, France
Oxytelinae	*Oxytelus ominosus* Förster, 1891	Kleinkembs, France
Oxytelinae	*Platystethus archetypus* Scudder, 1900	Florissant Fm., United States of America
Oxytelinae	*Platystethus carcareus* Scudder, 1900	Florissant Fm., United States of America
Paederinae	*Dysanabatium aenaum* Bogri et al., 2018	Baltic Amber, Russia
Paederinae	*Dysanabatium damgaardi* Bogri et al., 2018	Baltic Amber, Russia
Paederinae	*Dysanabatium johannesi* Bogri et al., 2018	Baltic Amber, Russia
Paederinae	*Dysanabatium kechrimparense* Bogri et al., 2018	Baltic Amber, Russia
Paederinae	*Lathrobium ambricum* Paśnik and Kubisz, 2002	Baltic Amber, Russia
Paederinae	*Lathrobium antediluvianum* Wickham, 1913	Florissant Fm., United States of America

Paederinae	*Lathrobium balticum* Paśnik and Kubisz, 2002	Baltic Amber, Russia
Paederinae	*Lathrobium jantaricum* Paśnik and Kubisz, 2002	Baltic Amber, Russia
Paederinae	*Lathrobium succini* Paśnik and Kubisz, 2002	Baltic Amber, Russia
Paederinae	*Lathrobium whitei* Abdullah and Abdullah, 1968	Baltic Amber, Russia
Paederinae	*Lithocharis scotti* Casey, 1886	Florissant Fm., United States of America
Paederinae	*Miolithocharis lithographica* Wickham, 1913	Florissant Fm., United States of America
Paederinae	*Orsunius electronefelus* Kypke and Solodovnikov, 2020	Baltic Amber, Russia
Paederinae	*Paederus adumbratus* Frank, 1988	Florissant Fm., United States of America
Phloeocharinae	*Charhyphus balticus* Shavrin, 2020	Baltic Amber, Russia
Phloeocharinae	*Charhyphus serratus* Yamamoto and Shavrin, 2022	Baltic Amber, Russia
Phloeocharinae	*Trigites coeni* Scudder, 1900	Florissant Fm., United States of America
Piestinae	*Eopiestus groehni* Cai and Lü, 2016	Baltic Amber, Russia
Pselaphinae	*Barybryaxis lata* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Batrisus antiquus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Batrisus pristinus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Bryaxis glabrella* Cockerell, 1983	Baltic Amber, Russia
Pselaphinae	*Bryaxis patris* Cockerell, 1983	Baltic Amber, Russia
Pselaphinae	*Bryaxis veterum* Cockerell, 1983	Baltic Amber, Russia
Pselaphinae	*Bythinus caviceps* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Bythinus foveopunctatus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Bythinus schaufussi* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Bythinus tenuipes* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Bythinus typicus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Ctenistodes claviger* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Cymbalizon tyroides* Schaufuss, 1890	Baltic Amber, Russia

Pselaphinae	*Dantiscanus costalis* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Deuterotyrus redivivus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Euplectus lentiferus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Euplectus mozarti* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Euplectus quadrifoveatus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Eupsinoides glabrellus* Motschulsky, 1856	Baltic Amber, Russia
Pselaphinae	*Europharinodes schaufussi* Yin and Cai, 2022	Baltic Amber, Russia
Pselaphinae	*Faronus porrectus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Faronus tritomicrus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Greys conciliator* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Hagnometopias pater* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Hetereuplectus retrorsus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Monyx spiculatus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Nugaculus calcitrans* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Nugator stricticolis* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Pammiges spectrum* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Pantobatrisus cursor* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Parabryaxis lata* Alekseev, 2013	Baltic Amber, Russia
Pselaphinae	*Rybaxis glabrella* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Rybaxis patris* Schaufuss, 1892	Baltic Amber, Russia
Pselaphinae	*Rybaxis veterum* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Tmesiphoroides cariniger* Motschulsky, 1856	Baltic Amber, Russia
Pselaphinae	*Tychus avus* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Tychus radians* Schaufuss, 1890	Baltic Amber, Russia
Pselaphinae	*Tyrus electricus* Schaufuss, 1890	Baltic Amber, Russia
Pseudopsinae	*Pseudopsis gansuensis* Zerche, 1998	Sichuan Fm., China
Pseudopsinae	*Pseudopsis gonggaensis* Zerche, 1998	Sichuan Fm., China
Pseudopsinae	*Pseudopsis pengi* Yin, 2021	Shanxi Fm., China
Pseudopsinae	*Pseudopsis serrata* Yin, 2021	Yunnan Fm., China
Pseudopsinae	*Pseudopsis suchi* Herman, 1983	Sichuan Fm., China
Scydmaeninae	*Aenictosoma doenitzi* Schaufuss, 1892	Baltic Amber, Russia
Scydmaeninae	*Baltostigus antennatus* Jałoszyński, 2015	Poland Amber, Poland
Scydmaeninae	*Baltostigus antennatus* Jałoszyński, 2015	Lithuania Amber, Lithuania

Scydmaeninae	*Cephennomicrus giganteus* Jałoszyński and Perkovsky, 2016	Rovno Amber, Ukraine
Scydmaeninae	*Clidicus balticus* Schaufuss, 1896	Baltic Amber, Russia
Scydmaeninae	*Clidicus qiuae* Cheng and Yin, 2019	Yunnan Fm., China
Scydmaeninae	*Clidicus yingjiangus* Cheng and Yin, 2019	Yunnan Fm., China
Scydmaeninae	*Cryptodiodon corticaroides* Schaufuss, 1890	Baltic Amber, Russia
Scydmaeninae	*Electroscydmaenus pterostichoides* Schaufuss, 1890	Baltic Amber, Russia
Scydmaeninae	*Euconnus fossilis* Franz, 1976	Baltic Amber, Russia
Scydmaeninae	*Euconnus liedtkei* Franz, 1976	Baltic Amber, Russia
Scydmaeninae	*Euconnus nathani* Alekseev, 2013	Rovno Amber, Ukraine
Scydmaeninae	*Euconnus palaeogenus* Jałoszyński and Perkovsky, 2016	Rovno Amber, Ukraine
Scydmaeninae	*Euconnus sucini* Franz, 1976	Baltic Amber, Russia
Scydmaeninae	*Euconnus wunderlichi* Franz, 1983	Baltic Amber, Russia
Scydmaeninae	*Euroleptochromus sabathi* Jałoszyński, 2012	Baltic Amber, Russia
Scydmaeninae	*Euroleptochromus tuberculatus* Yin and Cai, 2018	Baltic Amber, Russia
Scydmaeninae	*Glaesoconnus unicus* Jałoszyński and Perkovsky, 2016	Rovno Amber, Ukraine
Scydmaeninae	*Hetereuthia elegans* Schaufuss, 1890	Baltic Amber, Russia
Scydmaeninae	*Heuretus coriaceus* Schaufuss, 1890	Baltic Amber, Russia
Scydmaeninae	*Neuraphes fossilis* Franz, 1983	Baltic Amber, Russia
Scydmaeninae	*Palaeomastigus helmi* Schaufuss, 1890	Baltic Amber, Russia
Scydmaeninae	*Palaeothia tenuitarsis* Schaufuss, 1890	Baltic Amber, Russia
Scydmaeninae	*Rovnoleptochromus ableptonoide* Jałoszyński and Perkovsky, 2016	Rovno Amber, Ukraine
Scydmaeninae	*Rovnoleptochromus balticus* Jałoszyński and Yamamoto, 2019	Rovno Amber, Ukraine
Scydmaeninae	*Rovnoscydmus frontalis* Jałoszyński and Perkovsky, 2016	Rovno Amber, Ukraine
Scydmaeninae	*Rovnoscydmus microscopicus* Jałoszyński and Perkovsky, 2016	Rovno Amber, Ukraine

Scydmaeninae	*Scydmaenoides nigrascens* Motschulsky, 1856	Baltic Amber, Russia
Scydmaeninae	*Semnodioceras halticaeforme* Schaufuss, 1890	Baltic Amber, Russia
Scydmaeninae	*Stenichnus (Cyrtoscydmus) capucinus* Schaufuss, 1890	Baltic Amber, Russia
Scydmaeninae	*Stenichnus (Cyrtoscydmus) carinulatus* Schaufuss, 1890	Baltic Amber, Russia
Scydmaeninae	*Stenichnus (Cyrtoscydmus) laticlavus* Schaufuss, 1890	Baltic Amber, Russia
Scydmaeninae	*Stenichnus (Cyrtoscydmus) titubans* Schaufuss, 1890	Baltic Amber, Russia
Scydmaeninae	*Stenichnus proavus* Jałoszyński and Perkovsky, 2016	Rovno Amber, Ukraine
Scydmaeninae	*Taimyraphes microscopicus* Jałoszyński and Perkovsky, 2019	Santonian Taimyr Amber, Ukraine
Scydmaeninae	*Vertheia quadrisetosa* Jałoszyński and Perkovsky, 2016	Rovno Amber, Ukraine
Silphinae	*Eosilphites decoratus* Grebennikov and Newton, 2012	Geiseltal, Germany
Silphinae	*Necrodes primaevus* Toussaint and Condamine, 2016	Florissant Fm., United States of America
Silphinae	*Palaeosilpha fraasii* Flach, 1890	Baltic Amber, Russia
Silphinae	*Ptomascopus aveyronensis* Flach, 1890	Baltic Amber, Russia
Silphinae	*Silpha beutenmuelleri* Wickham 1913	Florissant Fm., United States of America
Silphinae	*Silpha colorata* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Acylophorus immotus* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Acylophorus hoffeinsorum* Żyła and Solodovnikov, 2019	Baltic Amber, Russia
Staphylininae	*Baltognathus aenigmaticus* Brunke et al., 2019	Baltic Amber, Russia
Staphylininae	*Bolitogyrus fragmentus* Brunke et al., 2019	Baltic Amber, Russia
Staphylininae	*Bembicidiodes inaequicollis* Schaufuss, 1888	Baltic Amber, Russia

Staphylininae	*Diochus electrus* Chatzimanolis and Engel, 2011	Baltic Amber, Russia
Staphylininae	*Eolophorus hoffeinsorum* Żyła and Solodovnikov, 2019	Baltic Amber, Russia
Staphylininae	*Hemiquedius europaeus* Brunke et al., 2019	Baltic Amber, Russia
Staphylininae	*Heterothops conticens* Shaw et al., 2020	Florissant Fm., United States of America
Staphylininae	*Laevisaurus gracilis* Brunke et al., 2019	Baltic Amber, Russia
Staphylininae	*Laevisaurus robustus* Brunke et al., 2019	Baltic Amber, Russia
Staphylininae	*Leistotrophus patriarchicus* Scudder, 1876	Green River Fm., United States of America
Staphylininae	*Leptacinus exsucidus* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Leptacinus fossus* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Leptacinus leidyi* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Leptacinus maclurei* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Leptacinus rigatus* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Platydracus breviantennatus* Cai et al., 2014	Florissant Fm., United States of America
Staphylininae	*Philonthus abavus* Wickham, 1913	Florissant Fm., United States of America
Staphylininae	*Philonthus horni* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Philonthus invelatus* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Philonthus macridulus* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Quedius breweri* Scudder, 1900	Florissant Fm., United States of America

Staphylininae	*Quedius chamberlini* Wickham, 1913	Florissant Fm., United States of America
Staphylininae	*Quedius cretaceous* Cai and Huang, 2013	Yixian Fm., China
Staphylininae	*Quedius mortuus* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Staphylinites obsoletum* Scudder, 1876	Green River Fm., United States of America
Staphylininae	*Staphylinus lesleyi* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Staphylinus vetulus* Scudder, 1900	Florissant Fm., United States of America
Staphylininae	*Staphylinus vulcan* Wickham, 1913	Florissant Fm., United States of America
Staphylininae	*Tympanophorus greenwalti* Chatzimanolis et al., 2017	Kishenehn Fm., North America
Staphylininae	*Xantholinus tenebrarius* Wickham, 1913	Florissant Fm., United States of America
Steninae	*Eocenostenus fossilis* Cai et al., 2014	Monteils Fm., France
Steninae	*Stenus abraham* Puthz, 2010	Baltic Amber, Russia
Steninae	*Stenus archetypus* Puthz, 2010	Baltic Amber, Russia
Steninae	*Stenus atavus* Puthz, 2010	Baltic Amber, Russia
Steninae	*Stenus avus* Puthz, 2010	Baltic Amber, Russia
Steninae	*Stenus groehni* Puthz, 2010	Baltic Amber, Russia
Steninae	*Stenus ketura* Puthz, 2010	Baltic Amber, Russia
Steninae	*Stenus methusalem* Puthz, 2010	Baltic Amber, Russia
Steninae	*Stenus morsei* Scudder, 1900	Florissant Fm., United States of America
Steninae	*Stenus noach* Puthz, 2010	Baltic Amber, Russia
Steninae	*Stenus priscus* Benick, 1943	Baltic Amber, Russia
Tachyporinae	*Atheta florissantensis* Wickham, 1913	Florissant Fm., United States of America
Tachyporinae	*Boletobius durabilis* Scudder, 1900	Florissant Fm., United States of America

Tachyporinae	*Boletobius funditus* Wickham, 1913	Florissant Fm., United States of America
Tachyporinae	*Boletobius iyelli* Scudder, 1900	Florissant Fm., United States of America
Tachyporinae	*Boletobius stygis* Wickham, 1913	Florissant Fm., United States of America
Tachyporinae	*Bolitobius groehni* Schülke, 2000	Baltic Amber, Russia
Tachyporinae	*Lordithon durabilis* Yamamoto, 2021	Florissant Fm., United States of America
Tachyporinae	*Lordithon funditus* Yamamoto, 2021	Florissant Fm., United States of America
Tachyporinae	*Lordithon iyelli* Yamamoto, 2021	Florissant Fm., United States of America
Tachyporinae	*Lordithon stygis* Yamamoto, 2021	Florissant Fm., United States of America
Tachyporinae	*Mycetoporus demersus* Wickham, 1913	Florissant Fm., United States of America
Tachyporinae	*Palaeosepedophilus succinicus* Paśnik and Kubisz, 2002	Baltic Amber, Russia
Tachyporinae	*Parabolitobius groehni* Alekseev, 2013	Baltic Amber, Russia
Tachyporinae	*Sepedophilus balticus* Paśnik and Kubisz, 2002	Baltic Amber, Russia
Tachyporinae	*Tachinus sommatus* Wickham, 1911	Florissant Fm., United States of America
Tachyporinae	*Tachyporus bicoloratus* Paśnik, 2005	Baltic Amber, Russia
Tachyporinae	*Tachyporus nigripennis* Campbell, 1991	Florissant Fm., United States of America
Incertae sedis	*Crytodiodon corticaroides* Scudder, 1876	Baltic Amber, Russia
Incertae sedis	*Euroleptochromus sbathi* Scudder, 1876	Baltic Amber, Russia
Incertae sedis	*Geodomicus abditus* Scudder, 1876	Florissant Fm., United States of America
Incertae sedis	*Pantobatrisus censor* Scudder, 1876	Baltic Amber, Russia
Incertae sedis	*Semnodioceras halticaeformis* Scudder, 1876	Baltic Amber, Russia

Oligocene

There are 44 staphylinids recorded during the Oligocene era.

Subfamily	**Taxa**	**Deposit**
Aleocharinae	*Hygronoma deleta* Goss 1878	Aix en Provence, France
Aleocharinae	*Paracyptus minutissima* Seevers, 1971	Chiapas Amber, Mexico
Oxyporinae	*Oxyporus vulcanus* von Heyden and von Heyden, 1866	Rott Bonn Fm., Germany
Paederinae	*Achenium ingens* Goss, 1878	Aix en Provence, France
Paederinae	*Lathrobium gensium* Zhang, 1989	Shanwang Fm., China
Paederinae	*Lathrobium homorum* Zhang, 1989	Shanwang Fm., China
Paederinae	*Lathrobium phthartum* Zhang, 1989	Shanwang Fm., China
Paederinae	*Lathrobium provinciale* Goss, 1878	Aix en Provence, France
Paederinae	*Lathrobium sobrinum* Zhang, 1989	Shanwang Fm., China
Paederinae	*Lathrobium xiejiaheense* Zhang, 1989	Shanwang Fm., China
Paederinae	*Lithocharis varicolor* Heer, 1856	Aix en Provence, France
Paederinae	*Lithoplanes deleta* Heer, 1856	Aix en Provence, France
Paederinae	*Lithoplanes elongata* Heer, 1856	Aix en Provence, France
Paederinae	*Lithoplanes elongata* Heer, 1856	Armissan, Aude, France
Paederinae	*Medon stenus* Zhang, 1989	Shanwang Fm., China
Paederinae	*Sunius demersus* von Heyden and von Heyden, 1866	Rott Bonn, Germany
Scydmaeninae	*Microlynx franzbesucheti* Newton and Franz, 1998	Sicilian Amber, Italy
Scydmaeninae	*Scydmaenus heeri* Heer, 1856	Aix en Provence, France
Silphinae	*Silpha statuum* von Heyden and von Heyden, 1866	Rott Bonn Fm., Germany
Silphinae	*Palaeosilpha fraasii* Flach, 1890	Caylux, France
Silphinae	*Ptomascopus aveyronensis* Flach, 1890	Caylux, France
Staphylininae	*Algon spatiosus* Zhang, 1989	Shanwang Fm., China
Staphylininae	*Ocypus atavus* Heer, 1856	Aix en Provence, France
Staphylininae	*Ocypus provincialis* Heer, 1856	Aix en Provence, France
Staphylininae	*Philonthus bituminosus* Goss, 1878	Rott Bonn Fm., Germany

Staphylininae	*Philonthus boyeri* Goss, 1878	Aix en Provence, France
Staphylininae	*Philonthus marcelli* Goss, 1878	Aix en Provence, France
Staphylininae	*Quedius flicheri* Goss, 1878	Cereste, France
Staphylininae	*Quedius loretii* Goss, 1878	Aix en Provence, France
Staphylininae	*Quedius reynesii* Goss, 1878	Aix en Provence, France
Staphylininae	*Quedius rhenanus* Förster, 1891	Kleinkembs, France
Staphylininae	*Staphylinus aquisextanus* Goss, 1878	Aix en Provence, France
Staphylininae	*Staphylinus calvus* Goss, 1878	Aix en Provence, France
Staphylininae	*Staphylinus germarii* Goss, 1878	Aix en Provence, France
Staphylininae	*Staphylinus priscus* Goss, 1878	Aix en Provence, France
Staphylininae	*Staphylinus prodromus* Goss, 1878	Aix en Provence, France
Staphylininae	*Xantholinus westwoodianus* Goss, 1878	Aix en Provence, France
Steninae	*Stenus gypsi* Oustalet, 1874	Aix en Provence, France
Steninae	*Stenus ornatus* Förster, 1891	Brunstatt, Alsace, France
Steninae	*Stenus prodromus* Heer, 1856	Aix en Provence, France
Steninae	*Stenus scribai* von Heyden and von Heyden, 1866	Rott Bonn Fm., Germany
Tachyporinae	*Tachyporus sepultus* Goss, 1878	Rott Bonn Fm., Germany
Trichophyinae	*Palaeopsenius mexicanus* Seevers, 1971	Chiapas Amber, Mexico
Incertae sedis	*Sinolispinoides torosus* Zhang, 1989	Shanwang Fm., China

Miocene

There are 51 staphylinids recorded during the Miocene era.

Subfamily	**Taxa**	**Deposit**
Aleocharinae	*Ambracyptus minutissima* Seevers, 1971	Mexican Amber, Mexico
Aleocharinae	*Pareburniola dominicana* Zilberman et al., 2022	Dominican Amber, Dominican Republic
Aleocharinae	*Prorhinopsenius alzadae* Kistner, 1998	Dominican Amber, Dominican Republic
Aleocharinae	*Prorhinopsenius mexicanus* Seevers, 1971	Mexican Amber, Mexico

Aleocharinae	*Prorhinopsenius poinari* Kistner, 1998	Dominican Amber, Dominican Republic
Aleocharinae	*Prorhinopsenius seeversi* Kistner, 1998	Dominican Amber, Dominican Republic
Aleocharinae	*Oxypoda bindosa* Seevers, 1971	Mexican Amber, Mexico
Omaliinae	*Homalium protogeae* Heer, 1862	Öningen Fm., Germany
Osoriinae	*Liberiana sp.* Irmler, 2003	Dominican Amber, Dominican Republic
Osoriinae	*Lispinomimus atavus* Irmler, 2003	Dominican Amber, Dominican Republic
Osoriinae	*Nacaeus dominicanensis* Irmler, 2003	Dominican Amber, Dominican Republic
Osoriinae	*Neosorius spp.* Irmler, 2003	Dominican Amber, Dominican Republic
Osoriinae	*Osoriellus sp.* Irmler, 2003	Dominican Amber, Dominican Republic
Osoriinae	*Thoracophorus palaeobrevicristatus* Irmler, 2003	Dominican Amber, Dominican Republic
Oxyporinae	*Oxyporus seuberti* Heer, 1862	Öningen Fm., Germany
Oxytelinae	*Bledius speciosus* Heer, 1862	Öningen Fm., Germany
Oxytelinae	*Dolichoxenus newtoni* Engel and Chatzimanolis, 2009	Dominican Amber, Dominican Republic
Oxytelinae	*Oxytelus proaevus* Heer, 1862	Öningen Fm., Germany
Paederinae	*Lathrobium oeningense* Heer, 1862	Öningen Fm., Germany
Paederinae	*Palaminus spp.* Seevers 1971	Mexican Amber, Mexico
†Protactinae	*Protactus erichsoni* Heer, 1862	Öningen Fm., Germany
†Protactinae	*Protactus minor* Heer, 1862	Öningen Fm., Germany
Pselaphinae	*Apharus sp.* Poinar and Poinar 1999	Dominican Amber, Dominican Republic
Pselaphinae	*Arthmius sp.* Poinar and Poinar 1999	Dominican Amber, Dominican Republic
Pselaphinae	*Brachygluta sp.* Poinar and Poinar 1999	Dominican Amber, Dominican Republic

Pselaphinae	*Barroeuplectoides sp*. Poinar and Poinar 1999	Dominican Amber, Dominican Republic
Pselaphinae	*Caccoplectus sucineus* Chandler and Wolda, 1986	Mexican Amber, Mexico
Pselaphinae	*Jubus sp.* Poinar and Poinar 1999	Dominican Amber, Dominican Republic
Pselaphinae	*Scalenarthrus sp.* Poinar and Poinar 1999	Dominican Amber, Dominican Republic
Pselaphinae	*Thesium sp*. Poinar and Poinar 1999	Dominican Amber, Dominican Republic
Pselaphinae	*Trimium sp*. Poinar and Poinar 1999	Dominican Amber, Dominican Republic
Scaphidiinae	*Euconnus sp*. Franz 1983	Dominican Amber, Dominican Republic
Scaphidiinae	*Homoconnus conservatus* Franz, 1983	Dominican Amber, Dominican Republic
Scaphidiinae	*Mastigus sp.* Poinar and Poinar 1999	Dominican Amber, Dominican Republic
Scaphidiinae	*Microscydmus sp.* Poinar and Poinar 1999	Dominican Amber, Dominican Republic
Scaphidiinae	*Neuraphes fossilis* Franz, 1983	Dominican Amber, Dominican Republic
Scaphidiinae	*Scaphidium deletum* Heer, 1862	Öningen Fm., Germany
Scaphidiinae	*Scaphisoma gracile* Heer, 1862	Öningen Fm., Germany
Silphinae	*Silpha obsolete* Heer, 1862	Öningen Fm., Germany
Silphinae	*Silpha tricostata* Heer, 1862	Öningen Fm., Germany
Staphylininae	*Heterothops cornelli* Chatzimanolis and Engel, 2013	Dominican Amber, Dominican Republic
Staphylininae	*Heterothops infernalis* Chatzimanolis and Engel, 2013	Dominican Amber, Dominican Republic
Staphylininae	*Holisus funeratus* Chatzimanolis and Engel, 2013	Dominican Amber, Dominican Republic

Staphylininae	*Neoxantholinus apolithomenus* Chatzimanolis and Engel, 2013	Dominican Amber, Dominican Republic
Staphylininae	*Philonthus hades* Chatzimanolis and Engel, 2013	Dominican Amber, Dominican Republic
Staphylininae	*Philonthus rhadamanthus* Chatzimanolis and Engel, 2013	Dominican Amber, Dominican Republic
Staphylininae	*Quedius electrodominicanus* Chatzimanolis and Engel, 2013	Dominican Amber, Dominican Republic
Staphylininae	*Sphingoquedius meto* Shaw et al., 2020	Foulden Maar, New Zealand
Staphylininae	*Staphylinus atavus* Heer, 1862	Öningen Fm., Germany
Tachyporinae	*Coproporus electron* Yamamoto, 2016	Dominican Amber, Dominican Republic
Tachyporinae	*Tachyporus sp.* Poinar and Poinar 1999	Dominican Amber, Dominican Republic

Pliocene

There are nine staphylinids recorded during the Pliocene era.

Subfamily	**Taxa**	**Deposit**
Micropeplinae	*Kalissus nitidus* Matthews, 1970	Lava Camp Mine (Alaska), United States of America
Micropeplinae	*Micropeplus hoogendorni* Matthews, 1970	Lava Camp Mine (Alaska), United States of America
Micropeplinae	*Micropeplus hopkinsi* Matthews, 1970	Lava Camp Mine (Alaska), United States of America
Micropeplinae	*Micropeplus macrofuvulus* Korge, 1967	Willerhausen, Germany
Micropeplinae	*Micropeplus tesserula* Matthews, 1970	Lava Camp Mine (Alaska), United States of America
Oxytelinae	*Pliosyntomium schmidti* Korge, 1967	Willerhausen, Germany
Silphinae	*Allopliosilpha inclavata* Madge, 1980	Willerhausen, Germany
Silphinae	*Nicrophorus pliozaenicus* Sikes, 2003	Willerhausen, Germany
Silphinae	*Pliosilpha strausi* Madge, 1980	Willerhausen, Germany

Pleistocene and Holocene

There are 44 staphylinids recorded during the Pleistocene era.

Subfamily	**Taxa**	**Deposit**
Aleocharinae	*Gymnusa antiqua* Lomnicki, 1894	Boryslawia, Czech Republic
Euaesthetinae	*Euaesthetus laeviusculus* Keen et al., 1997	Frog Hall, England
Glypholomatinae	*Glypholoma chepuense* Thayer 1997	Chiloe, Chile
Glypholomatinae	*Glypholoma germaini* Thayer 1997	Chiloe, Chile
Glypholomatinae	*Proglypholoma aenigma* Thayer 1997	Chiloe, Chile
Micropeplinae	*Micropeplus cribratus* Ashworth and Nelson, 2014	Magnolia Bluff, United States of America
Micropeplinae	*Micropeplus laticollis* Miller et al., 1985	Quadra Sand Fm., British Columbia
Micropeplinae	*Micropeplus punctatus* Ashworth and Nelson, 2014	Magnolia Bluff, United States of America
Omaliinae	*Acidota (Omalium) quadrata* Campbell, 1982	Titusville Site, Canada
Omaliinae	*Acidota (Omalium) quadrata* Campbell, 1982	Two Creeks Forest Bed, Canada
Omaliinae	*Microedus austinianus* Campbell, 1982	Titusville Site, Canada
Omaliinae	*Olophrum (Omalium) consimile* Campbell, 1983	Titusville Site, Canada
Omaliinae	*Olophrum henryi* Elias 1980	Sangamon River, Canada
Omaliinae	*Olophrum latum* Campbell, 1983	Titusville Site, Canada
Omaliinae	*Olophrum (Omalium) rotundicolle* Campbell, 1983	Titusville Site, Canada
Omaliinae	*Omalium fuscum* Campbell, 1983	Thrupp House Farm, Canada
Omaliinae	*Omalonomus relictus* Campbell and Peck, 1990	Cypress Hills, Canada
Omaliinae	*Pycnoglypta campbelli* Campbell, 1983	Magnolia Bluff, United States of America
Omaliinae	*Pycnoglypta lurida (Omalium luridum)* Shavrin, 2016	Titusville Site, Canada
Oxyporinae	*Oxyporus stiriacus* Scudder, 1890	Scarborough Fm., United States of America

Oxytelinae	*Anotylus (Oxytelus) gibbulus* Hammond et al., 1979	Four Ashes, Toronto, Canada
Oxytelinae	*Anotylus (Oxytelus) gibbulus* Hammond et al., 1979	Seisdon, Toronto, Canada
Oxytelinae	*Anotylus (Oxytelus) gibbulus* Hammond et al., 1979	Brandon Wood, Toronto, Canada
Oxytelinae	*Osorius brunnicornis* Hope, 1837	Gum Animè Amber, Madagascar
Oxytelinae	*Oxytelus (Anotylus) gibbulus* Hammond et al., 1979	Four Ashes, Toronto, Canada
Oxytelinae	*Oxytelus (Anotylus) gibbulus* Hammond et al., 1979	Seisdon, Toronto, Canada
Oxytelinae	*Oxytelus (Anotylus) gibbulus* Hammond et al., 1979	Brandon Wood, Toronto, Canada
Oxytelinae	*Oxytelus laqueatus* Ashworth and Nelson, 2014	Magnolia Bluff, United States of America
Oxytelinae	*Oxytelus laqueatus* Ashworth and Nelson, 2014	Magnolia Bluff, United States of America
Oxytelinae	*Oxytelus niger* Ashworth and Nelson, 2014	Magnolia Bluff, United States of America
Oxytelinae	*Oxytelus niger* Ashworth and Nelson, 2014	Magnolia Bluff, United States of America
Paederinae	*Pinostygus galapagoensis* Campbell and Peck, 1989	Galapagos Islands, Ecuador
Pseudopsinae	*Pseudopsis sulcata* Herman, 1983	Highbury Silts and Sands Fm., United States of America
Pseudopsinae	*Pseudopsis sulcata* Herman, 1983	Kalaloch Site, Browns Point, United States of America
Steninae	*Stenus comma* Miller et al., 1985	Port Moody Disposal Pit, British Columbia
Steninae	*Stenus comma* Miller et al., 1985	Mary Hill Gravel Pit, British Columbia
Tachyporinae	*Lordithon longiceps* Elias, 2014	Ziegler Reservoir, United States of America

Tachyporinae	*Tachinus angustatus* Elias, 2014	Ziegler Reservoir, United States of America
Tachyporinae	*Tachinus apertus* Matthews, 1977	Western shore of Baldwin Peninsula, United States of America
Tachyporinae	*Tachinus elongatus* Elias, 2014	Ziegler Reservoir, United States of America
Tachyporinae	*Tachinus fimbratus* Elias, 2014	Ziegler Reservoir, United States of America
Tachyporinae	*Tachinus lanei* Elias, 2014	Ziegler Reservoir, United States of America
Tachyporinae	*Tachinus schwartzi* Elias, 2014	Ziegler Reservoir, United States of America
Tachyporinae	*Tachyporus nimbicola* Elias, 2014	Ziegler Reservoir, United States of America

6.4 Selected subfamilies of the Staphylinidae

The purpose of this section is to provide context for fossil staphylinids that have been formally described in chapter 8, and have been assigned to three subfamilies of the Staphylinidae.

6.4.1 Fossil Steninae

Two species of *Stenus* Latreille, 1797 have been described from the Mesozoic: *Stenus inexpectatus* Schlüter, 1978 from Bezonnais amber, France (99.7-94.3 Ma) (Schlüter 1978), and *Stenus imputribilis* Ryvkin, 1988 from Magadanskaia, Russia (84.9-70.6 Ma) (Ryvkin 1988). Żyła et al. (2017) also described *Festenus* with two species; viz. *F. gracilis*, and *F. robustus* from Burmese amber, Myanmar. Cai et al. (2019) also mentioned an undescribed genus from Australia. There are also nine other descriptions of fossil *Stenus* that have been discovered in the Cenozoic. These are from the Baltic amber of France (55-34 Ma), some of which were described by Puthz (2010). Clarke and Chatzimanolis (2009) reviewed the fossil record of the ‘euaesthetine subgroup’, which includes Euaesthetinae and Steninae. Thereafter, Cai et al. (2014) described a Steninae from the Eocene of France, *Eocenostenus fossilis* (Alés-Monteils, 37.2-33.9 Ma).

6.4.2 Fossil Tachyporinae

The oldest Tachyporinae may be *Protostaphylinus mirus* Lin, 1976, which was described from the Jurassic of Jiulongshan in China (164.7-155.7 Ma) (Cai and Huang 2010). Henceforth, the fossils belonging to the genera *Mesotachinus (M. major, M. medius and M. minor), Abscondus (A. regularis and A. similis)* and *Tachyporoides (T. villosus)* that have been described from Karatau, Kazakhstan (174.1-163.5 Ma) are, with confidence, currently considered to be the oldest Tachyporinae (Tikhomirova 1968). There are also other tachyporines that have been described from Talbragar Beds in Australia (155.7-150.8 Ma) (Cai et al. 2013), and from Daya Formation in Russia (145.1-100.5 Ma) (Ryvkin 1990).

There are numerous Cretaceous compression fossils of Tachyporinae that have been reported. Examples include *Glabrimycetoporus amoenus* Yue et al. 2009; and *Hesterniasca lata* Cai et al. 2011 form the Yixian Formation of China (126.2-124.6 Ma). The most prominent tachyporine is *Protachinus minor* Cai et al. 2013, and it is known to provide an early calibration point for the tribe Tachyporini (Cai et al. 2013). There are also tachyporines that have been described from Cretaceous ambers, such as: *Mesotachyporus puer* Gusarov, 2000, from New Jersey amber of the United States of America (94.3-89.3 Ma) (Gusarov 2000), and *Procileoporus burmiticus* Yamamoto, 2016, from Burmese amber of Myanmar (99.7-94.3 Ma) (Yamamoto 2016).

Yamamoto (2016, 2021) recently reviewed both the Mesozoic and Cenozoic Tachyporinae. Tachyporinae known from the Cenozoic epoch are listed in Table 8.2 of this study. Tachyporinae known from the Cenozoic epoch include compression fossils that have been recovered from the Green River Formation of the United States of America (50.3-46.2 Ma), Florissant Formation of the United States of America (1.4-1.1 Ma) (Scudder 1900), Baltic amber of Russia (55-34 Ma) and Dominican amber of the Dominican Republic (20.4-13.8 Ma) (e.g., Paśnik and Kubisz 2002; and Yamamoto and Takahashi 2016).

6.4.3 Fossil Paederinae

The earliest Paederinae are described from the Yixian Formation, and were initially assigned as '*incertae sedis*' to the genus *Mesostaphylinus* (Chatzimanolis 2018). These have since been described as *M. antiquus*, *M. elongatus*, and *M. yixianus* Solodovnikov et al., 2013 (Solodovnikov et al. 2013). There are also several described Mesozoic Paederinae such as *Apticax solidus* and

Apticax volans Schomann and Solodovnikov, 2012 known from the Crato Formation of Brazil (122.46-112.6 Ma) (Chatzimanolis 2018). Many species of Paederinae are described from Cenozoic deposits such as the Florissant Formation of the United States of America and Baltic amber of Russia and many other countries, and it is thought that the majority have been incorrectly assigned to the genus *Lathrobium* Gravenhorst, 1802 (Chatzimanolis 2018).

Furthermore, there are other notable Cenozoic taxa belonging to the above-mentioned genus, such as *Lathrobium provinciale* Goss, 1878 that has been formally described from the Aix-en-Provence of France (11.6-7.2 Ma) and several *Lathrobium* sp. from Shanwang of China (Chatzimanolis 2018). According to Betz et al. (2018), there are taxa that continue to await description from the Burmese amber of Myanmar, and from the Green River Formation of the United States of America, and Kishenehn Formation of the United Stated of America.

Despite studies by Bogri et al. (2020); Shaw et al. (2020); Schomann and Solodovnikov (2017); and Żyła et al. (2019, 2021), more effort is still needed regarding the classification and phylogeny of Paederinae, before new descriptions can be reliably assigned to existing tribes, subtribes and genera of the subfamily. For example, the genus *Apticax* proposed by Schomann and Solodovnikov, 2012 was formally described from the Crato Formation, and it was assigned to Paederinae + Staphylininae lineage; whereas more phylogenetic analysis by Solodovnikov et al. (2013) argued that the genus is missing some of its key features, and could therefore, not be assigned with much confidence in any subfamily of the Staphylinidae. To try and resolve this issue, perhaps it would also be helpful if a more rigorous classification is updated to the latest hypothesis by Cai et al. (2021), where Silphidae are actually downgraded to the subfamily within Staphylinidae.

CHAPTER SEVEN

MATERIALS AND METHODS

7.1 Deposit

The material used in this study was collected from ODM in Botswana, the only established Cretaceous deposit in Africa that has compression and impression fossil insects and plants. The material was collected from eighteen locations inside the ODM. The collection involved several visits to ODM by invited researchers from the then Bernhard Price Institute (BPI) of Palaeontology (now known as the Evolutionary Studies Institute - ESI) at the University of the Witwatersrand, Johannesburg, South Africa, between 1983 and 1988. The dates of the collections, GPS co-ordinates and approximate altitudes of the localities during the 1980's are given in PhD theses by McKay (1990), Bamford (1990) and Waters (1990), respectively.

In addition, another visit was made by Dr I.J. McKay and Mr. S. Mnguni, in June 2018. The purpose of this trip was to collect material from the waste heap, where all the fine-grained sediments collected from the ODM pit are dumped. There are still many fine-grained sediments that were left behind. More collecting trips are planned, to salvage more of the remaining sediments, and preserve the remaining heritage of ODM, which is threatened by mining activities. The unique deposit has been described in greater detail in chapters 2, 3 and 4 of this study.

7.2 Collecting method

In all the visits, the first collection step involved going around the mine, looking for an ideal working spot. When a fossil-rich bedding plane in the mudstone was found, it was followed laterally, and split with a hammer and a chisel. A 2.5 centimeter long chisel and geologist pick were best for breaking the rocks into pieces. Finer implements were used to expose small, and delicate specimens. Large rocks were split with a larger hammer, and a crowbar was used to remove blocks of rock from the rock face.

The Orapa kimberlitic mudstone weathers very rapidly, and it was these partially weathered rocks, which had been exposed by mining, that were the easiest to work with. Most specimens were

collected as rubble at the base of the rock face, making it difficult to work out an accurate origin of any specimen. Most localities were dynamited and bulldozed before specimens were collected (McKay 1990), and some were also collected from the spoil heap in 1983.

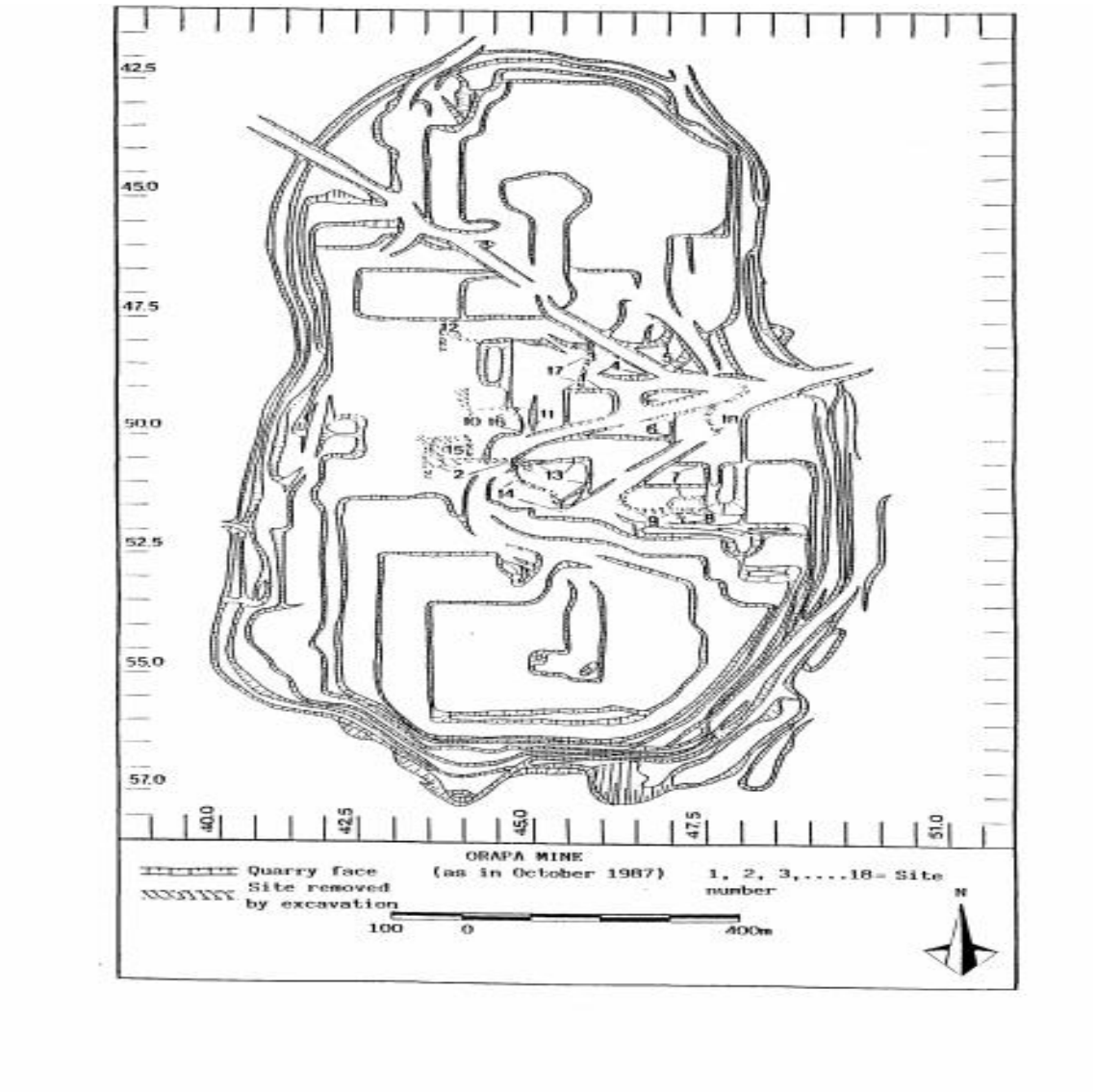


Figure 7.1 The positions of fossil site within the ODM. Taken from McKay (1990, page 252).

The collected lacustrine mudstone blocks from ODM were neatly wrapped in newspaper to prevent them from drying too quickly. These were then packed with foam in cardboard boxes to prevent

damage during transportation. Once the fossil insects and plants had arrived in the Herbarium of the ESI of the University of the Witwatersrand, Johannesburg, in South Africa; they were unwrapped, sorted, numbered, and curated accordingly.

7.3 Storage

Specimens were sorted on metal trays covered with foam. The fossil material collected from ODM is currently housed in the Herbarium of the ESI, at the University of the Witwatersrand, Johannesburg, in South Africa. Others are housed in the National Museum of Botswana (NMB), Gaborone, in Botswana, to form part of an exhibition, for tourism and heritage purposes. The ESI collection is currently housed in 20 cupboards that have 356 trays with about 6800 lacustrine mudstone blocks. The NMB collection has about 220 lacustrine mudstone blocks (Brothers and Rasnitsyn 2003). The plan in the future is to return to the mine and collect more specimens from the waste dump. The most recent visit to ODM by Dr I.J. McKay and Mr. S. Mnguni in June 2018 produced about 100 blocks which still need to be unwrapped sorted, numbered, curated, and studied accordingly. This is a painstaking process, which must be conducted with care, as every single block is fragile and delicate, and may contain dozens of specimens.

7.4 Taphonomy

This study involved randomly selecting 60 blocks; 20 brown sediments , 20 red sediments and 20 green sediments, respectively. One of each of these is illustrated in chapter 2 (Figures 2.3-2.6). Results from brown and red sediments were summed, since it was established that they were probably both deposited in similar, near-shore environments, and the different shades of color were difficult to tell apart. The summation meant that 40 brown-red sediments were compared with 20 green sediments. In total, 238 specimens were counted on the brown-red sediments, while 59 were counted on the green sediments. These were converted into percentages. It was expected that these would possibly indicate different depositional environments. The data was collected by recording the block color, specimen number, shelf/cupboard number, tray number, and temporal block number (which were assigned by me, so that I can easily locate the specimens). For the taphonomic analyses of this study, insect order and their relative abundances, insect body part preservation and their relative abundances, as well as the size of the insects and their relative abundances, were all recorded. Sedimentary features were also tentatively recorded, but are not presented in this study.

The presence of plant fragments, seeds, leaves, charcoal, and other non-insects material were also tentatively recorded as additional information. Some of these are presented in this study.

At this moment, the taphonomy section of this study offers preliminary results. Henceforth, the conclusions drawn are tentative. Future studies should perhaps increase the sample size, and also measure the relative abundance of the following variables: beetle suborders, families, habitat preferences, diet types and sizes. A similar study that can be used as a model was conducted by Smith (2000) at Willcox Playa, an ephemeral lake from south-eastern Arizona. This study compared relative abundance of the diet, feeding habitat, and size in live and dead assemblages. Due to time constraints, it was not possible to measure some of those variables in this study.

All the statistical analyses of the taphonomy section were done using PAST4PROJECT Statistical Software (https://folk.uio.no/ohammer/past/). The data was checked for normality in the brown-red and green lacustrine mudstone blocks from ODM. Twenty blocks were selected for each color. In other words, a total of 60 lacustrine mudstone blocks were selected, 40 brown-red and 20 green colored sediments. Insect orders, insect body parts and insect sizes were compared between the two differently-colored lacustrine mudstone blocks from ODM, which were thought to indicate different deposition environments. Only measures from the brown-red sediments were combined and subsequently compared against measures from green sediments. The data which were initially collected from cream and pink lacustrine mudstone blocks from ODM were excluded in the analysis altogether. It has been established that they indicate post-depositional alteration. As such, they did not fall into the scope of the study, which strictly involved investigating nearshore and offshore environments, assessing the taphonomic processes as discussed in chapter 5 of this study.

The data was analysed using Chi-Square Analysis of Contingency Tables (50:50 distribution) Tests. Measures that recorded zero were excluded in the statistical analysis. Chi-Square Analysis of Contingency Tables (50:50 distribution) Tests are said not to work well with recorded frequencies that are less than five. As a solution to this, Zar (1996) cited in Smith (2000), strongly suggested combining or completely removing rows and columns that have extremely low frequencies. Even with this removal of the measures with low frequencies, in the end, a lot of measures were still included in the statistical analysis of this study.

7.5 Taxonomic techniques

The identification and interpretation of fossils was challenging, because many structures were only visible under polarized light, or light from certain angles. Some of these structures are key for identification purposes, especially at family, subfamily, tribes, subtribe and genus taxonomic levels. Observation and photography were made with a combination of an Olympus SZX7 binocular microscope (with Olympus U-TV0.36XC camera), and an Olympus DSX 110 digital microscope. The binocular microscope (Olympus S7X7, SZX2-TR30, 4B06426, Japan) had an optical lens (Olympus DF PLAPO 1X-4, Schott EasyLED RL, Japan). Polarising filters (Model: Olympus KL 1600 LED, Schott Puravis; Japan) were attached to the swan necked light sources, and the objective lenses of the microscope. The scale in all the photographs is 1 mm.

Multiple images were stacked and measured using an Olympus Stream 2.4. All the images were edited in Adobe Photoshop version 5.6.5.58 (Adobe Creative Cloud, University of the Witwatersrand, Johannesburg, South Africa). The specimen was examined under cross polarised light to clarify the outlines, and non-polarised light was used at various angles to show relief. Using ethanol as an alternative to non-polarised light would have dissolved the material from the ODM.

When using the stereo microscope, non-polarised light was used to examine the relief of the specimen. However, excess reflection brought difficulties in viewing the outline and fine details. For this, polarised light was used. Polarising filters were attached to the swan necked lights, the objective lens of the microscope, and were rotating to polarise the light and remove reflection.

As a solution to the above-mentioned problem, an Olympus DSX 110 digital microscope, which shows the relief of the specimens in greater detail than the stereo or dissecting microscope was later used to interpret the fossils. It had a higher resolution than the binocular microscope. Therefore, this machine in particular made it possible to observe features that were not visible with the binocular microscope. The DSX 110 digital microscope is housed at the South African National Biodiversity Institute (SANBI) (Pretoria, South Africa). The DSX 110 digital microscope allowed us to take multiple images of sections of each specimen under high magnification, increase depth of field for each section using vertical stacking, and then stitch the images together for a complete image. Unfortunately, while the technique had many advantages, the stitching was not always seamless, resulting in distortion of the images, which could not be corrected (e.g., Fig 8.14). However, this was compensated for by taking separate images of the affected area.

7.6 Selection of specimens

The lacustrine mudstone blocks housed at the ESI were viewed with the naked eye, and interesting-looking specimens were examined under the microscope. Using this technique, the Staphylinidae described here were located and identified. All of them were tentatively assigned to a subfamily. All the specimens presented in this study have been assigned to a subfamily of the Staphylinidae, namely: Steninae, Tachyporinae, and Paederinae. After an intense literature search, a combination of observations and morphological character matrices by Clarke and Grebennikov (2009) (Steninae), Grebennikov and Newton (2009) (Tachyporinae),and Żyła et al. (2019), Bogri et al. (2020), and Shaw et al. (2020) (Paederinae) were used to assign the fossils into the subfamilies.

There are also other Staphylinidae that have been photographed in various research articles from ODM, which could not be located in the Herbarium of the ESI (e.g., McKay and Rayner 1986; Rayner and McKay 1986; and McCarthy and Rubidge 2005). These are possibly housed in the collection at Gaborone Museum in Botswana, or might still be in the ESI collection, but were not detected in the preliminary scan. Nevertheless, it is unlikely that the conclusions drawn in this study will be altered. However, locating the missing specimens in the future is warranted, since they are also in need of a proper taxonomic and systematic evaluation.

7.7 Taxonomy and Systematics

Taxonomy and systematics were restricted to the following subfamilies of the Staphylinidae: Steninae, Tachyporinae, and Paederinae. The staphylinids described in chapter 8 of this study were in the 60 lacustrine mudstone blocks that were selected for the taphonomical analyses of this study. This is why taphonomic results precede taxonomic and systematic results in chapter 8 of this study.

7.7.1 Classification system

The hierarchical nomenclature used in this study followed that of Bouchard et al. (2011), as it was the most comprehensive and recently-updated classification scheme. The classification system used followed the standard procedure in taxonomy and systematics research articles. In describing the fossils belonging to the Steninae, Tachyporinae, and Paederinae, online resources like LUCID (https://keys.lucidcentral.org/keys/v3/botw/) were used in the classification methodology.

7.7.2 Identification methodology

The fossil insects were examined, photographed, identified, and described. They were compared to extant southern African species housed at the Ditsong National Museum of Natural History (DNMNH) (Pretoria, South Africa). Diagrams and descriptions of taxa not represented in the collection were also used. In addition, the fossils were also compared with extant species from other regions using a variety of references and online resources such as BugGuide.Net (https://bugguide.net/node/view/126/bgpage) and Coleoptera,Org (http://www.coleoptera.org/). This comparative approach was paramount since most key features used to identify extant staphylinids; such as labium, antennae, eyes, coxae, paratergites, genitalia, and other group-specific features, are in most cases often missing, or poorly preserved, making it challenging to identify the fossils using a taxonomic dichotomous key, especially one based on extant taxa.

Regrettably, many tribes, subtribes and genera of Staphylinidae share similarities when it comes to traditional morphological characters. This poses identification challenges when using the general shape and size of the fossil. In addition, convergence may have produced forms in the Mesozoic, which resemble extant forms (i.e., bradytely), but have unique combinations of characters, which cannot be detected because they are not preserved.

The terminology of Bouchard et al. (2011) was used to describe the specimens. Specimens that could not be confidently assigned within a broader taxon were classified as '*incertae sedis*'.

It may be possible in the future, with improved techniques and technologies (e.g., image analysis, synchrotron, etc.), to identify more key features on the fossils that were not visible when using the available resources. The Olympus DSX 110 digital microscope offered the best opportunity of seeing the features clearly. However, it had its own difficulties, such as when stacking and stitching the images together, which often resulted in blurred photographs (e.g., Fig 8.14). It is possible that better technology will enable more features to be distinguished.

An additional research possibility will be to create a morphological character matrix for each of the assigned specimen. This will assist in comparing them with other taxa, and conducting a phylogenetic analysis, to see if it supports the conclusions drawn in this study. Not all the specimens are equally preserved, but the paederine may be well-enough-preserved to attempt this approach. Furthermore, a staphylinid specialists with a more intimate knowledge of the world taxa,

and this hyper-diverse group, may recognise features and similarities which have been missed here. Moreover, these fossils offer an opportunity for collaboration with staphylinid experts.

7.7.3 Terminology

The standard terminology from the literature which is commonly used to describe extant staphylinids was followed to describe the fossils. Palaeoentomologists are often faced with a challenge of coming across fossils preserved in two halves, from either side of the bedding plane where they were preserved.

The images are termed ‘part’ and the ‘counterpart’. In fossils, we established that it was usually possible to see both the dorsal and ventral features together, thereby causing confusion in the interpretation of the specimens. For example, in the staphylinids, it was difficult to distinguish the tergites and the sternites on the abdomen.

When a fossil insect is preserved in both ‘part’ and ‘counterpart’, the term refers to the ‘part’ preservation of the specimen. The terms ‘left’ and ‘right’ refer to the sides of the specimen; and, if the specimen was preserved in ‘part’ and ‘counterpart’, then the term refers to the ‘part’ preservation of the specimen, unless otherwise stated.

7.7.4 Measurements

Measurements were taken using Olympus Stream Essential. Ink, Olympus Soft Imaging Solution, XV Image Processing Software that is connected to the Olympus SZX7 binocular microscope.

The following measurements are given in the Systematic Palaeontology section (all in millimeters): (1) head width, which was measured at the widest point, (2) head length, which was measured from the anterior margin of the head to the anterior margin of the nuchal constriction; (3) neck width, which was measured across the narrowest part of the nuchal constriction; (4) pronotal length, which was measured mid-longitudinally from the anterior to the posterior margin; (5) pronotal width, which was measured across the widest place, usually at about the anterior third of the specimen; (6) elytral length, which was measured mid-longitudinally from the anterior to the posterior margin; and (7) elytral width, which was measured across the widest place, usually at about the anterior third of the specimen. Further details are given in the descriptions.

7.8 Distortion of the fossils during fossilisation

It is challenging to interpret fossils accurately without knowing the many processes by which they would have been distorted during fossilisation. Some of the relevant taphonomical processes have been described in greater detail in chapter 5 of this study.

The first distortion may occur when an organism is undergoing decomposition. For example, Ponomarenko (1977) cited in McKay (1990) suggested that it was difficult to interpret the hind coxae of fossil assigned to Archostemata and Adephaga, due to possible alteration of the specimen during decomposition. Importantly, no example of this type of distortion were noted in this study.

Distortion may also occur during fossilisation, as the specimen is compressed by sediments, resulting in some unusual effects:

i) Both dorsal and ventral surface of the specimens can be observed simultaneously, making it difficult to distinguish between them. The difficulty experienced in distinguishing between abdominal tergites and sternites has already been mentioned.
ii) Internal structures can become visible.
iii) The pressure on the fossil may possibly suggest that a specimen has distinct lateral margins that are absent in the living organism (Darlington 1967, cited in McKay 1990).

McKay (1990) cited Walton (1936), who argued that significant changes in the shape of an organism may not occur when it is subjected to high pressure. The author argues that theoretically, pressure from sediments during lithification should be equal from all sides. The Tachyporinae and Paederinae described in this study have identical shapes to their extant relatives. However, the Steninae is more difficult to interpret, because it is not lying straight. Constancy in morphology is called morphological stasis (Chatzimanolis 2018). It is possible that a beetle lying at an angle in the sediments may not collapse symmetrically, and have its shape and proportions changed during preservation. Rasnitsyn and Quicke (2007) also suggest that insects buried in the same layer with different cuticle thicknesses might be distorted differently.

CHAPTER EIGHT

TAPHONOMY AND TAXONOMY RESULTS

8.1 Taphonomy

This study involved randomly selecting 20 brown sediments, 20 red sediments and 20 green sediments, respectively. The brown and red sediments were combined, since it was established that they would have been recovered from the same depositional environment. Moreover, it was difficult to tell them apart. Therefore, the combination of the brown-red sediments was compared with green sediments. These were hypothesised to represent different environments, with the brown-red sediments hypothesised to represent a shallow-water oxidising environment, and the green sediments representing a deep-water anoxic environment. The study has a small sample size. Thus, the results reported here are only preliminary, and the conclusions drawn are tentative.

8.1.1 Insect orders

The majority of the insects were unknown specimens, in both brown-red and green sediments. The brown-red sediments had more unknown specimens than the green sediments. However, the results were not statistically significant ($\chi 2 = 10.92$, df = 7, p = 0.1288). There were differences in the representation of the different insect orders which could be identified. However, they were not statistically significant because of the small numbers of specimens ($\chi 2 = 10.92$, df = 7, p = 0.1288). The Coleoptera had the biggest contribution in both brown-red and green sediments, and they are considered to be medium to strong fliers in Smith (2012). However, their greatest percentage was found in the green sediments, which were hypothesised to represent a deep-water anoxic environment. Furthermore, brown-red sediments contained a slightly larger proportion of Diptera, Hymenoptera and Blattodea than green sediments, and these are considered to be strong fliers in Smith (2012). The situation for the Hemiptera was reversed, and it was rather identical to that of the Coleoptera. Another notable finding is that green sediments (in category none) had more blocks with no specimens than red-brown sediments.



Figure 8.1 The relative abundance of insect orders and unknown specimens in percentages (n = 20) ($\chi 2 = 10.92$, df = 7, p = 0.1288).

8.1.2 Insect body parts

The greatest percentage of the insects were preserved whole-bodied, almost 50%, in both brown-red and green sediments ($\chi 2 = 26.56$, df = 8, p = 0.0004). The brown-red sediments had slightly more whole-bodied insects than green sediments. In addition, in terms of disarticulation, brown-red sediments had more ¾ bodies, fragments and antennae, which were either non-existent, or minimal in green sediments. In contrast, green sediments had more wings, abdomen and elytra than brown-red sediments. Another notable finding is that there was almost an equal number of legs and paired elytra preservation in both brown-red and green sediments.

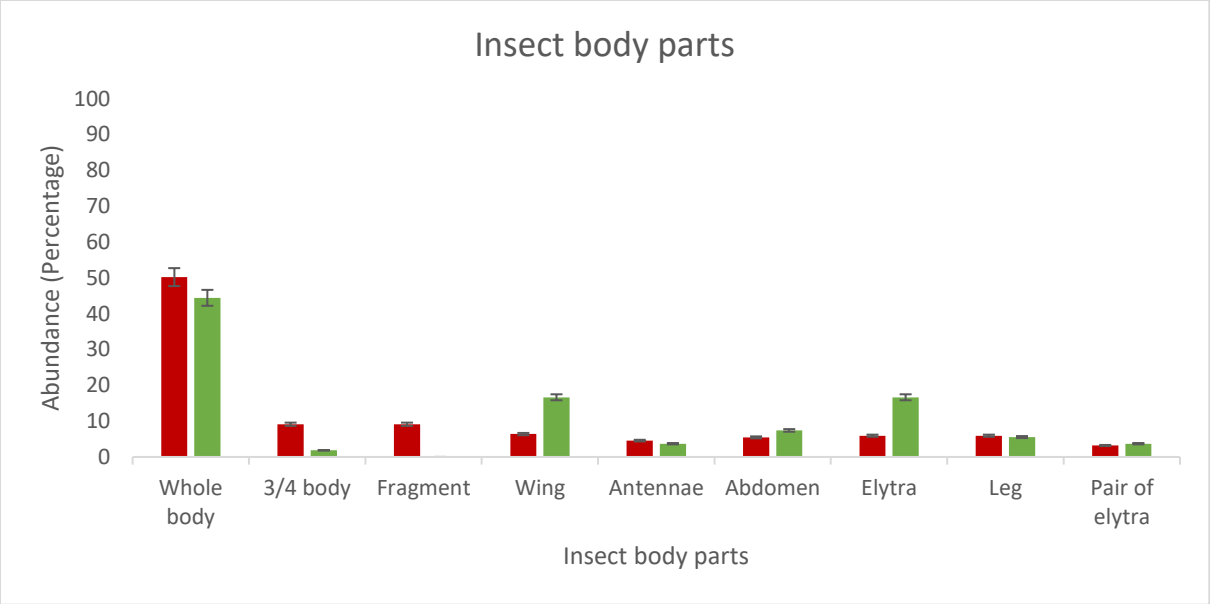


Figure 8.2 The relative abundance of insect body parts in percentages (n = 20) ($\chi 2 = 26.56$, df = 8, p = 0.0004).

8.1.3 Insect size

Although not significant, the majority of the insects were of small size, ranging between 1-5 mm, in both brown-red and green sediments ($\chi 2 = 3.07$, df = 4, p = 0.6114). There was also a fair number of medium size insects, ranging between 5-10 mm, in both brown-red and green sediments. A few large insects ranging between 10-15 mm were found in both brown-red and green sediments. Another notable finding is that the number of insects is equal in all the ranges reported here.

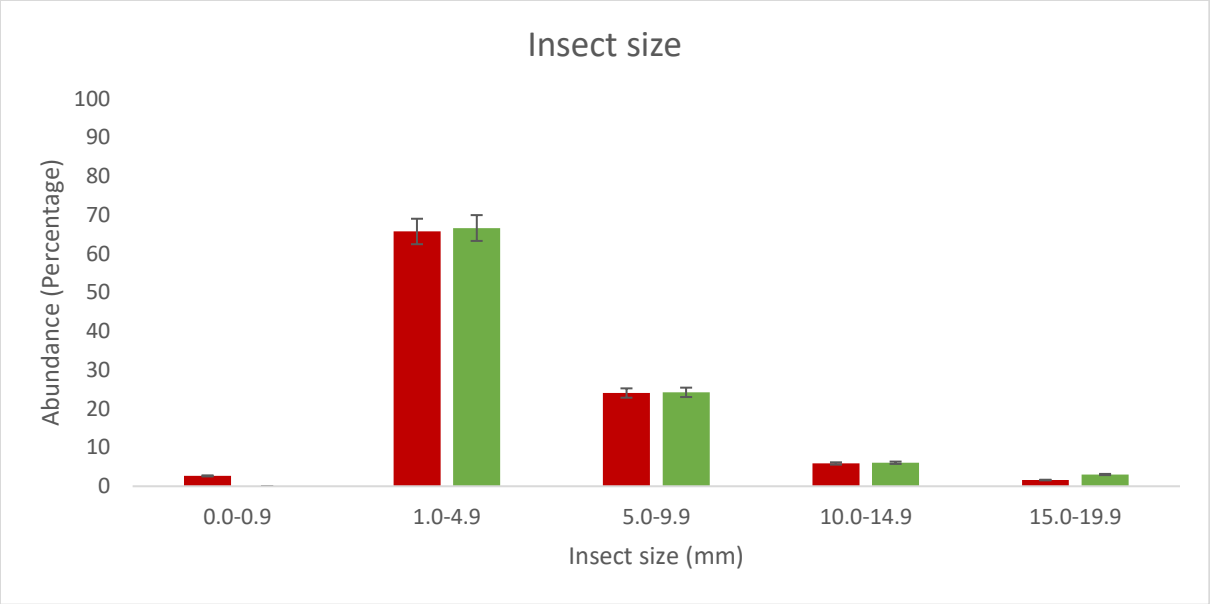


Figure 8.3 The relative abundance of insect sizes in percentages (n = 20) ($\chi 2 = 3.07$, df = 4, p = 0.6114).

8.1.4 Plant fragments

Generally, the majority of the blocks had a low amount of plant fragments ($\chi 2 = 33.16$, df = 3, p = 0.0001). The brown-red sediments had a slightly higher abundance of low amount of plant fragments compared to green sediments. The green sediments had the highest abundance of high amount of plant fragments Nonetheless, both brown-red and green sediments had over 50% of the blocks with a low amount of plant fragments. Finally, about 35% of brown-red sediments had no plant fragments whatsoever, compared to only 10% of green sediments.

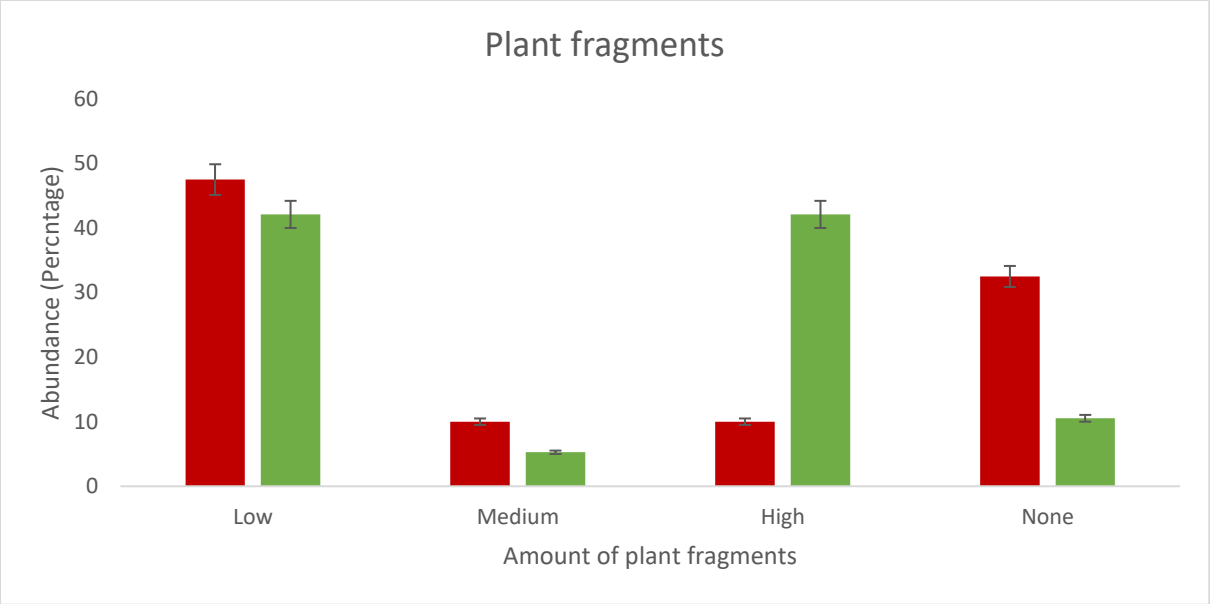


Figure 8.4 The relative abundance of plant fragments in percentages (n = 20) ($\chi 2 = 33.16$, df = 3, p = 0.0001).

8.1.5 Plant stems

In total, brown-red sediments had far fewer stems than green sediments ($\chi 2 = 33.16$, df = 3, p = 0.0001). In the low to high categories, green sediments had more stems than brown-red sediments. The green sediments had the highest abundance of high amount of plant stems. The green sediments also had the highest abundance of medium amount of plant stems. Moreover, the green sediments also had the highest abundance of low amount of plant stems Blocks with a high abundance of stems were estimated to have over 50% of the surface covered with stems, a medium abundance was between 25 and 50%, and low abundance was under 25%. Finally, over 70% of brown-red sediments contained no stems at all.

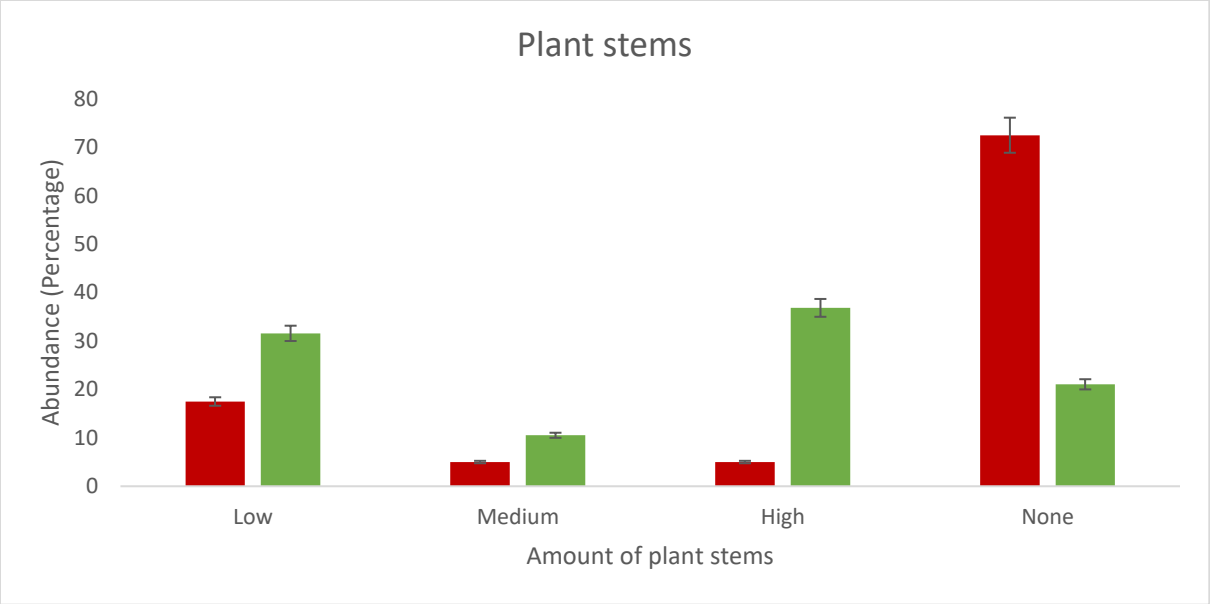


Figure 8.5 The relative abundance of plant stems in percentages (n = 20) ($\chi 2 = 57.15$, df = 3, p = 0.0001).

Summary of the taphonomy results

The surprising result of this analysis was that there were statistically more plant fragments and stems in green sediments than in brown-red sediments. This is a reverse of what would be expected, if brown-red sediments were nearshore, and green sediments were offshore. Perhaps there was some unknown mechanism carrying the plant material to the centre of the lake. The differences between the insect taxa were in general not significant. However, this would have to be confirmed by a larger sample size. Notably, there was a greater proportion of both medium and stronger fliers in the brown-red sediments, than in the green sediments, which had a higher proportion of Coleoptera and Hemiptera. The strong fliers included Diptera (which were described by Waters 1990), Hymenoptera (which were studied by Brothers and Rasnitsyn 2003) and Blattodea. The presence of Diptera and Hymenoptera, together with an abundance of Coleoptera in the brown-red sediments, supports the notion that these were shallow-water, oxidising, nearshore sediments. Strong fliers belonging in the above-mentioned insect orders would have landed in the water, spread their wings, and become rafted to shore (Smith 2012).

However, the presence vs. absence of surface mats can potentially have a crucial impact on these findings, and may strongly affect the conclusions drawn in this study concerning the nearshore vs. offshore hypothesis.

Table 8.1: Actual numbers showing the relative abundances of the orders, body parts and sizes of fossil insect specimens preserved in five differently-colored-mudstone-blocks that have been discovered from Orapa Diamond Mine (ODM) in Botswana (*n* = 20).

Mudstone blocks	**Brown**	**Red**	**Green**	**Pink**	**Yellow**	**Total**
Insect orders						
Unknown	100	48	29	13	23	213
Coleoptera	25	20	17	16	6	84
Diptera	10	5	2	1	0	18
Hymenoptera	7	0	1	1	2	11
Hemiptera	4	3	3	3	0	13
Blattodea	4	4	1	2	1	12
Orthoptera	3	0	1	1	0	5
None	2	3	5	5	8	23
Total	**155**	**83**	**59**	**42**	**40**	**379**
Insect body parts						
Whole body	61	49	24	26	24	184
¾ body	14	6	1	0	1	22
Wing	13	1	9	2	1	26
Antennae	7	3	2	1	2	15
Abdomen	6	6	4	1	3	20
Elytra	11	2	9	3	3	28
Pair of elytra	7	0	2	0	0	9
Leg	12	1	3	1	1	18
Fragment	11	7	0	2	1	21
Total	**142**	**75**	**54**	**36**	**36**	**343**

Insect size (mm)						
0.0-0.9	5	0	0	1	2	8
1.0-4.9	78	45	22	30	17	192
5.0-9.9	27	18	8	9	10	72
10.0-14.9	5	5	2	3	3	18
15.0-19.9	1	2	1	3	1	8
Total	**116**	**70**	**33**	**46**	**33**	**298**

8.2 Taxonomy

8.2.1 Steninae

The subfamily Steninae contains the most speciose staphylinid genus in *Stenus*, which is also the second largest animal genus, with approximately 3 000 species (Betz et al. 2018). Although the higher order systematics of the Staphylinidae is continuously being revised, there is some consensus that the Steninae are a monophyletic group, and are the sister group of the Euaesthetinae (Clarke and Chatzimanolis 2009; Clarke and Grebennikov 2009; McKenna et al. 2015; and Żyła et al. 2017), although Lü et al. (2020) could not find compelling evidence for this relationship.

The Steninae comprise of only four genera. The two extant genera are *Stenus* (with > 3000 species) and *Dianous* (with > 300 species) (Clarke and Grebennikov 2009; Cai et al. 2014; McKenna et al. 2015; Żyła et al. 2017; and Betz et al. 2018). There are also two extinct genera; the monospecific *Eocenostenus fossilis* from the Eocene Alés-Monteils of France (Cai et al. 2014), and the Cretaceous genus *Festenus* with two species; viz. *F. gracilis*, *F. robustus* (Żyła et al. 2017).

In addition to *Eocenostenus* and *Festenus*, there are 24 specimens ascribed to *Stenus* (Betz et al. 2018). Schlüter (1978) described *Stenus inexpectatus* using one specimen from a Cretaceous Bezonnaise amber in France, and Ryvkin (1988) described a single rock fossil as *Stenus imputribulus* from a Cretaceous Obeschayushchiy locality in Russia. These specimens are figured in Puthz (2010). While the details are hard to make out, both specimens have the typical habitus of *Stenus*, which includes a large pair of protruding eyes, a cylindrical prothorax, and a flattened or cylindrical abdomen. *Stenus inexspectatus* Schlüter 1978 (Figure 18; Puthz 2010) has an

antennae with a 3-segmented club originating from just behind the anterior margins of the eyes, but poor preservation makes a more accurate determination impossible.

Except for *Eocenostenus* Cai et al. 2014, which differs from other stenines because it combines a strongly transverse prothorax with anterolateral projections, together with anteriorly placed antennal insertions (Cai et al. 2014), all Cenozoic fossils of stenines belong to the genus *Stenus* (Żyła et al. 2017). Żyła et al. (2017) have, therefore, questioned whether *Eocenostenus* might not be a typical *Stenus* species that was deformed during fossilisation. A more thorough interpretation of the fossil suggests that this is not likely.

Nine of the Cenozoic *Stenus* fossils are known from Baltic amber of Russia (Alekseev 2013). Some of them have typical features of the genus, such as the characteristic elongated mouthparts (Żyła et al. 2017). The remaining *Stenus* species are from Eocene, Oligocene, Miocene, and Pleistocene rock fossils (Smith and Marcot 2015; and Żyła et al. 2017).

The Steninae have been studied since the 19th century. Their biology is relatively well-known, and has recently been reviewed by Betz et al. (2018) who, along with Żyła et al. (2017) and Cai et al. (2019) have described one of the unique features of the taxa (their "harpoon-like" mouthparts) in some detail. The genus *Stenus* has a unique elongated labium tipped by an adhesive covered cushions called paraglossae, which can be protruded to trap and retrieve small prey invertebrate animals such as Collembola (i.e., springtails) within a few milliseconds (Cai et al. 2019). The complex mechanism of the rapid protrusion of the labium includes an increase in haemolymph pressure coordinated with the contraction and relaxation of various muscles (Betz et al. 2018).

Aside from the genus *Stenus*, these "harpoon-like" mouthparts have also been identified in an undescribed Australian species (Clarke and Grebennikov 2009), and in fossil specimens of the genus *Festenus* (Cai et al. 2019). The mouthparts are also common in *Stenus* specimens described in Cenozoic ambers (Żyła et al. 2017). On the other hand, the mouthparts appear to have been secondarily lost from the paraphyletic genus *Dianous*, which arose from the genus *Stenus* (Koerner et al. 2013; and Żyła et al. 2017). Also, perhaps the mouthparts possibly evolved more than once in the Steninae (Żyła et al. 2017). Given the discovery of the specialised mouthparts in *Festenus*, it seems most likely that they are part of the ground plan of the Steninae, and that perhaps the loss in *Dianous* is secondary (Cai et al. 2019). Moreover, it should be noted that the development of

the mouthparts is variable across the family, and even the genus *Dianous* can slightly protrude the labium, but do not have sticky-pad paraglossae like *Stenus* (Żyła et al. 2017).

Another intriguing characteristic, which may be a synapomorphy of the subfamily Steninae (Clarke and Grebennikov 2009) are the secretions of the larger set of paired pygidial glands that empty into the rectum, and which, in addition to a defensive and anti-microbial function, can be squirted onto the water surface, where they spread rapidly, propelling the stenine beetles forward at high speed on their hydrophobic tarsi. This is termed skimming behavior (Lang et al. 2012; and Betz et al. 2018).

Clarke and Grebennikov (2009) identified 23 larval and adult synapomorphies for the family Staphylinidae. Of these, they selected 12 features (6 adult and 6 larval) as being most significant because they have the highest consistency index. The 6 adult features are: antennae inserted into the frons between the eyes, although the genera *Festenus, Eocenostenus,* and one specimen of an unidentified Australian species have the antennae inserted at the front of the eyes (Żyła et al. 2017); apodemes arising from interantennal pits; labial palps situated close together at the anterior margin of labrum; pronotal marginal carina meeting pronotosternal suture laterally, although in *Festenus,* the pronotal marginal carina does not meet the pronotosternal suture (Żyła et al. 2017); apex of mesoventral intercoxal process abuts metaventral intercoxal process, although in *Festenus* these two processes overlap; and paired pygidial glands opening into the rectum.

It is not surprising that some of the most consistent synapomorphies are associated with the specialized "harpoon-like" labial feeding apparatus. For example, Żyła et al. (2017) lists several of these characteristics: adhesive cushions of labium present, with labial palps almost contiguous and nearer to medial apex than sides of labium; lateral palpomere rests on mentum divided by medial longitudinal carina; and prementum modified, elongated into eversible rod-like structure. Given that these are traditionally accepted as properties of *Stenus*, and are now known to be properties of *Festenus* (Żyła et al. 2017; and Betz et al. 2018) and the undescribed Australian genus (Clarke and Grebennikov 2009), it seems safe to confidently state they are synapomorphies of the basal Steninae, and that most, except for the "almost contiguous labial palps", have been secondarily lost in the genus *Dianous* (Żyła et al. 2017; and Cai et al. 2019).

The habitat preference of the Steninae have been reviewed by Betz et al. (2018). They are associated with damp environments ranging from waterside, moist tree litter, forest canopy,

foliage, grass clumps, moss, dung, ant nests and mammal middens. A few are found in dry habitats; and on the other extreme, *Dianous* can be found in the water spray associated with rapids and cascades. *Stenus* may be found in all continents except for Antarctica. It is also absent from New Zealand. Although *Stenus* is relatively common in almost all the regions, there are only 29 species recorded in Australia. Meanwhile, *Dianous* is restricted to Palaearctic, Nearctic and Oriental geographical localities (Betz et al. 2018).

Systematic Palaeontology.

Order: Coleoptera Linnaeus, 1758

Series: Staphyliniformia Latreille, 1802

Superfamily Staphylinoidea Latreille, 1802

Family: Staphylinidae Latreille, 1802

Subfamily: Steninae MacLeay, 1825

Genus: ***Afristenus* gen. nov.**

Afristenus orapensis species nov. (Figures 8.6 and 8.7 show the specimen in polarised and non-polarised lights from different angles).

Etymology: The generic epithet is a combination of "Afri", from Africa, and the generic name *Stenus*.

Type species. *Afristenus orapensis* species nov., by monotypy and present designation.

Composition. Only type species *Afristenus orapensis* species nov.

Diagnosis. With harpoon-like labial feeding apparatus which is characteristic of the Steninae. It can be distinguished from all the other Steninae by: transverse pronotum distinctly broader than the head (although this character could be caused by the distortion of the specimen), eyes cover two thirds of the sides of the head, antennae with a five-segmented-club originating on frons between the eyes.

Afristenus orapensis species nov.

Etymology: The epithet of the new species is a combination of Orap- from 'Orapa', the deposit where the specimen described here was recovered, which is derived from Sesarwa (a San or Bushmen language), and is named after a nearby cattle post, meaning 'the resting place of lions' (https://www.debeersgroup.com/about-us/our-operations/our-mines/), and also see Brook, (2019); and -ensis, a Latin suffix meaning 'from'.

Material studied: Holotype, adult, (specimen number BP/2/25912, Herbarium, Evolutionary Studies Institute (ESI), University of the Witwatersrand, Johannesburg, South Africa). A single specimen of an adult with carbonaceous material preserving dorsal and ventral structures is described. The antennae, eyes, legs, and mouthparts are only partially preserved in a large, triangular piece (205 x 115 x 45 mm) of brown lacustrine mudstone block. Originally, the specimen had the tip of the abdomen preserved (Fig. 8.6). Due to the fragile nature of the mudstone, the tip was accidentally lost (Fig. 8.7).

Type locality and horizon: Botswana, Orapa Diamond Mine, lacustrine deposit, Upper Cretaceous (Davis, 1977 and Haggerty et al., 1983).

Diagnosis: As for the genus ***Afristenus*** **gen. nov.**.

Description: Body small measuring 2.33 mm in length (from tip of mandibles to tip of abdomen) (Figures 8.6 and 8.7).

Head (Figure 8.8, 8.9 and 8.10) angled to right, prognathous, longer than wide, 0.33 mm long (from tip of mandible to anterior margin of pronotum) and 0.30 mm wide at posterior of eyes. Eyes large, slightly protruding, cover 2/3 of side of head, with pair of dark internal ring structures, possibly representing entrance of optic nerve into head capsule. Distinct ridge runs across head between posterior margins of eyes, presumably representing negative of nuchal depression found in some Steninae, lacks nuchal constriction behind eyes, head and neck broader than eyes. Antenna (Figures 8.7, 8.7 and 8.9) with 5-segmented club and stem of indistinct elongated antennomeres. Antennal insertions on frons between eyes. Protruding labium with connecting tube (mentum), elongated prementum and paired paraglossae (Figure 8.10). Two segments of maxillary palps indistinctly preserved on left hand side, possibly one segment of right maxilla or right mandible. Mandibles indistinct.

Pronotum transverse (Figure 8.6, 8.7, 8.8, 8.9, and 8.10), rectangular, finely punctate, possibly representing compressed tube, wider than long with rectangular anterior and posterior margins, lateral margins slightly curved (greatest width medially), anteriorly distinctly wider than head. Left elytron (Figures 8.6, 8.7, 8.8, and 8.9) well preserved with circular clear area which may be a pigment spot, right elytron indistinct. Elytron rectangular, broader distally than at base, coarsely punctate, longer than wide (0.60 mm long, from posterior margin of pronotum to posterior margin of elytron, 0.30 mm wide at base and 0.37 mm wide distally), together distinctly wider than abdomen, slightly wider than pronotum, lacks epipleural ridge and fold. Legs poorly preserved (Figures 8.6, 8.7, 8.8, 8.9, and 8.10), procoxae not preserved, mesa and metacoxae indistinct, legs on left side of body represented by indistinct meso-femur and compact tarsus, difficult to distinguish individual segments, legs on right side of body represented by three pairs of femora and tibia.

Abdomen apparently flat (Figures 8.6 and 8.7), flexible, and gradually tapering posteriorly (1.22 mm long, 0.34 wide at base and 0.41 mm wide distally), with six visible abdominal segments, distinctly narrower than elytra; widest around segments III-V, first four visible abdominal segments with paratergites, segment VIII at tip of the abdomen with a V-shaped imagination posteriorly, possibly representing male sternite.

Taphonomical remarks: It appears as though it is about to sting. Perhaps becoming trapped in the mud would have activated a flight/fight behavioral response. Alternatively, this could be an artefact from a post-mortem distortion. In addition, according to Frank and Thomas (2002), for fossil insects, the length of adults is an inaccurate matric of size since abdominal segments can be telescoped, making the body misleading longer when the insect is alive under moist conditions, but shorter when it is dried, dead and buried.

Palaeoenvironment: Stenus is commonly found in waterside situations and wet places such as marshes, while some are found in forest litters (Betz et al. 2018). The discovery of *Afristenus orapensis* at Orapa Diamond Mine in Botswana supports the current model of the deposit as being a well vegetated crater with a lake in the centre. The preservation of a labium with paraglossae is remarkable, and confirms that the new species (and possibly other members of *Afristenus*) were also predacious, similarly to all the other members of the Steninae that have been discovered.

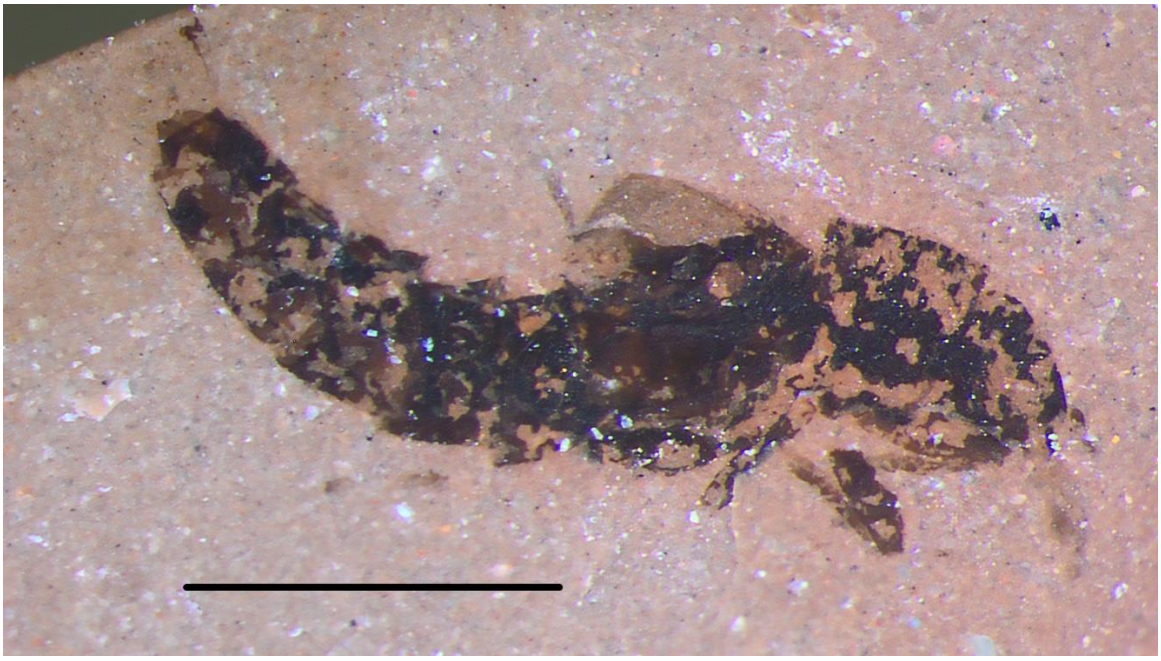


Figure 8.6 The whole body of *Afristenus orapensis* gen. nov., species nov. holotype, BP./2/25912, Orapa Diamond Mine, polarised light, scale bar = 1 mm.

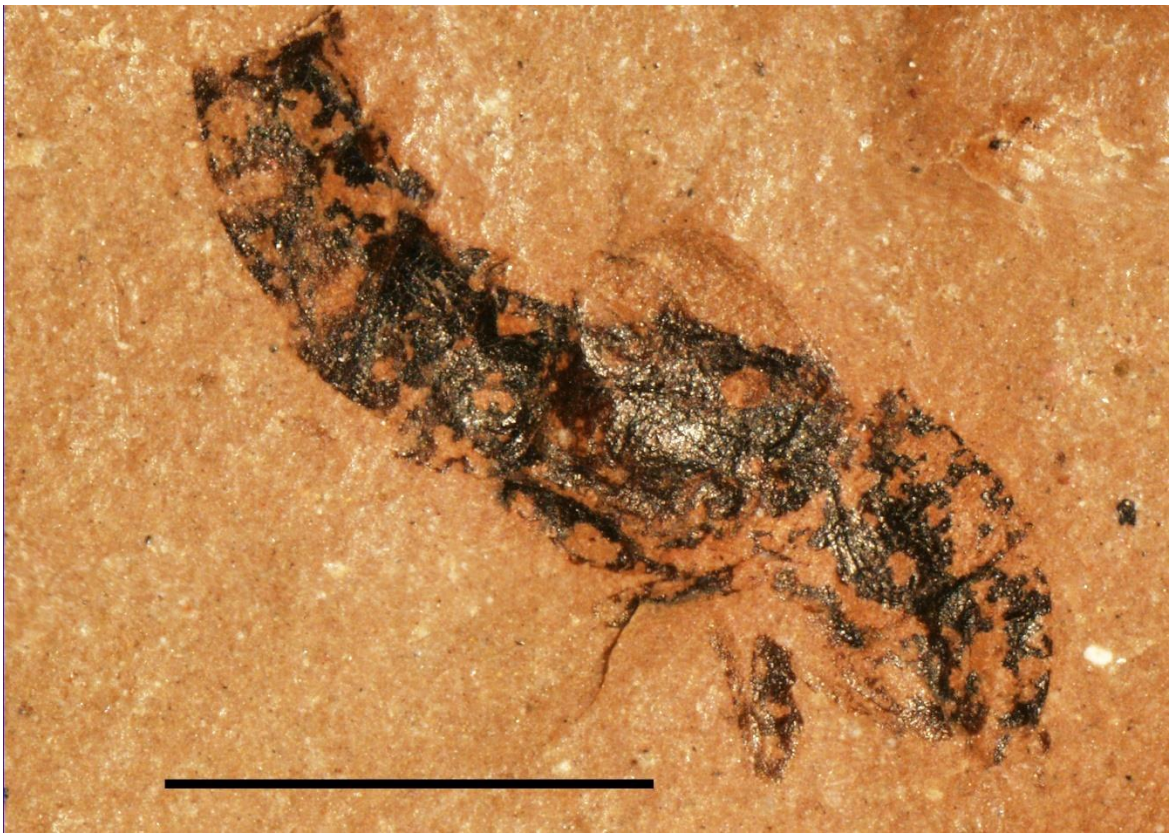


Figure 8.7 The whole body of *Afristenus orapensis* gen. nov., species nov. holotype, BP./2/25912, Orapa Diamond Mine, non-polarised light, scale bar = 1 mm.

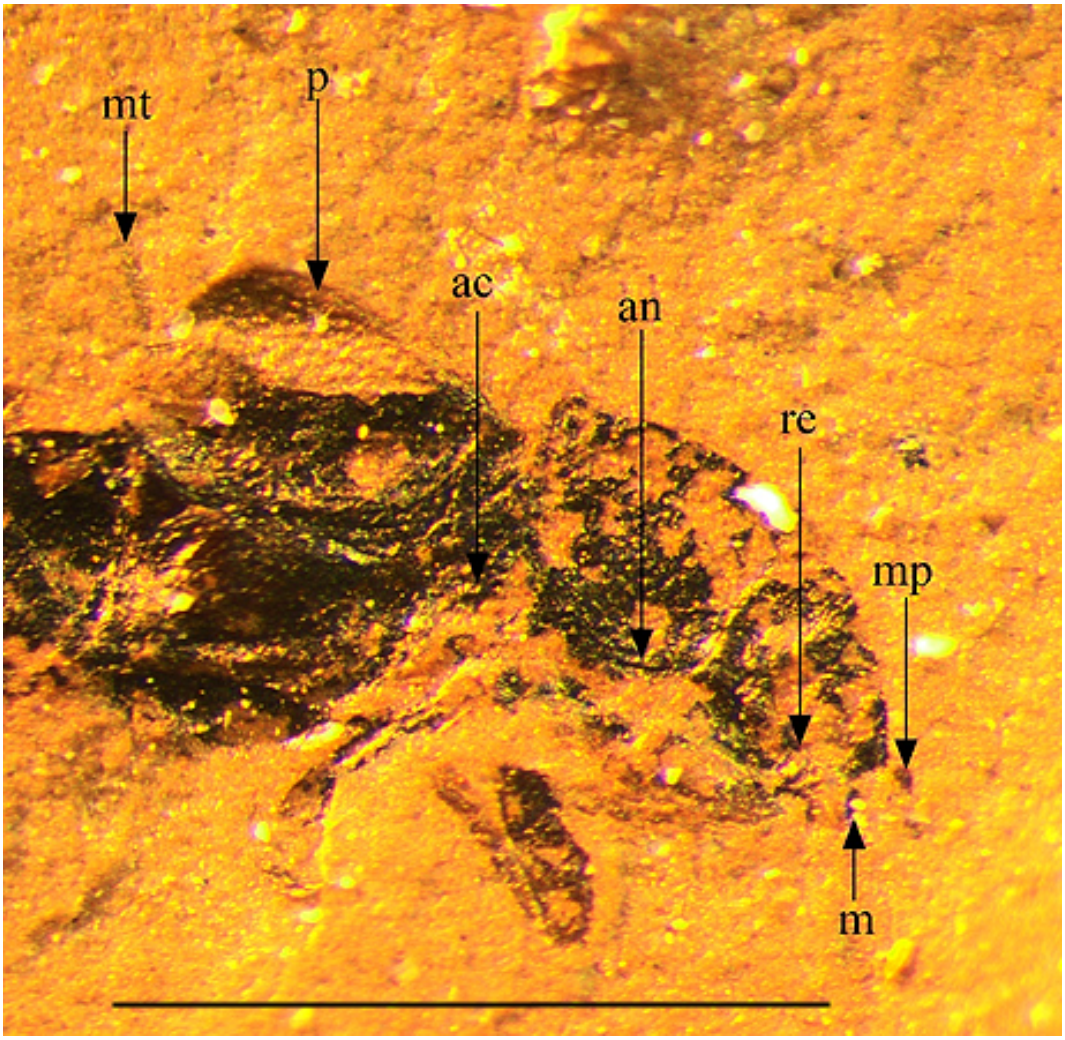


Figure 8.8 The body parts of *Afristenus orapensis* gen. nov., species nov. holotype, BP./2/25912, scale bar = 1 mm. Abbreviations: mt = metatarsus, p = punctuation, ac = antennal club, an = antennomere, re = right eye, mp = maxillary palp and m = mandible.

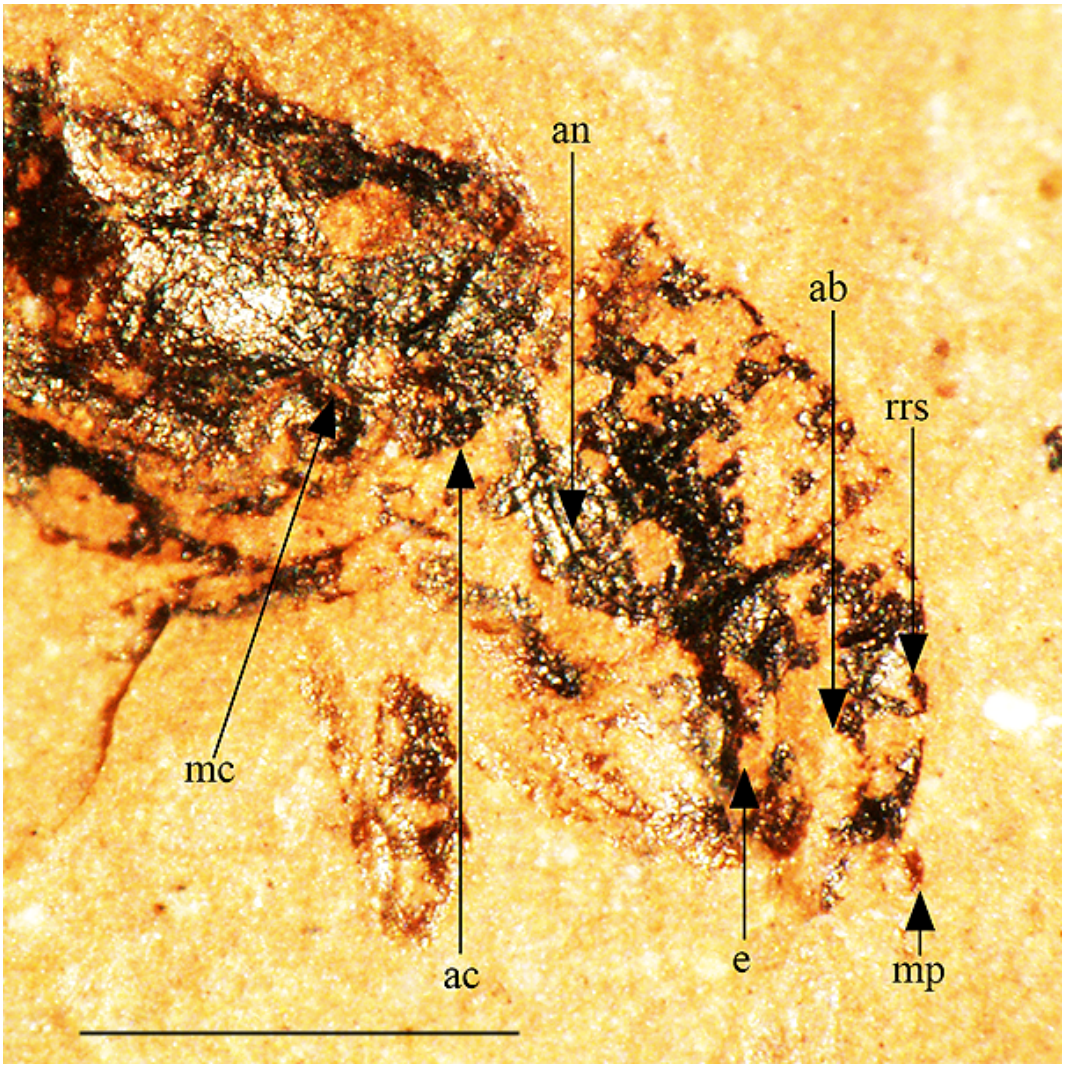


Figure 8.9 The body parts of *Afristenus orapensis* gen. nov., species nov. holotype, BP./2/25912, scale bar = 1 mm. Abbreviations: an = antennomere, ab = antennal base, rrs = right ring structure, mc = mesocoxae, ac = antennal club, e = eye and mp = maxillary palp.

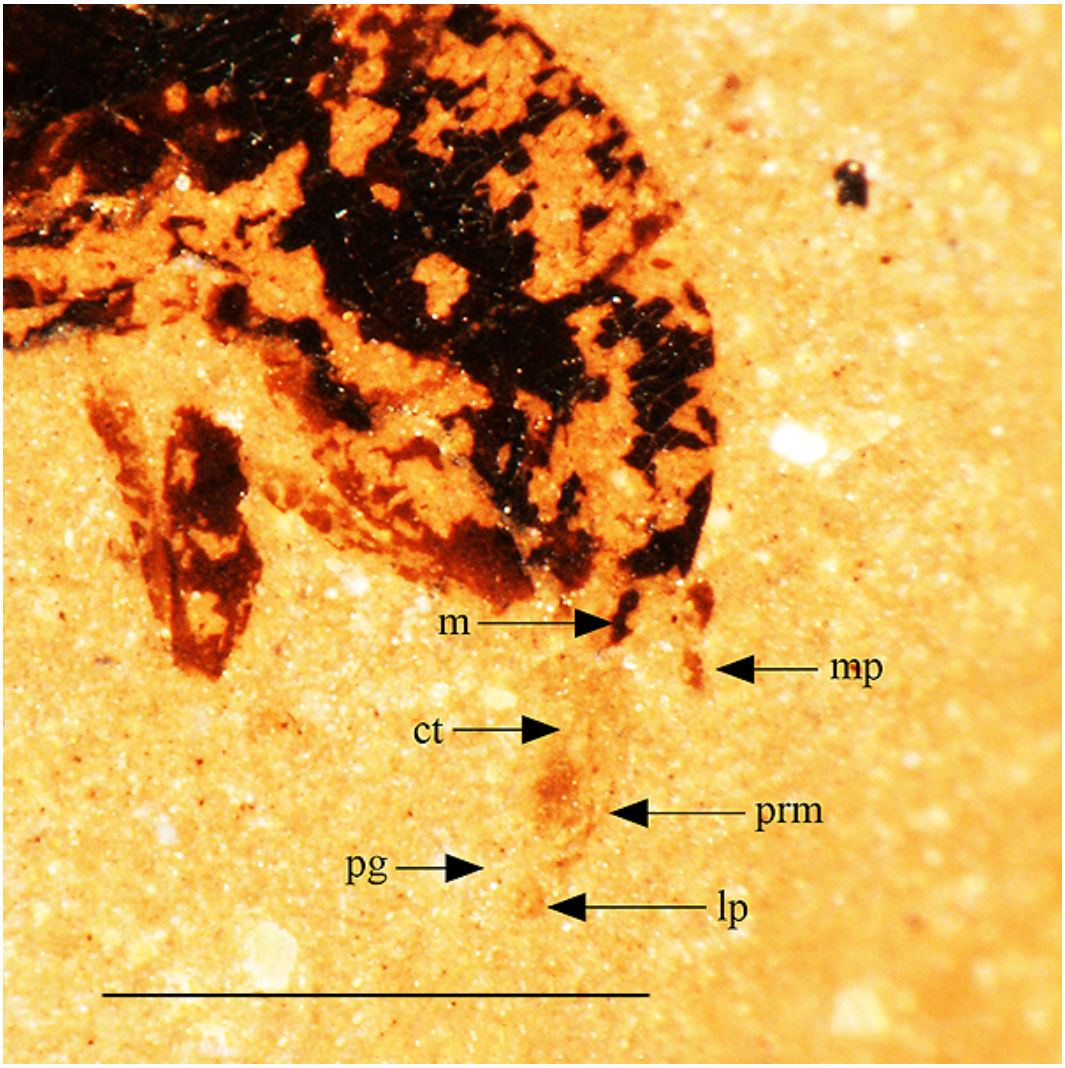


Figure 8.10 The body parts of *Afristenus orapensis* gen. nov., species nov. holotype, BP./2/25912, scale bar = 1 mm. Abbreviations: m = mandible, mp = maxillary palp, ct = connecting tube, prm = prementum, pg = paraglossae and lp = labial palp.

8.3.2 Tachyporinae

The Tachyporinae have recently been reviewed by Yamamoto (2021). According to Yamamoto (2021), the Tachyporinae is one of the most phylogenetically problematic subfamilies of the Staphylinidae. They currently have approximately 1 638 species (28 extinct) of 40 genera (12 extinct) in five tribes (Yamamoto 2021). Their descriptions are given from Table 6.3 to Table 6.6 of this study. The Tachyporinae are commonly distinguished by having a typically much more tapered abdomen compared to other staphylinids, and are frequently found in forest-associated

environments, being distributed in all zoogeographical regions, except the Antarctica (Yamamoto 2021). The Tachyporinae are thought to be one of the older lineages of the Staphylinidae (Yamamoto 2016). Their first record dates back to the Jurassic (Table 8.2). So far, very little work has been done on their biology and ecological habits, especially beyond the subfamily level.

Research done thus far has shown tachyporines to be largely mycophagous, or predators of other arthropods, especially those found in wet conditions, such as along rivers and streams (Cai et al. 2013). A recent study has even suggested that some tachyporines are effective pollinators (Yamamoto 2021). According to Newton et al. (2000), many tachyporines are small to medium in size, inhabiting micro-habitats such as leaf litter, bark, dead wood or fungi. Some tachyporines have a myrmecophilous relationship with army ants, which has resulted in an evolution of the limuloid and sublimuloid body forms, with a hugely expanded pronotum (Yamamoto 2021). The limuloid body form is one of the diagnostic features of the group (Yamamoto 2021).

The Tachyporinae is said to be polyphyletic in lineage, on the basis of both larval and adult morphological characters (Yamamoto 2021). The non-monophyly of the Tachyporinae was first proposed around the mid-1970's (Yamamoto 2021). Since then, many phylogenetic analyses have not supported the monophyly of the tachyporine subgroup, Tachyporinae, and Tachyporini at large (e.g., McKenna et al. 2015; and Lü et al. 2020).

Until recently, Deropini Smetana 1983, Megarthropsini Cameron 1919, Mycetoporini Thomson 1859, Tachyporini MacLeay 1825, and Vatesini Seevers 1958 were the 5 tribes that make up the Tachyporinae (Yamamoto 2021). According to Yamamoto (2021), Herman (2001) recognised two more tribes, Symmixini Bernhauer 1915 and Cordobanini Bernhauer 1910, respectively. The Symmixini Bernhauer 1915 are now synonymised under Tachyporini, while the Cordobanini Bernhauer 1910 have now been transferred to the Aleocharinae (Yamamoto 2021). To date, the maximum parsimony phylogenetic analysis of the Tachyporinae by Yamamoto (2021) is the most recent and comprehensive phylogenetic analysis of the group. In the analysis, Yamamoto (2021) divides the family into two monophyletic units, the Mycetoporinae and the Tachyporinae. In Yamamoto (2021), the Tachyporinae are further subdivided into the Tachyporini, Vatesini, Tachinusini and the Deropini. The Mycetoporinae are reported to be distantly related to the Tachyporinae, and are most likely the sister taxon of the Olisthaerinae (Yamamoto 2021).

Yamamoto (2021) summarised the fossil record of the Tachyporinae. According to Yamamoto (2021), six species in three genera were described by Tikhomirova (1968) from the Upper Jurassic Karabastau Formation in Kazakhstan. These were *Abscondus regularis*, *Abscondus similis*, *Mesotachinus major*, *Mesotachinus medius*, *Mesotachinus minor*, and *Tachyporoides villosus*. The Jurassic fauna is completed by other two monotypic genera, *Prototachinis minor* Cai et al. 2013 from Australia (Cai et al., 2013), and *Protostaphylinus mirus* Lin 1976 (Lin, 1976) from Middle Jurassic in China. According to Yamamoto (2021) six tachyporine "rock fossils" are known from the Cretaceous; and include 3 monotypic genera from Russia (Ryvkin 1988), and 3 species in two genera from China (Zhang et al. 1992; Yue et al. 2009, and Cai et al. 2011). Finally, according to Yamamoto (2021), two fossil tachyporines are described from a Turonian-aged Upper Cretaceous New Jersey amber, *Mesotachyporus puer* Gusarov 2000 (Gusarov 2000), and *Procileoporus burmiticus* Yamamoto 2016 (Yamamoto 2016). A brief summary of these discoveries is provided in Table 8.2 below.

Table 8.2: Species list of the Mesozoic Tachyporinae (Coleoptera: Staphylinidae). Modified from Yamamoto (2016).

Taxon	**Stratigraphy**	**Deposit**	**Preservation**
Abscondus regularis	Upper Jurassic	Karatau, Kazakhstan	Rock
Abscondus similis	Upper Jurassic	Karatau, Kazakhstan	Rock
Cretatachinus orapensis	Upper Cretaceous	Orapa, Botswana	Rock
Cuneocharis elongates	Early Cretaceous	Daya Fm., Russia	Rock
Glabrimycetoporus amoenus	Early Cretaceous	Yixian Fm., China	Rock
Hesterniasca lata	Early Cretaceous	Yixian Fm., China	Rock
Hesterniasca obesa	Early Cretaceous	Laiyang Fm., China	Rock
Mesotachinus major	Upper Jurassic	Karatau, Kazakhstan	Rock
Mesotachinus medius	Upper Jurassic	Karatau, Kazakhstan	Rock
Mesotachinus minor	Upper Jurassic	Karatau, Kazakhstan	Rock
Mesotachyporus puer	Upper Cretaceous	New Jersey, USA	Amber
Procileoporus burmiticus	Upper Cretaceous	Hukwang, Myanmar	Amber

Protachinus minor	Upper Jurassic	Talbragar, Australia	Rock
Protostaphylinus mirus	Middle Jurassic	Jiulongshan, China	Rock
Ryvkinius gracilis	Early Cretaceous	Daya Fm., Russia	Rock
Tachyporoides villosus	Upper Jurassic	Karatau, Kazakhstan	Rock
Undiatina pilosa	Early Cretaceous	Semyon Fm., Russia	Rock

*The author + year of publication of these descriptions is already given in Table 6.3 of this study.

Systematic Palaeontology.

Order: Coleoptera Linnaeus, 1758

Series: Staphyliniformia Latreille, 1802

Superfamily Staphylinoidea Latreille, 1802

Family: Staphylinidae Latreille, 1802

Subfamily: Tachyporinae MacLeay, 1825

Incertae sedis

Genus: ***Cretachinus* gen. nov.**

Cretatachinus orapensis species nov. (Figures 8.11, 8.12 and 8.13 show the specimen in polarised and non-polarised lights from different angles).

Etymology: The generic epithet is a combination of Creta-, from 'Cretaceous', and tachinus, from genera suffix '*Coprotachinus*'.

Type species: Cretatachinus orapensis species nov., by monotype and present designation.

Composition: Only type species *Cretatachinus orapensis* species nov.

Diagnosis: Sublimuloid body form, head triangular, almost the same length as the transverse prothorax, lacking a neck, abdomen with six tapering visible segments, antennal insertions located anterior of the eye.

Cretatachinus orapensis species nov.

Etymology: The epithet of the new species is a combination of Orap- from 'Orapa', the deposit where the specimen was discovered, which in itself is derived from Sesarwa (a San or Bushmen language), and is named after a nearby cattle post, meaning 'the resting place of lions'; and -ensis, a Latin suffix meaning 'from'.

Material studied: Holotype, adult (specimen number BP./2/27480, Herbarium, Evolutionary Studies Institute (ESI), University of the Witwatersrand, Johannesburg, South Africa). The fossil beetle is a well preserved but incomplete impression fossil, both dorsal and ventral structures can be seen. It is preserved in pink lacustrine mudstone block (135 x 65 x 30 mm). The specimen is accompanied by a small coleopteran (0.9 mm) with an attenuated elytra, possibly a myxophagan, belonging to the family Hydroscaphidae.

Type locality and horizon: Botswana, Orapa Diamond Mine, lacustrine deposit, Upper Cretaceous (Davis, 1977 and Haggerty et al., 1983).

Diagnosis: As for the genus ***Cretatachinus*** **gen. nov.**.

Description: The body is sublimuloid in shape, measuring 4.92 mm in length (from tip of mandibles to tip of abdomen) and 1.58 mm in width (from exterior edge of one elytron to edge of another elytron) (Figures 8.11, 8.12 and 8.13).

Head triangular (Figures 8.11, 8.12 and 8.13), 0.82 mm long (from tip of mandible to anterior margin of pronotum) and posteriorly 0.76 mm wide at base. Longer than wide, widest posteriorly at base, narrower than pronotum (Figure 8.12), without neck constriction. Eyes (Figures 8.11, 8.12 and 8.13) small, oval, 4 times as long as wide, located anteriorly on head covering $^{1}/_{3}$ of temple (Figure 8.13). Antennae probably with 11 segments, left antennae represented by 7 distal antennomeres. Antennomere 11 oval, almost twice as long as wide; antennomere 10 slightly longer than wide; antennomeres 6, 7, 8, 9 rectangular, slightly wider than long; antennomere 5 roughly twice as long. Right antenna represented by at least 7 poorly preserved antennomeres. Antennal insertions not preserved. Anterior position of antennae, and lack of evidence for insertions between eyes suggest they were located anterior of eyes. Mandibles and palps visible, but poorly preserved, details indistinct. Pair of parallel dark pigmented structures running down either side of head, terminating in dark oval structure, connected posteriorly. These are interpreted as possibly being dorsal tentorial arms, and ovals as dorsal tentorial pits (Blackwelder 1936). Similar structures are figured in Yamamoto (2021, Figure 9A, page 18).

Pronotum (Figures 8.11, 8.12 and 8.13) difficult to interpret as margins are partially covered with sediment. Appears transverse, rectangular (1.08 mm wide, 0.69 mm long); greatest width about halfway down length; anterior margin concave, with pair of poorly preserved triangular corner projections. However, left side distinctly curved, probably representing original condition. Right side straight from right angles to posterior margins, forming rectangular corner. However, this may be due to poor preservation. Hypomeron visible on left side, lacks postcoxal bar, or transverse ridge at apical $^{1}/_{3}$ to ¼ (Yamamoto 2021); procoxal cavities large and open posteriorly. Fore legs not preserved. Elytra slightly less than $^{1}/_{3}$ of the body, exposing 6 abdominal segments, oval (each is 1.37 mm long, from posterior to anterior margin; 0.76 mm wide, from left to right margin); with epipleural keel and fold; no prominent punctations or stria, some evidence of setae, posterior edges, poorly preserved, appear somewhat sinuate. Mesocoxae large, oval, diverging, contiguous or almost contiguous, metacoxae contiguous, not reaching edge of body. Mesothoracic leg preserved on right side, tibia slightly more robust than lift tibia, no prominent setae or spurs, tarsal segments

elongated, slender tarsomeres impossible to count. Metathoracic leg preserved on left side, portion of femur projecting on right side, left tibia long and slender, with setae on left margin, faint metatibial spur, tarsi long and slender, tarsomeres impossible to count. Metafemora visible as impressions in body.

Abdomen (Figures 8.11, 8.12 and 8.13) gradually tapering posteriorly, 1.44 mm wide and 2.55 mm long; with segments III-VIII preserved, segments VII and VIII partially fused, junction indistinct, no evidence of brick-wall pattern between intersegmental segments (but this may possibly be due to poor preservation), tip of abdomen incomplete, seemingly dissected, segments IV-VIII with paratergites, segments IV and V apparently with two pairs on either side, segments VI-VIII with a single pair, segment III probably with paratergites, but obscured by legs on one side and incomplete on other. Abdomen covered with coarse setae.

Palaeoenvironment: The specimen would have been found in dead-decaying organic matter such as dung, carrion, rotting mushrooms, and compost. The specimen described here has brush-like mouthparts, which would definitely have been used for sifting. This suggests that it was mycophagous. Alternatively, it would have been found in leaf litter and moist debris near streams.

Remarks: The tapering abdomen of the specimen and moderately sublimuloid shape suggest that it belongs to the Tachyporine subgroup of subfamilies (Lawrence and Newton 1982, 1995). Among these, it can be separated from two closely related subfamilies, Habrocerinae and Trichophyinae, because it lacks the characteristic verticillate antennae. It lacks the slender shape of the Olisthaerinae, and characteristic pronotal postcoxal process (Cai et al. 2015), which would be probably visible on this specimen if it was present. Separating the specimen from the Phloeocharinae is rather difficult, since the diagnostic features such as the procoxae lacking mesal groove, antennal insertions concealed from above, and cuticular combs on the abdominal terga (Chatzimanolis et al. 2013) are not preserved. Finally, separating the specimen from the Aleocharinae is an even more challenging task, because the taxonomy of the hyper-diverse group is in disarray (Orlov et al. 2021), and the few features that distinguish this group are associated with genitalia that are not visible on the fossil (Orlov et al. 2021). It can be distinguished from the

newly recognised subfamily Mycetoporinae by having a robust body with a triangular head, instead of a slender, fusiform body with a small head.

Nonetheless the fossil is tentatively placed within the Tachyporinae based on sublimuloid body shape, antennal insertions probably located anterior of the eyes, absence of distinct neck, and gradually tapering abdomen with six visible abdominal segments. In addition, the specimen has long, thin setae on the elytra and abdomen which are commonly found on some Tachyporinae (Yamamoto 2021). However, it is distinct from other Tachyporinae because of its triangular head, which is almost the same length as the transverse thorax. However, the thoracic margins are not well preserved and were probably more curved than apparent on the fossil.

The specimen has a spiral structure resembling a gastropod on the right side of its abdominal segment 2 (Ss, Fig 8.12). It is possible that this is a gastropod. Efforts to identify it are underway.

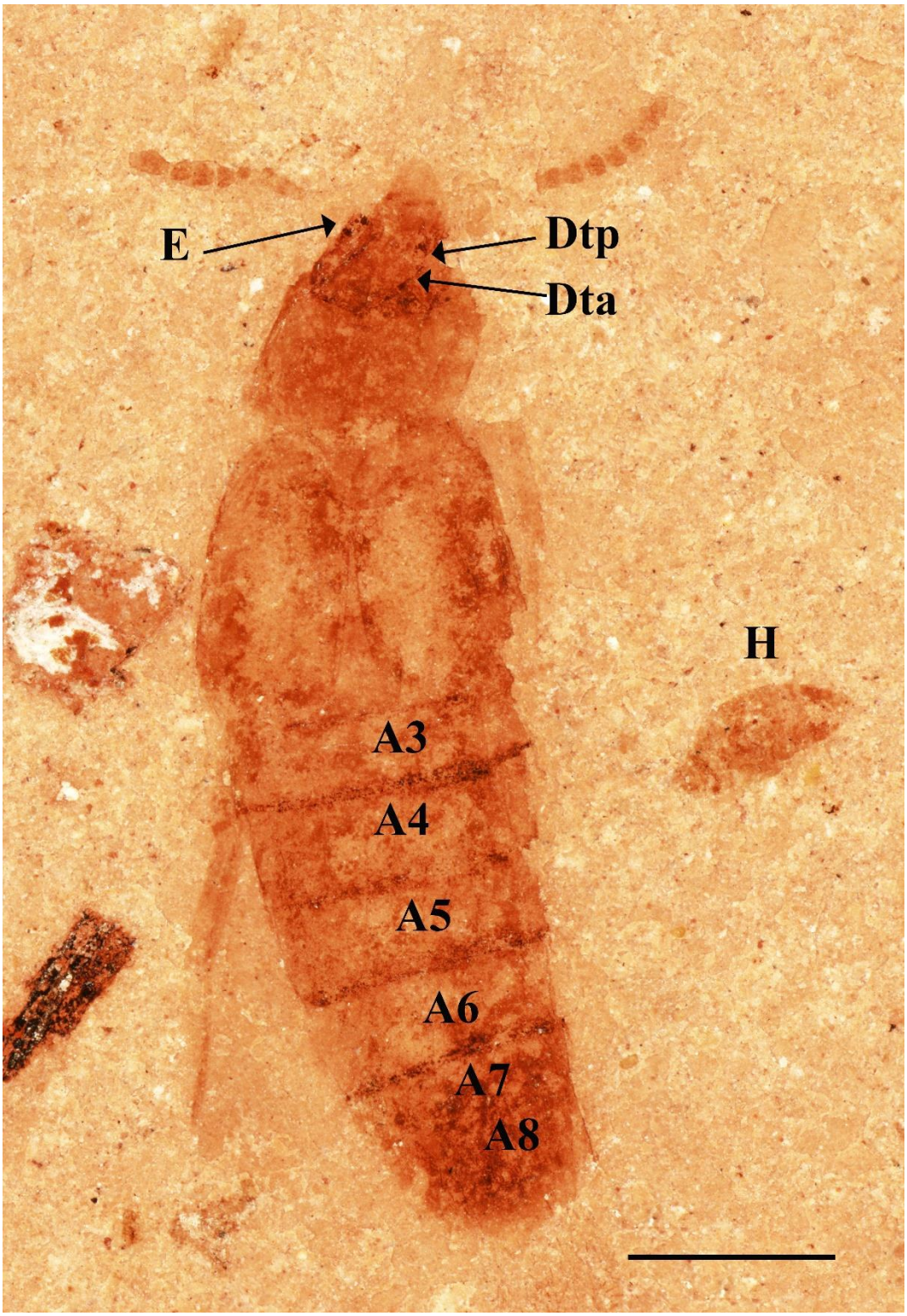


Figure 8.11 The whole body of *Cretatachinus orapensis* gen. nov., species nov. holotype, BP./2/27480, Orapa Diamond Mine, polarised light, scale bar = 1 mm. Abbreviations: A3-A8 =

abdominal segments, dta = dorsal tentorial arms, dtp = dorsal tentorial pits, e = eye, h = Hydradephaga.

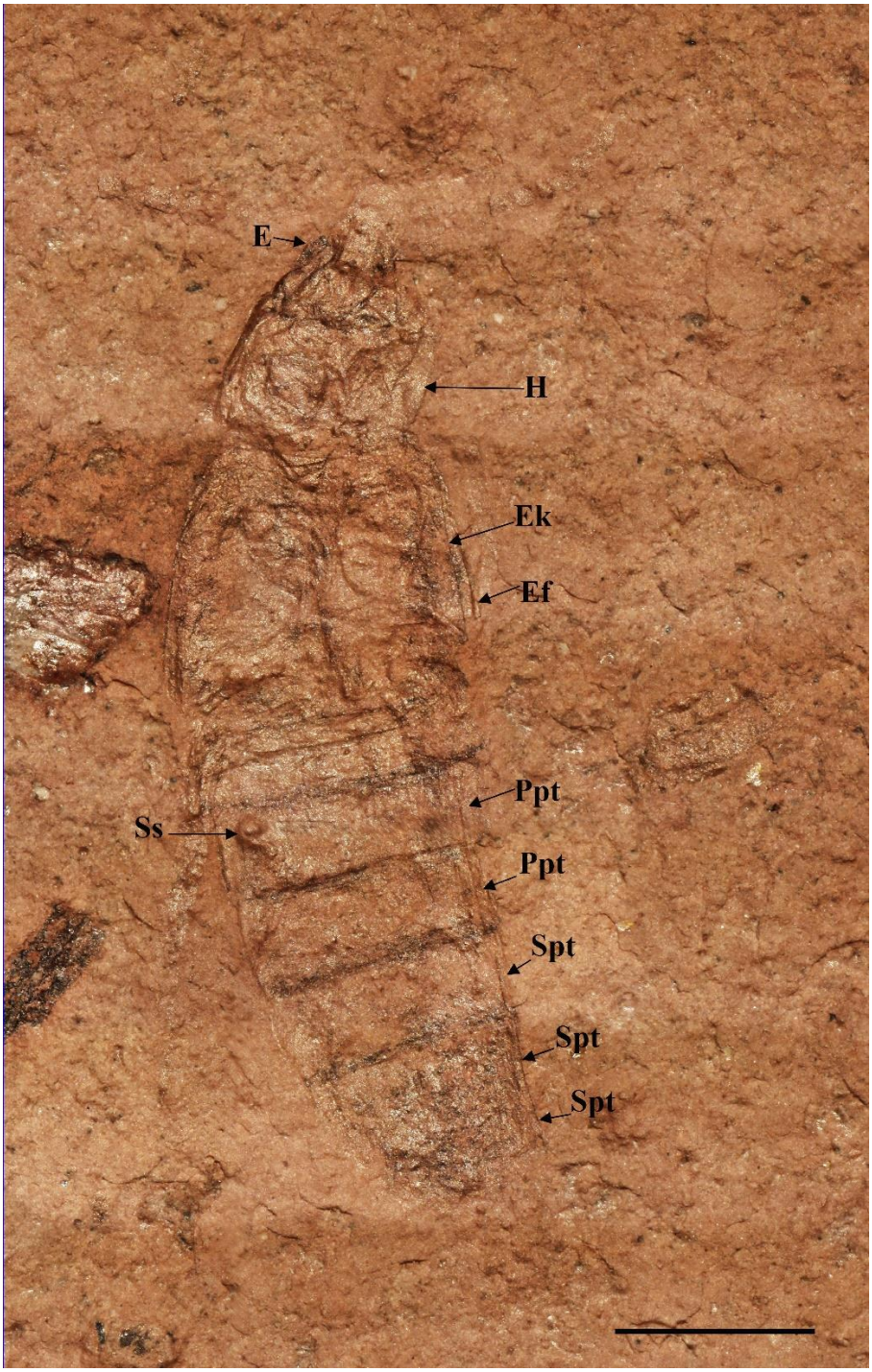


Figure 8.12 The whole body of *Cretatachinus orapensis* gen. nov., species nov. holotype, BP./2/27480, Orapa Diamond Mine, non-polarised light from top right, scale bar = 1 mm. Abbreviations: e = eye, h = hypomeron, ek = epipleural keel, ef = epipleural fold, ppt = paired paratergite, spt = single paratergite, ss = spiral structure.



Figure 8.13 The whole body of *Cretatachinus orapensis* gen. nov., species nov. holotype, BP./2/27480, Orapa Diamond Mine, non-polarised light from top left, scale bar = 1 mm.

8.3.3 Paederinae

The Paederinae is one of the hyper-diverse subfamilies of the Staphylinidae with 225 (1 extinct) genera and approximately 7 584 extant, and 35 extinct species (Bogri et al. 2020). This subfamily remains largely unexplored, both phylogenetically and taxonomically (Schomann and Solodovnikov 2017), and is known as one of the most challenging subfamilies to work on (Żyła et al. 2021). This is due to the shortage of the number of phylogenetic studies that have been undertaken. In addition, its phylogeny-based tribal and subtribal classification schemes still need extensive work, while many genera await proper delimitation (Bogri et al. 2020). The difficulty is exacerbated by the high species diversity and the few experts working on this group (Żyła et al. 2021).

Until recently, the Paederinae was divided into four tribes, namely: Pinophilini, Paederini, Cylindroxystini, and Lathrobiini (Schomann and Solodovnikov 2017). Recently, Żyła et al. (2021) conducted the most comprehensive phylogenetic analysis of the Paederinae, based on multiple genetic loci, and the broadest sampling of the taxa. The study reduced the Paederinae to three monophyletic tribes, the Pinophilini and Paederini which are sister groups of one another, and which together are the sister group of the Lathrobiini. The Cylindroxystini are demoted to a group within the Lathrobiini (Żyła et al. 2021). According to Żyła et al. (2021), the sister clades of the Paederinae are either the Euasthetinae or Staphylininae. However, most phylogenetic studies (e.g., Grebennikov and Newton 2009; Solodovnikov et al. 2013; and McKenna et al. 2015) find that the Paederinae and Staphylininae are sister groups. Surprisingly, this relationship only finds weak support in a recent study by Lü et al. (2020).

The split between Paederinae and Staphylininae is estimated to have occurred during the Late Jurassic (156.6 Ma), from mitogenomic sequencing (Hernando and Andujar 2020). More recently, this notion has been supported by Lü et al. (2020), who analysed four nuclear gene and two mitochondrial gene sequences, and estimated that the two subfamilies originated between 147.58 and 160.41 million years ago, respectively.

The Mesozoic fossil record of the Paederinae is restricted to the Cretaceous. None are recorded between the Triassic and Jurassic ages. Schomann and Solodovnikov (2012) assigned *Apticax* and *A. solidus* from the Nova Olinda Member of the Crato/Santana Formation in north-eastern Brazil to the Staphylininae + Paederinae lineage, but disclosed that the small number of diagnostic features do not allow definite placement in any of the 33 subfamilies of the Staphylinidae.

Solodovnikov et al. (2013) described *Mesostaphylinus elongatus*, *M. yixianus*, and *M. antiquus* from the Early Cretaceous deposit, Yixian Formation of China, based on the phylogenetic analysis of both extinct and extant taxa. These were placed "*incertae sedis"* within the Paederinae, due to lack of diagnostic features. Żyła et al. (2019) described two inclusions from the Cretaceous Burmese amber of Myanmar, *Diminudon schomannae* and *D. kachinensis* (Lathrobiini), by a combination of morphological and molecular datasets. Shaw et al. (2020) described the earliest record of the Pinophilini, *Cretoprocirrus trichotos* from Burmese amber of Myanmar. Bogri et al. (2018) described *Dysanabatium kechrimparense*, *D. aenaum*, *D. damgaardi*, and *D. johannesi* from the Baltic amber of Russia, to address a connection between co-occurrence of thermophilic and temperate insect taxa and Eocene climate change. In addition, Bogri et al. (2020) described *Micrillus electrus* and *Scymbalium phaethoni* from the Baltic amber of Russia, based on diagnostic morphological character matrices of both genera.

In total, the fossil record of the Paederinae encompasses 33 species from 12 genera: *Achenium* Curtis 1826, *Apticax* Schomann and Solodovnikov 2012, *Cretoprocirrus* Shaw et al. 2020, *Diminudon* Żyła et al. 2019, *Dysanabatium* Bernhauer 1915, *Lathrobium* Gravenhorst 1802, *Lithocharis* Dejean 1833, *Miolithocharis* Wickham 1913, *Medon* Stephens 1833, *Mesostaphylinus* Zhang 1988, *Orsunius* Assing 2011 and *Paederus* Lin et al. 2018; ranging from Cretaceous to Miocene. This summary is given by Żyła et al. (2019), with an addition of *Cretoprocirrus* (Shaw et al. 2020). All the Cenozoic fossils have been assigned to the tribe Lathrobiini (Żyła et al. 2019). All the descriptions assigned to Paederinae are listed between Table 6.3 and Table 6.6.

The terminology in the description below follows the terminology of Herman (2010) and Bogri et al. (2020), who recently conducted phylogenetic analyses of the Procirrina and Paederinae respectively. The morphological character matrix by Bogri et al. (2020) was used for this fossil.

Systematic Palaeontology.

Order: Coleoptera Linnaeus, 1758

Series: Staphyliniformia Latreille, 1802

Superfamily Staphylinoidea Latreille, 1802

Family: Staphylinidae Latreille, 1802

Subfamily: Paederinae Fleming, 1821

Tribe: Pinophilini Nordmann, 1837

Subtribe: Pinophilina Nordmann, 1837

Genus: ***Afrinophilina* gen. nov.**

Afrinophilina species nov. (part and counterpart) (Figures 8.14, 8.15 and 8.16 show the specimen in polarised and non-polarised lights from different angles).

Etymology: The generic epithet is a combination of Afri-, from 'Africa', and nophilina, from subtribe suffix 'Pinophilina'.

Type species: Afrinophilina orapa species nov. by monotype and present designation.

Composition: Only type species *Afrinophilina orapa* species nov.

Diagnosis: The new species is placed in Paederinae using the following diagnostic characters of the subfamily: antennal insertions concealed under "shelf" (and therefore not visible from above), and hypomeron of prothorax with well-developed postcoxal process. It is placed among the Pinophilini based on an elongated procoxae, as long as the profemora; broad, oval, fully exposed

trochantin and a conspicuous second abdominal segment. The Pinophilini has two tribes Pinophilina and the Procirrina, and the new species is placed in the Pinophilina, rather than the Procirrina, based on the presence of well-preserved pair of paratergites on segments III-VIII. Characters which may distinguish this new species from all the other Pinophilina includes the lack of an emarginate shape of the rear margins of the elytra (Herman 2010) and having two pairs of paratergites in each sides of segments III-VIII.

Afrinophilina orapa species nov.

Etymology: The epithet of the new species is a name of the deposit where the specimen was discovered, which in itself is derived from Sesarwa (a San or Bushmen language), and is named after a nearby cattle post, meaning 'the resting place of lions'.

Material studied: Holotype, adult (specimen number BP./2/27586a (part) and BP./2/27596 (counterpart), Herbarium, Evolutionary Studies Institute (ESI), University of the Witwatersrand, Johannesburg, South Africa). The fossil beetle is an adult, carbonaceous, compression fossil, both dorsal and ventral structures can be seen, and the body parts are well preserved, but incomplete. The part (120 x 90 x 38 mm) and counterpart (73 x 62 x 30 mm) are preserved in brown-red lacustrine mudstone block.

Type locality and horizon: Botswana, Orapa Diamond Mine, lacustrine deposit, Upper Cretaceous (Davis, 1977 and Haggerty et al., 1983).

Diagnosis: As for the genus ***Afrinophilina*** **gen. nov.**.

Description: Body slender measuring 8.81 mm in length (from tip of mandibles to tip of abdomen) and 1.82 mm in width (from exterior edge of one elytron to edge of another elytron, at widest point). Covered with setae of variable length throughout (Figures 8.14, 8.15 and 8.16)

Head (Figures 8.17 and 8.18), 1.44 mm long (from tip of mandible to anterior margin of pronotum) and 1.01 mm wide at widest point just anterior to neck, making it approximately 1.4 x longer than wide, with distinct curved temples; width of head 1.4 x broader than width of neck constriction. Eyes (Figures 8.17 and 8.18) not well preserved, circular to slightly oval, located closer to base of antennae, covering $^1/_3$ of temple. Antennae filiform (Figures 8.14 and 8.15), approximately length of head and prothorax, bases hidden under "shelf" at base of head; proximal antennomers incomplete, but antennomere 1 appears less or equal length as antennomers 2 and 3, antennomeres 9 and 10 funnel shaped, antennomere 11 poorly preserved, but with funnel shape, suggesting that it had spiniform pencil of setae, in general antennae have setae visible in many places. Mandibles curved, sickle-shaped, left mandible curved over right, possibly with traces of teeth posteriorly. Pair of long setae posterior to base of the mandibles. Labial palps, represented only by indistinct segment or portion of segment adjacent to left mandible. Labrum poorly preserved. Several indistinct structures in region which could be interpreted as spines or lobes. Margin between labrum and frontoclypeus apparently straight, curved at corners. Gular sutures fully separate, apparently reaching posterior margin of head.

Pronotum (Figure 8.21) rectangular with curved sides, longer than wide (0.59 mm wide, 0.68 mm long); length 0.9 times less than that of head, width 1.1 x broader than head, anterior margin forms slight collar around the neck, posterior margin gently curved. Superior marginal line apparently deflexed, reaching from anterior margin of prothorax and tapering off just before posterior corner of prothorax. Distinct postcoxal process of hypomeron. Longitudinal carina of furcasternum present, sharp, apparently extending anterior as longitudinal carina of basisternum, crossed by transverse basisternum carina anteriorly.

Elytra (Figure 8.22) jointly 1.57 times wider than pronotum, and 1.1 times longer. Elytra oval, collectively wider than long (each 1.61 mm long, from posterior margin of pronotum to anterior margin of elytron, 1.76 mm wide); with epipleural margin; no obvious epipleural ridge, separated at base by small scutellum, with faint striae. Elytral posterior margins curved evenly, non-emarginate, apparently with long setae.

Prothoracic legs (Figure 8.20) with broad coxa, long as femur, trochanter large and oval, tibia narrow at base, becoming broader distally, lacking any distinct medial expansion, with row of setae in posterior mid-section, possibly representing longitudinally arranged protibial combs, distal

surface with grooves which also represent ctenidium, only tarsomere 1 preserved, moderately expanded, triangular, with curved distal surface fringed with short setae. Mesocoxae indistinct, remainder of leg represented by poorly preserved fragments of femur and tibia, femur covered with long setae, remnants of tarsal segments visible. Thorns on mesotibia not visible. Metathoracic coxae apparently large and extended, trochanter difficult to discern, femurs projecting beyond side of body, tibia 1 ½ x long as femur, with tibial thorn at apex, tarsomere 1 about 1 ½ x longer than tarsomere 2, approximately same for tarsomere 5, although with poorly-preserved distal segments.

Abdomen (Figure 8.23) approximately 3 x length of elytra and 1.2 x elytral width, 3 ½ x as long as wide (4.96 mm long, 1.56 mm wide), apparently with waist (but this may be due to poor preservation of first visible segment), tapering strongly from segment VI. Segments II-VIII visible, segment II distinct, without paratergites, with intercoxal carina in the middle that extends into segment III, segments III-VIII each with pair of paratergites, segments III-VI each with two pairs of paratergites, segment IX with pair of setiferous latero-apical processes, separated by pair of pointed structures interpreted as tergal plates (Naomi 1989).

Palaeoenvironment: There has been no recent review of the Pinophilina. Apparently, most of them prefer wet tropical forest environments (Campbell and Peck 1989), a view shared with the Procirrina (Herman, 2010). Extant paederines are commonly found in damp places, under logs, litter or foliage, while some have been recorded in caves and ant nests.

Remarks: The maxillary palps crucial for differentiating the tribes within the Paederinae are not preserved. Its first protarsal segment (protibial combs) is dilated with a setiferous margin as for the Pinophilini. However, there are other taxa, for example, *Scymbalium* and *Micrillis* (classified as Lathrobiini *incertae sedis*), with similar features (Bogri et al. 2020). The tribe Lathrobiini was ruled out based onan invisible typical small and unmodified apical (fourth) maxillary palpomere . Interestingly, this species shares the characters of having an elongated procoxae and well-developed abdominal segment II with *Mesostaphylinus* (Solodovnikov et al. 2013), but is more similar to the Pinophilina in other features. The elongated procoxae are not typical for

Mesostaphylinus. The abdominal segment II is not usually preserved in fossils, as it is mostly hidden behind the elytra, making it difficult to compare this character.

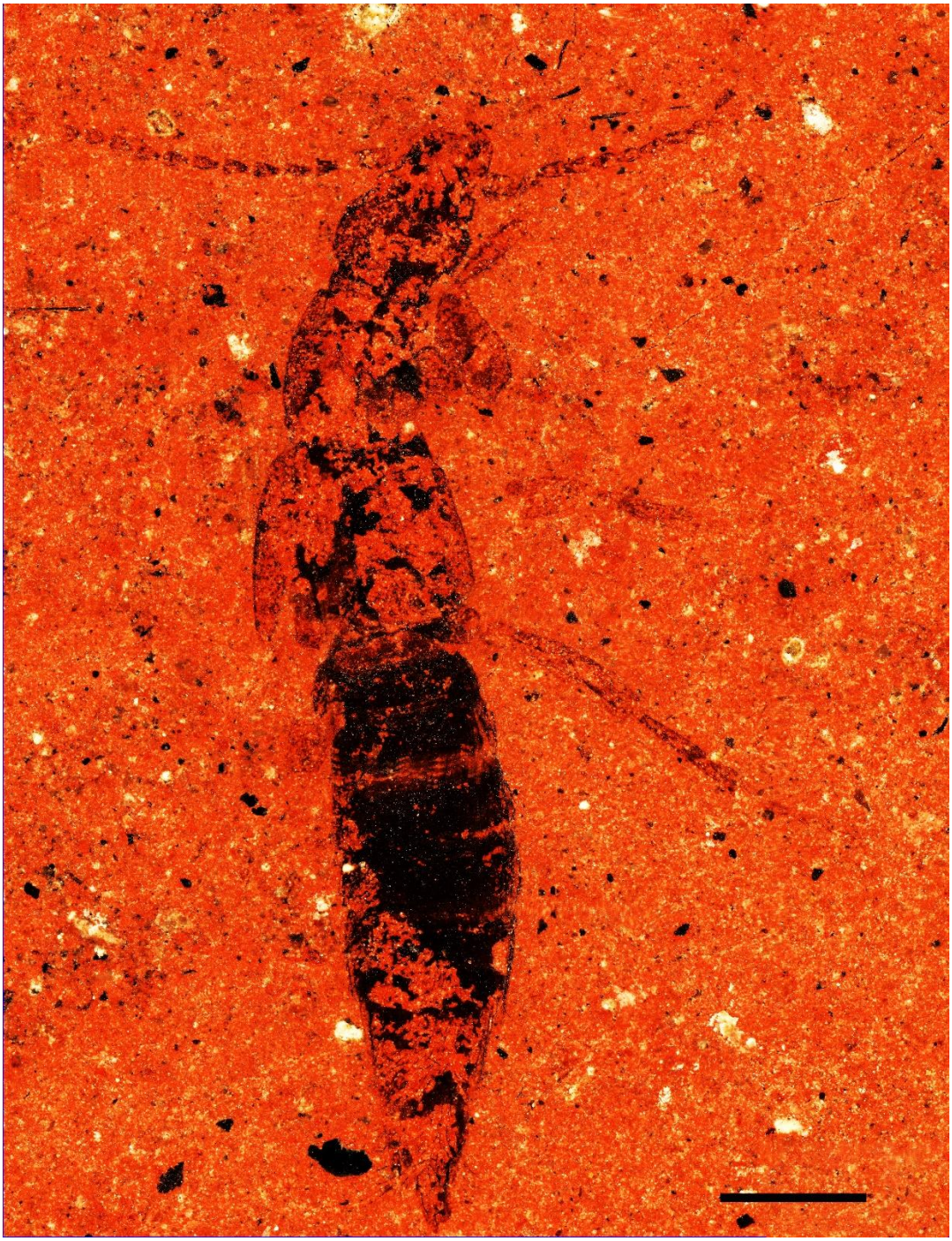


Figure 8.14 The whole body of *Afrinophilina orapa* gen. nov., species nov. holotype, part, BP./2/27586a, Orapa Diamond Mine, polarised light, scale bar = 1 mm.



Figure 8.15 The whole body of *Afrinophilina orapa* gen. nov., species nov. holotype, counterpart BP./2/27596, Orapa Diamond Mine, polarised light, scale bar = 1 mm.



Figure 8.16 The whole body of *Afrinophilina orapa* gen. nov., species nov. holotype, counterpart, BP./2/27596, Orapa Diamond Mine, non-polarised light, scale bar = 1 mm.

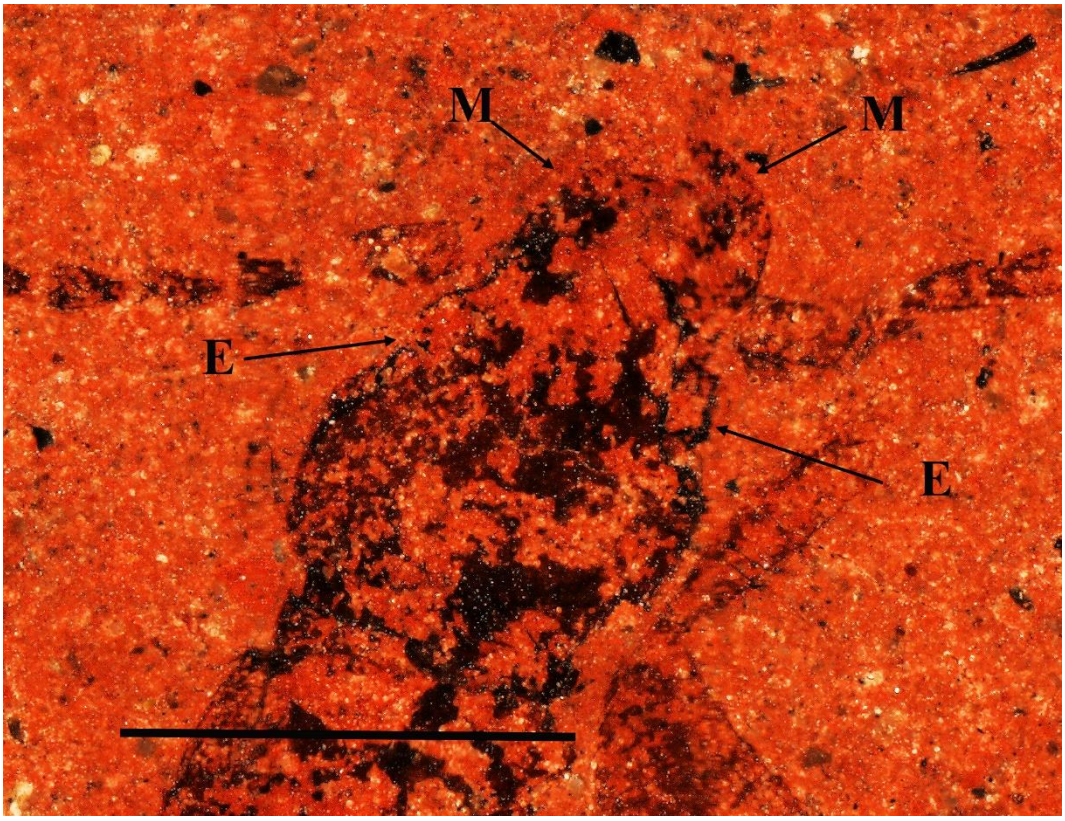


Figure 8.17 *Afrinophilina orapa* gen. nov., species nov. holotype, part, BP./2/27586a, scale bar = 1 mm. Details of head. Abbreviations: m = mandible, e = eye.

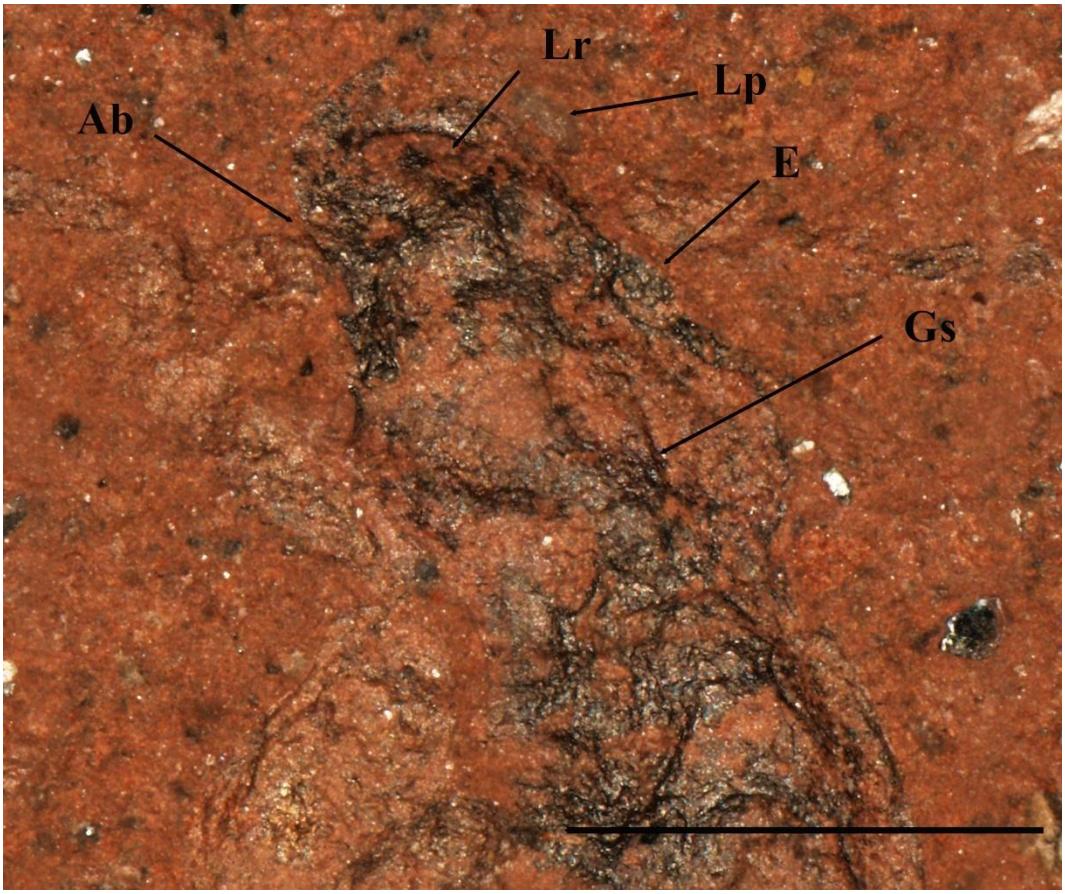


Figure 8.18 Details of head of *Afrinophilina orapa* gen. nov., species nov. holotype, counterpart, BP./2/27596, scale bar = 1 mm. Abbreviations: ab = antennal base, e = eye, gs = gular suture, lp = portion of labial palp, lr = possible labrum.

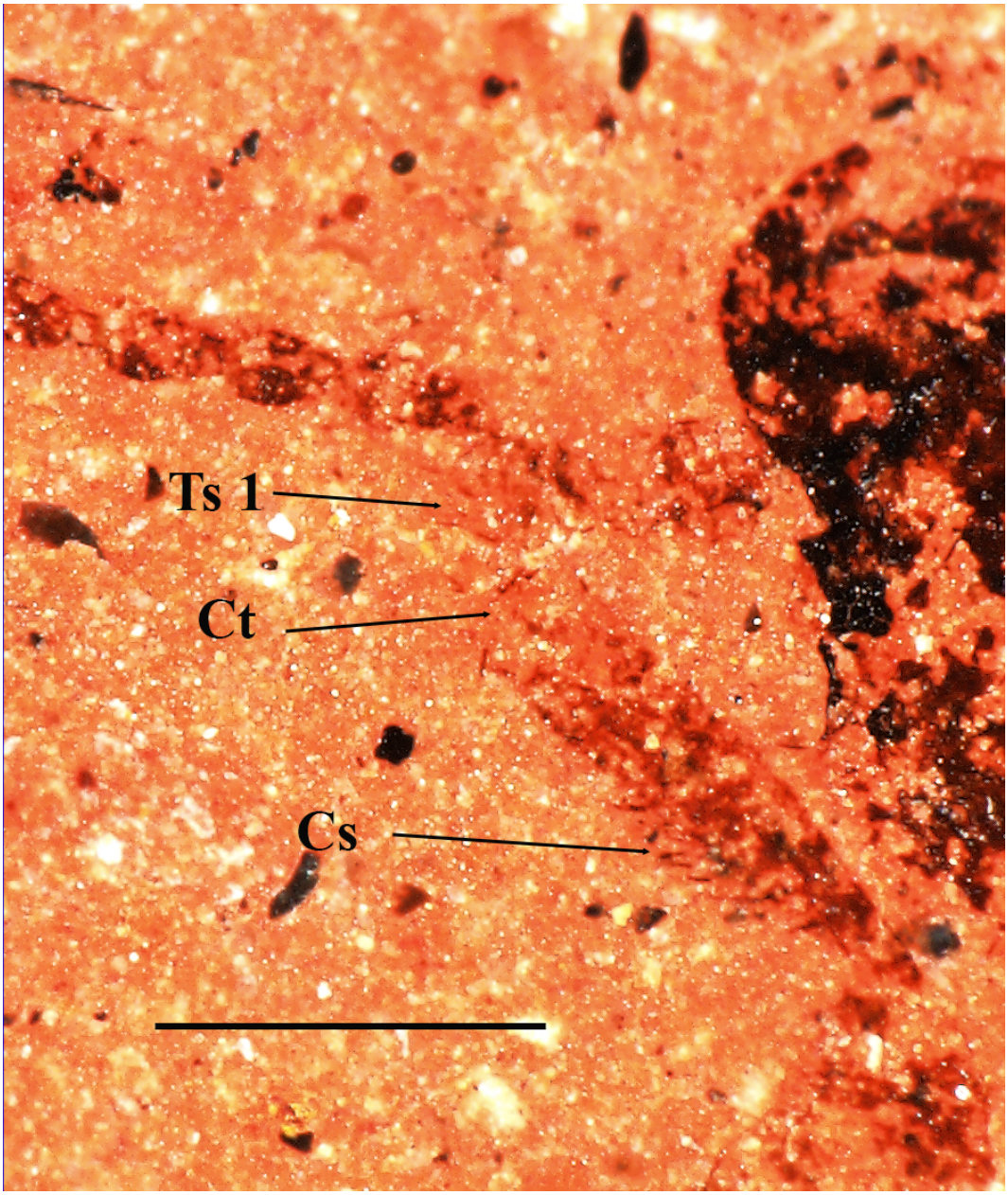


Figure 8.19 Details of protibia, *Afrinophilina orapa* gen. nov., species nov. holotype, counterpart, BP./2/27596, scale bar = 0.5 mm. Abbreviations: cs = ctenidial setae, ct = ctenidial comb.

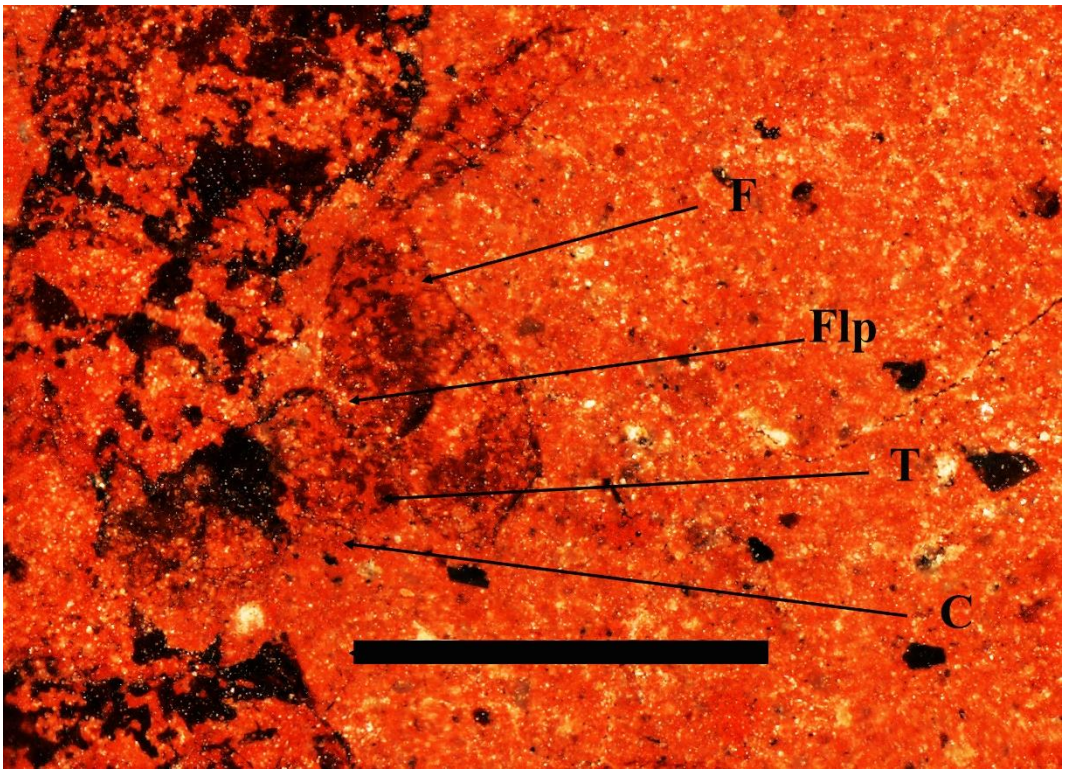


Figure 8.20 Details of prothoracic leg. *Afrinophilina orapa* gen. nov., species nov. holotype, part, BP./2/27586a, scale bar = 1 mm. Abbreviations: c = coxa, f = femur, flp = femoral projection, t = trochanter.

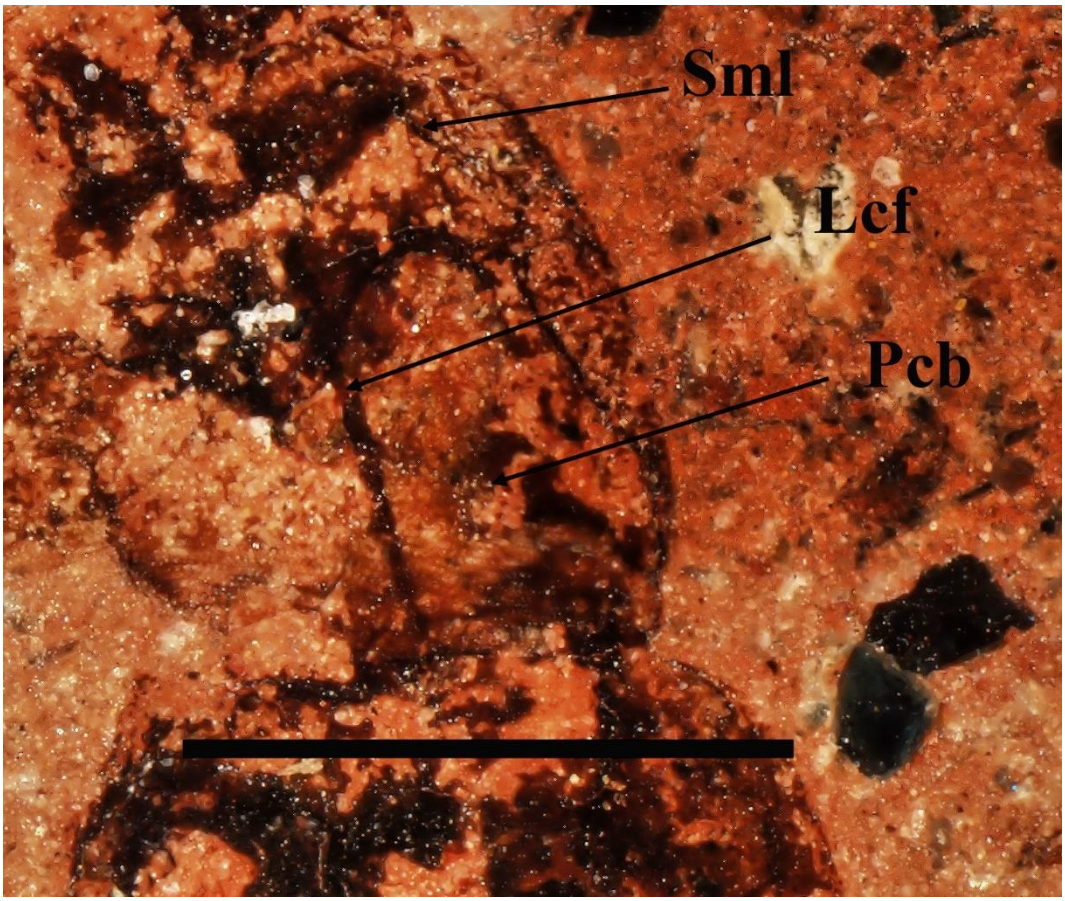


Figure 8.21 Some details of the thorax of *Afrinophilina orapa* gen. nov., species nov. holotype, counterpart, BP./2/27586a, scale bar = 1 mm. Abbreviations: lcf = longtitudinal carina of furcasternum, sml = superior marginal line, pcb = postcoxal process.

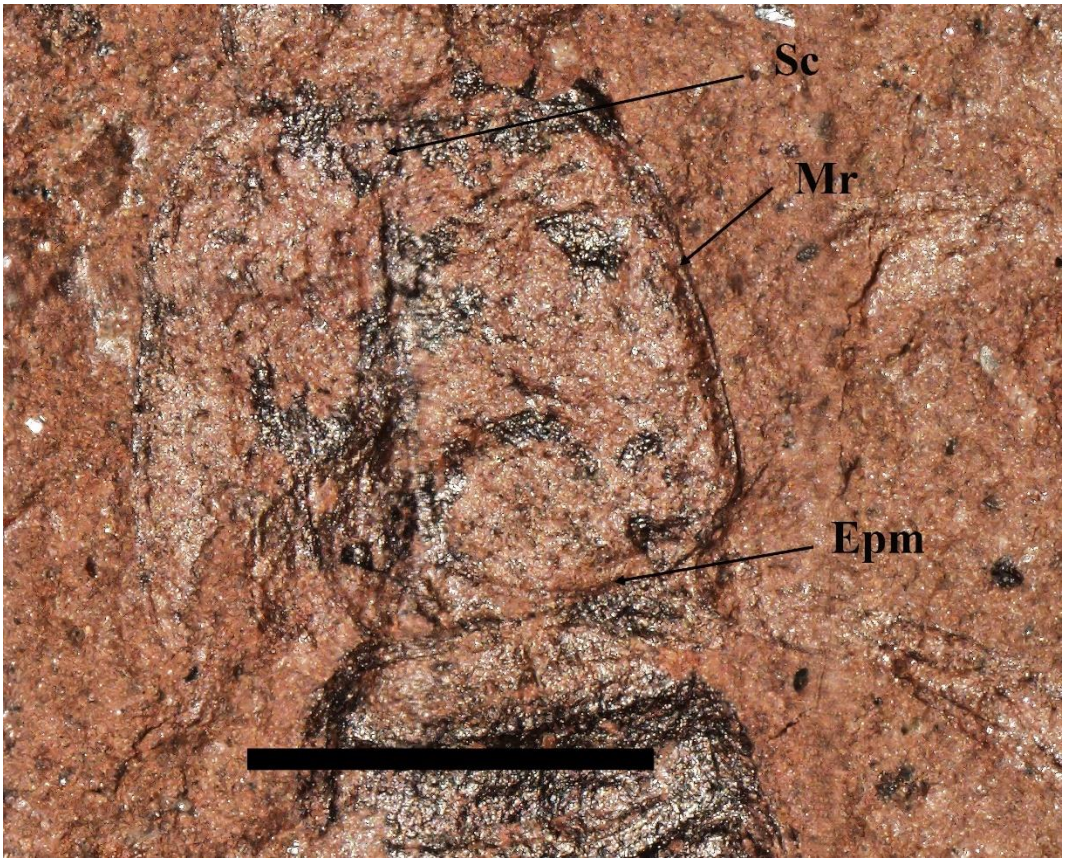


Figure 8.22 Some details of the elytra, *Afrinophilina orapa* gen. nov., species nov. holotype, counterpart, BP./2/27586a, scale bar = 1 mm. Abbreviations: epm = elytral posterior margin, mr = marginal ridge, sc = scutellum.

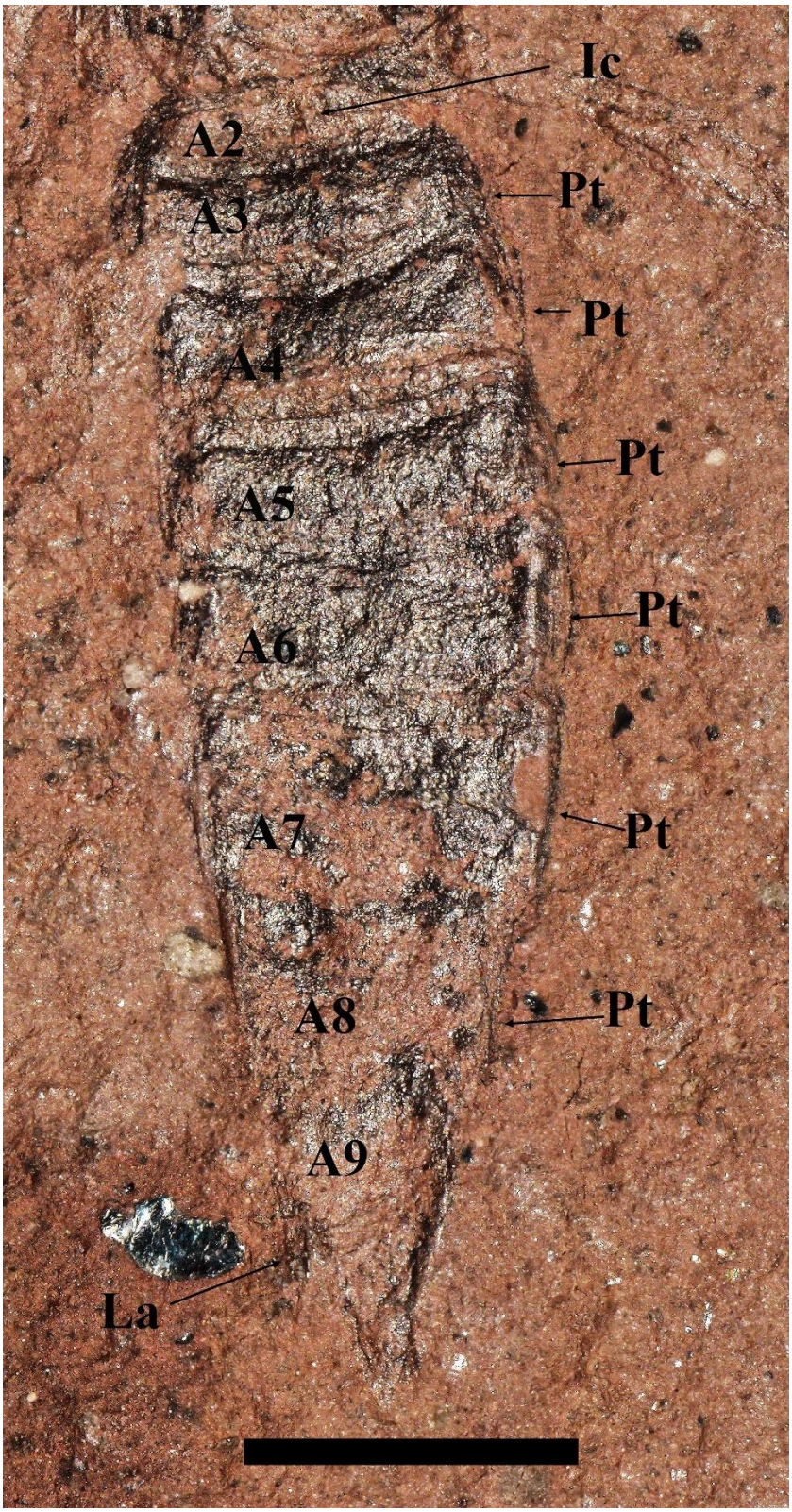

Ic
A2
Pt
A3
Pt
A4
Pt
A5
Pt
A6
Pt
A7
Pt
A8
A9
La

Figure 8.23 Some details of the abdomen, *Afrinophilina orapa* gen. nov., species. nov. holotype, counterpart, BP./2/27586a, scale bar = 1 mm. Abbreviations: A1-A9 = abdominal segments, ic = intercoxal carina, pt = paratergites, la = latero-apical process.

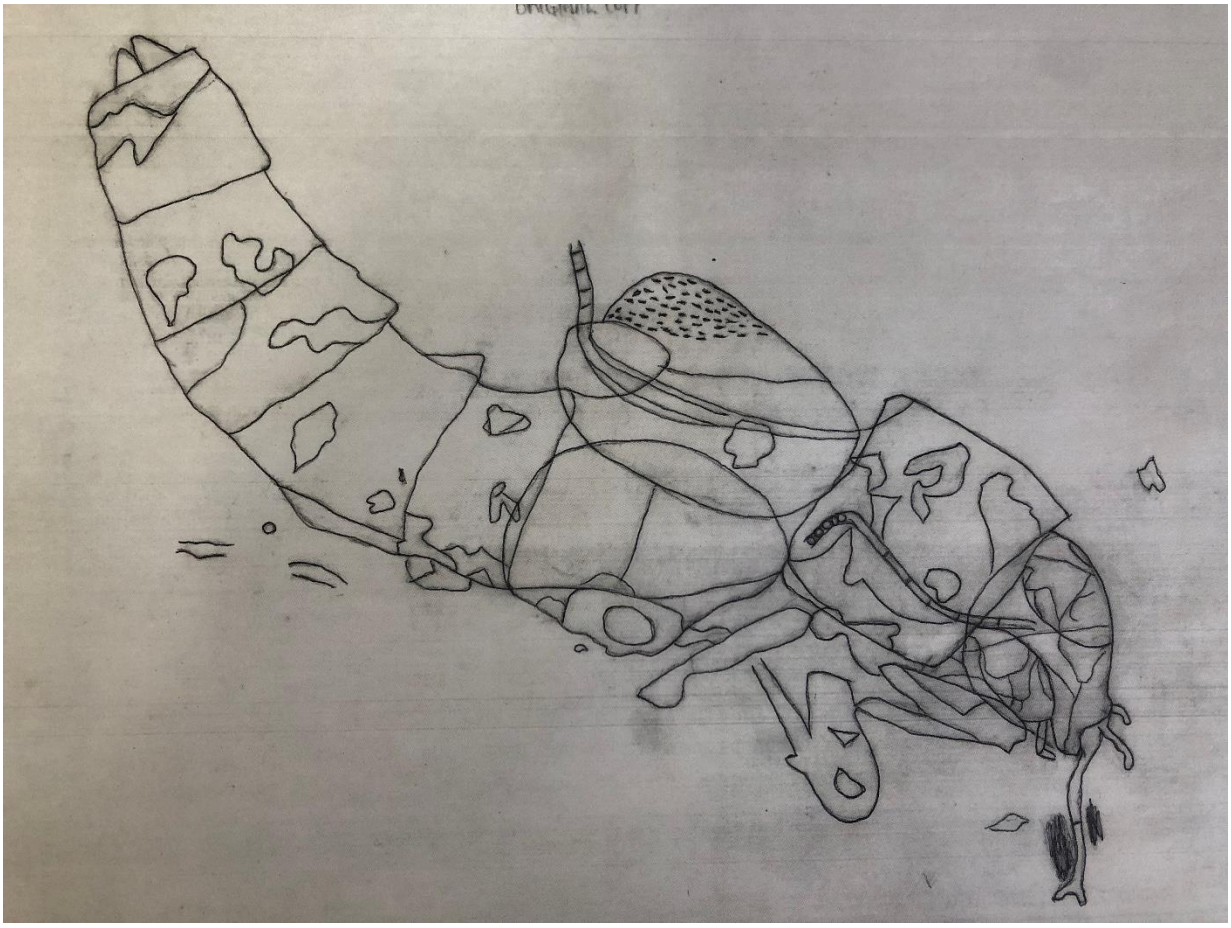


Figure 8.24. The whole body of *Afristenus orapensis* gen. nov., species nov. holotype, BP./2/25912, Orapa Diamond Mine, drawing, scale bar = 1 mm.

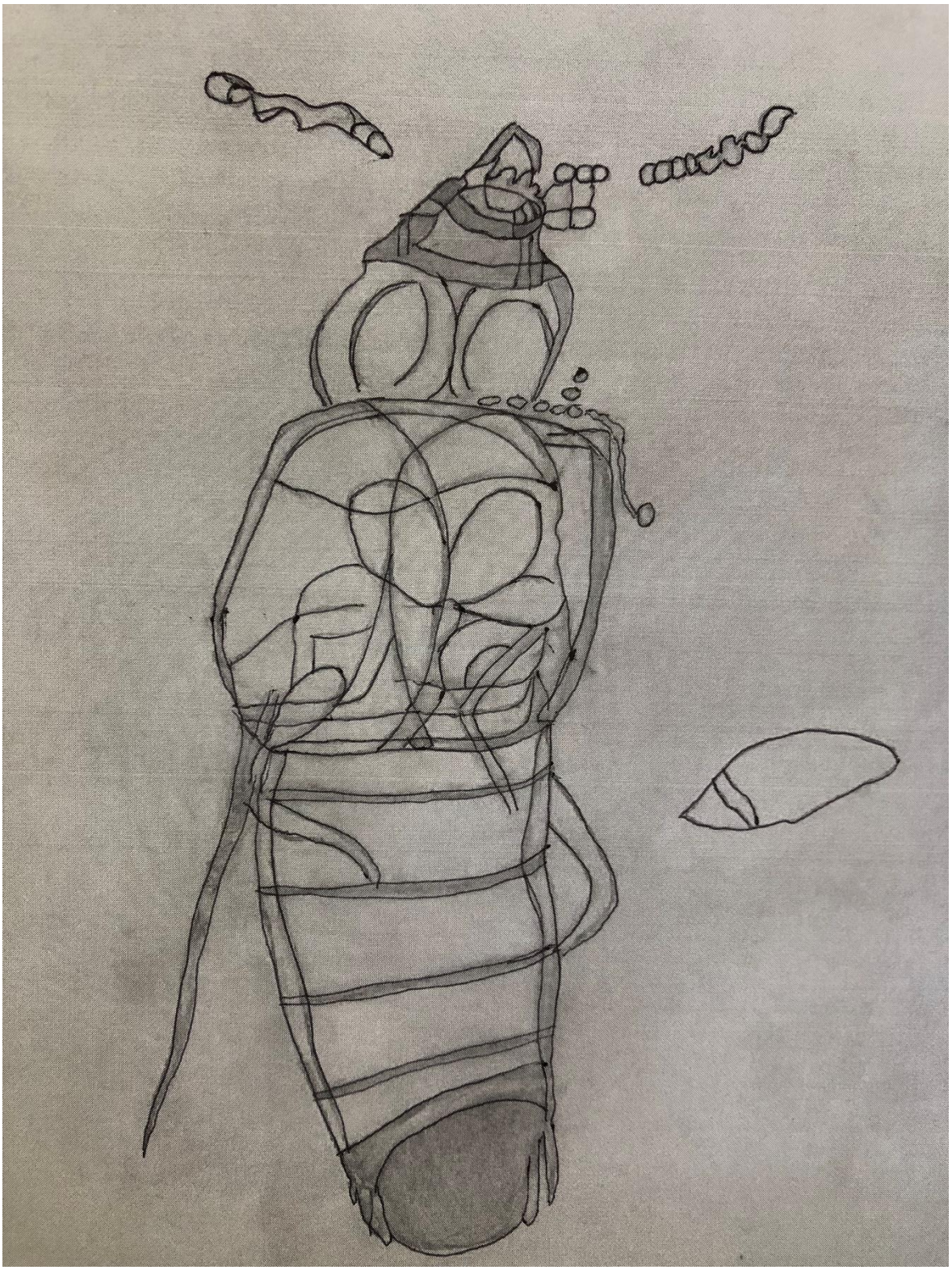


Figure 8.25. The whole body of *Cretatachinus orapensis* gen. nov., species nov. holotype, BP./2/27480, Orapa Diamond Mine, drawing, scale bar = 1 mm.

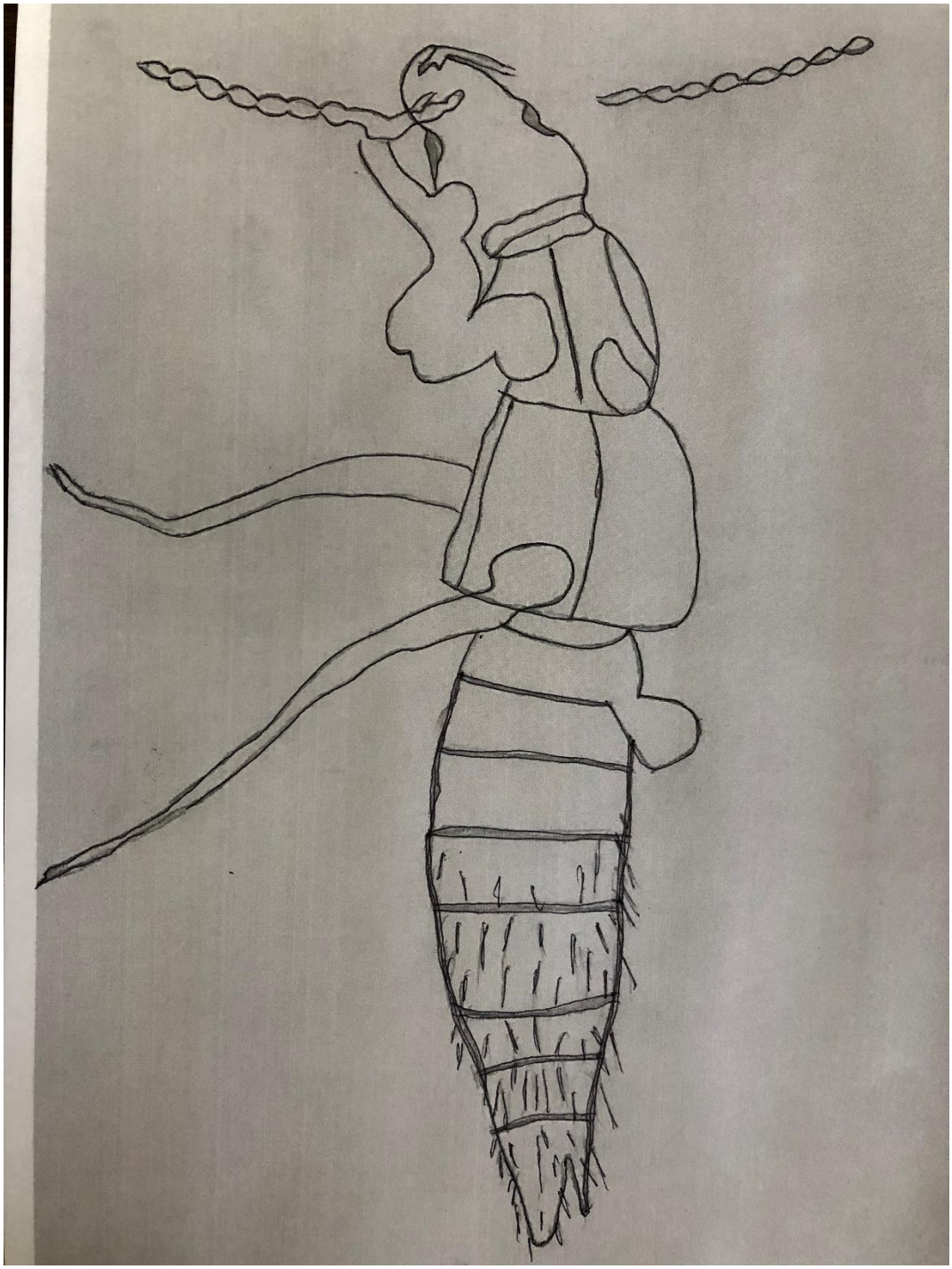


Figure 8.26. The whole body of *Afrinophilina orapa* gen. nov., species nov. holotype, part, BP./2/27586a, Orapa Diamond Mine, drawing, scale bar = 1 mm.

CHAPTER NINE

DISCUSSION

In this chapter, the taxonomic and systematic implications of the individual taxa of Staphylinidae that were described in chapter 8 of this study are discussed. These include the Steninae, Tachyporinae and Paederinae. This is followed by a discussion of the contribution of all the ODM fossils pertaining to our knowledge of the staphylinid evolution, and their use in reconstructing the palaeoenvironment at ODM during the Cretaceous age. Finally, the limitations of this study are discussed, and recommendations to address these limitations in the future are proposed.

9.1 Steninae

The specimen (*Afristenus orapensis* species nov., Figures 8.6-8.10) preserves a delicate "harpoon-like" extended labial mouthpart, which is a characteristic (if variably developed), of all the Steninae, except *Dianous*, where it appears to have been secondarily lost (Koerner et al. 2013; and Żyła et al. 2017). On the other hand, the unique characters which separate the specimen from other stenines are more difficult to interpret. These characters include: apodemes arising from interantennal pits; labial palps situated close together at the anterior margin of labrum; pronotal marginal carina meeting pronotosternal suture laterally; apex of mesoventral intercoxal process abuts metaventral intercoxal process; and paired pygidial glands opening into the rectum. The 'transverse' prothorax is also a characteristic of *Eocenostenus* (Cai et al. 2014). However, it has been proposed that the feature was produced by distortion during fossilisation (Żyła et al. 2017).

The specimen also lacks a lateral constricted neck, another feature shared by *Eocenostenus* Cai et al. 2014. However, in the latter specimen, the head appears almost retracted into the prothorax, possibly making it difficult to see the neck. Morphological characteristics such as the presence or absence of a distinct neck, or nuchal constriction, are commonly used in adult staphylinid taxonomy/systematics (Clarke and Grebennikov 2009). However, the posterior portion of the head of the fossil (which is broader than the eyes) is regarded to be unique for this group by Clarke and

Grebennikov (2009). Nonetheless, *Stenus gypsi* Oustalet, 1874 figured in Puthz (Fig. 22; 2010) may have a similar neck, although it is more typically narrower than the eyes.

The antenna with a 5-segmented-club, and an elongated stem, which originates more-or-less distinctly on the frons between the eyes is clearly visible on the right-hand side. The compound eyes are also interesting because they have a faint but distinct outline running up the side of the head, and within each eye there is a circle with a dark ring around it. These are clearly associated with the eyes, and may be openings in the head capsule where the optic nerve enters. Perhaps the faint outline of the eyes has been misinterpreted, and the rings are the actual eyes. An examination of the specimen with better equipment, or with different techniques, may resolve the problem.

Afristenus gen. nov. is the second fossil stenine described from the southern hemisphere and the first from the African continent. With a median age of 90.1 Ma (Davis 1977; and Haggerty 1983), the Orapa deposits are slightly younger than the other Cretaceous stenines from Burmese amber of Myanmar (Żyła et al. 2017; and Cai et al. 2019), Bezonnaise resin of France (Schlüter 1978), and Obeschayushchiy locality of Russia (Ryvkin 1988), which are all Cenomanian in age (Shi et al. 2012; Kopylov 2010; and Rasnitsyn et al. 2016). Interestingly, the molecular clock analysis of Lü et al. (2020) estimated that the Steninae diverged 122.22 Ma ago, in the Aptian. It seems reasonable to conjecture, therefore, that the presence of *Afristenus* with its elongated protrusible labium supports the evidence provided by *Festenus* (Cai et al. 2019), and possibly the other two *Stenus* fossils from France and Russia (which presumably had the same mouthparts), that the "harpoon-like" mouthparts would have evolved by the Mid-Cretaceous.

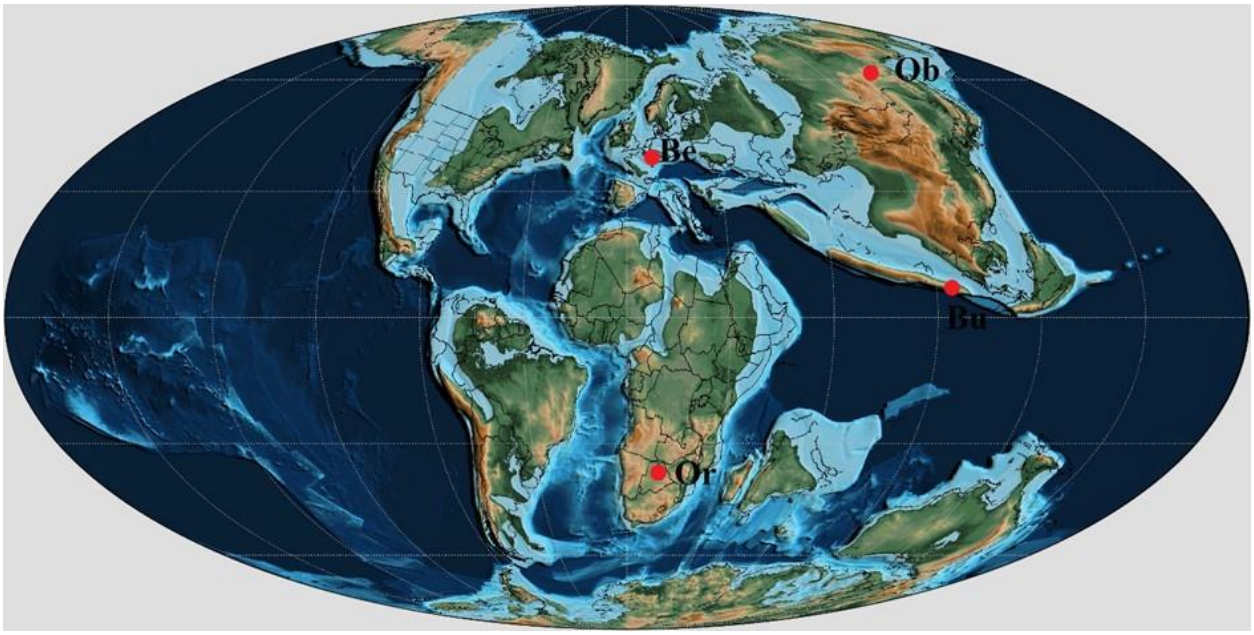


Figure 9.1 Global palaeogeographic reconstruction of the Mid-Cretaceous (100 Ma) showing the localities where the fossil Steninae have been recovered. The map in Mollweide projection was sourced from Scotese (2021). The position of the localities was adapted from Rasnitsyn and Quicke (2002). Abbreviations: Be = Bezonnaise amber, France; Bu = Burmese amber, Myanmar; Or = Orapa, Botswana; Ob = Obeschayushchiy locality, Russia.

At the time, the Steninae were present in widely distributed localities, spanning approximately 60º N and 35 º S (Figure 9.1), suggesting a much earlier, common point of origin. This is supported by the occurrence of the crown group Euaesthetinae in the Early Cretaceous age (125-135 Ma) Lebanese amber (Lefebvre et al. 2005). Given that euaesthatines and stenines are monophyletic and sister clades (Clarke and Chatzimonolis 2009; Clarke and Grebennikov 2009; Grebennikov and Newton 2009; McKenna et al. 2015; Żyła et al. 2017; and Liu et al. 2021), it is likely that the stenines would have been present at the same time as the euaesthatines. Since there are no staphylinine group fossils known from the Jurassic (Liu et al. 2021), and that the first unambiguous staphylinines belonging to the Oxyporinae, Paederinae, Staphylininae and Euaesthetinae (Liu et al. 2021), are known from the 125 Ma Lower Cretaceous Yixian Formation (Liu et al. 2021), in addition to the euaesthetine from the Lebanese amber (Lefebvre et al. 2005), it is likely that the Steninae may have also appeared around this time. However, it is equally probable that they

appeared even earlier than the proposed 125-135 Ma, but not as early as the Jurassic age (145-200 Ma), because they are deeply nested within the staphylinines in most phylogenies (e.g., Clarke and Chatzimonolis 2009; Clarke and Grebennikov 2009; Grebennikov and Newton 2009; McKenna et al. 2015; Żyła et al. 2017; Lü et al. 2020; and Liu et al. 2021).

9.2 Tachyporinae

The revision by Yamamoto (2021) of the subfamily greatly assisted with the description, and placement of this specimen (*Cretatachinus orapensis* species. nov., Figures 8. 11-8.13). The tachyporine described in this study has a sublimuloid body and preserves a tapering abdomen, which are characteristic of all the Tachyporinae (Yamamoto 2021). The species also has setae on the elytra and abdomen, a trait that is commonly found in extant species. However, it lacks the small head and curved prothorax, which are also characteristics of the subfamily (Yamamoto 2021). In fact, the head is triangular, and is almost the same length as the prothorax. However, there are suggestions that the prothorax may be curved in a more characteristic tachyporine shape, and that its rectangular appearance is an artefact of preservation. The shape of the posterior margin of the elytra is also difficult to interpret, because it coincides almost exactly with the metathoracic femora. Separating the specimen from the Aleocharinae is challenging because the Aleocharinae themselves are presently under revision (Orlov et al. 2021 a and b), and the few features that distinguish this group are associated with genitalia, which are not visible on the fossil (Orlov et al. 2021b). Its synapomorphies: laterotergal struts of male tergum IX present; lateral lobe of aedeagus (paramere) broad, enclosing median lobe; and apical lobe of female gonocoxites reduced or absent (Orlov et al. 2021 a and b) are also not visible on the fossil. Nonetheless, Aleocharinae remains one of the most taxonomically challenging groups of Coleoptera (Klimaszewski et al. 2018).

The Tachyporinae are an ancient group, originating in the Jurassic age (Yamamoto 2016, 2021), suggesting that they would have had a Pangean distribution. Therefore, it is expected to find one at ODM. Tachyporines have also been found in New Jersey amber of the United States of America and Burmese amber of Myanmar, which are of similar age (Gusarov 2000; and Yamamoto 2016).

Given the general placement of this specimen as a tachyporine, which, according to Yamamoto (2021), are found in forested environments, it unequivocally supports all previous research at ODM, which suggests a moist, well-vegetated habitat within the volcanic crater (Rayner 1987; Bamford 1990; McKay 1990; Waters 1990; Kuschel et al. 1994; and Rayner et al. 1991, 1997).

9.3 Paederinae

The Paederinae is also one of the most challenging subfamilies to work on (Żyła et al. 2021). However, Bogri et al. 2020, and Żyła et al. 2019, 2021, have made considerable progress in creating a well-supported phylogenetic framework for the subfamily. The species (*Afrinophilina orapa* species. nov., Figures 8.14-8.23) described in this study has antennae that are concealed under the "shelf", and a hypomeron with postcoxal process, which are both diagnostic features for this group (Herman 2010, Bogri et al. 2020, and Żyła et al. 2019, 2021). Unfortunately, the characters which are generally used to separate Paederinae tribes, in particular, such as the maxillary palps, are poorly preserved. This hinders the interpretations of the mouthparts.

The specimen has its first protarsal segment (protibial combs) dilated with a setiferous margin as for the Pinophilini (Herman 2010). However, it is perhaps not as dilated as in extant pinophilines that I have examined at Ditsong National Museum of Natural History (DNMNH) (Pretoria, South Africa). It would be useful to have the rest of the tarsus preserved, because there are other genera, for example, *Scymbalium* Erichson, 1839 and *Micrillis* Raffray, 1873, with similar features (Bogri et al. 2020). Other characters which may be important in assisting to place this specimen among the Pinophilini are the elongate procoxae, which are as long as the profemora (Campbell and Peck 1989; Herman 2010, and Shaw et al. 2020); broad, oval, fully exposed trochantin, and a conspicuous second abdominal segment (Campbell and Peck 1989). As previously mentioned, the Pinophilini only has two tribes, the Pinophilina, and the Procirrina (Herman 2010).

The new species is placed in the Pinophilina, rather than the Procirrina, based on the presence of well-preserved pair of paratergites on segments III-VIII (Campbell and Peck 1989; Herman 2010, and Shaw et al. 2020). One character which may distinguish this new species from all the other Pinophilina is the lack of an emarginate shape of the rear margins of the elytra, and having two pairs of paratergites on each side of segments III-VIII (Herman 2010). It is also interesting to note, however, that two recent matrices of morphological characters (i.e., Bogri et al. 2020; and Żyła et al. 2021), do not include characters like extended procoxae, and fully exposed protrachantin. Is this because these characters are not useful for distinguishing amongst other tribes of the Paederinae? However, it is equally probable that the characters could be widely variable among the Paederinae genera. It is unclear at the moment, and needs further investigation.

Finding a paederine fossil at ODM is not surprising, given that Schomann and Solodovnikov (2012) suggest that the genus *Apticax*, from the Lower Cretaceous Crato/Santana Formation of Brazil, is possibly, if not conclusively, a paederine. In addition, Solodovnikov et al. (2013) described the genus *Mesostaphylinus* from the Lower Cretaceous, Yixian Formation of China as "Paederinae, *incertae sedis*". These were later described as *M. elongatus*, *M. yixianus*, and *M. antiquus*. There is also *M. laiyangensis* Zhang, 1988 from Laiyang Formation of China. Solodovnikov et al. (2013), which suggests that the discovery of Lower Cretaceous Paederinae and Staphylininae sets the split between these groups back to the Jurassic. Interestingly, *Mesostaphylinus* Solodovnikov et al., 2013 shares two characters with *Afrinophilina* Mnguni et al. in preparation, in a form of an extended procoxae, well-developed segment II of the abdomen, and seemingly dilated protarsi.

However, *Afrinophilina* Mnguni et al. in preparation resembles the Pinophilina based on the shape of the antennae and arrangement of the setae on the protarsi. Perhaps it would be worth reanalysing *Mesostaphylinus* for affinities with Pinophilina. Shaw et al. (2020) have recently described the first fossil Procirrina, *Cretoprocirrus trichotos* from the Burmese amber of Myanmar, and it is therefore to be expected that the sister group of this taxon would have appeared around this time. *Afrinophilina orapensis* Mnguni et al. in preparation is therefore the first fossil paederine belonging to the subtribe Pinophilina from the Cretaceous, and the first fossil Paederine from Africa, the second in the southern hemisphere.

9.4 Implication for staphylinid evolution

Other than *Apticax* (Schomann and Solodovnikov 2012), from the Lower Cretaceous Crato/Santana Formation of Brazil, *Afristenus orapensis*, *Cretatachinus orapensis* and *Afrinophilina orapensis* are the only fossil staphylinids described from the southern hemisphere. However, none of the fossils described here change the existing picture of staphylinid evolution that has emerged in the past few years. It is interesting, that we have a new genus of Steninae in the Cretaceous, which supports the proposition by Żyła et al. (2017), who suggested, with the description of *Festenus*, that there was a greater, unexpected diversity of the Steninae in the Cretaceous. Originating in the Jurassic, the tachyporines would certainly have been present in the Upper Cretaceous, and it would possibly be more interesting if the specimen turned out to be an aleocharine, since only three other fossils of this extremely diverse subfamily from the Burmese

amber of Myanmar have been described during this time (Cai and Huang 2015; Yamamoto et al., 2016 ; and Cai et al., 2017).

Finally, we have described the first member of the Pinophilina (*Afrinophilina orapensis* Mnguni et al. in preparation) from the Cretaceous, as would be predicted, because a fossil member of its sister taxa, the Procirrina, was recently described by Shaw et al. (2020) from the Burmese amber of Myanmar. Nonetheless, the specimen also shares intriguing similarities with the genus *Mesostaphylinus* described by Solodovnikov et al. (2013) as *M. elongatus*, *M. yixianus*, and *M. antiquus* in terms of the structure of the coxa, suggesting that either the *Mesostaphylinus* may have some relationship with the Pinophilina, or that the interpretation of the characteristic in the fossil described in this study as being diagnostic of the tribe (Shaw et al. 2020) need to be re-evaluated.

9.5 Palaeoenvironment reconstruction at ODM

The staphylinids described above [stenine, tachyporine and paederine (pinophiline)] are mostly found in well-vegetated wet environments. Individually, taxa within these groups may have other habitat preferences; but together, they suggest an abundance of vegetation and humid conditions. However, the fossils are not accurately placed in the crown groups of the subfamilies, and could have had very different biology from the extant taxa. This corroborates data from the other fossils from ODM, which also suggest wet, well-vegetated conditions. A summary of the publications that have offered a palaeoenvironmental reconstruction of the ODM is presented in the table below.

The publications from ODM included below highlight the palaeoenvironment reconstructions.

Table 9.1: Research published on the fossils from the Orapa Diamond Mine in Botswana.

Environment reconstruction	**Source**
Angiosperms, well vegetated successional vegetation.	Bamford 1990
Wet, well vegetated, perhaps forested.	Kuschel et al. 1994
Well vegetated crater, moist, possibly seasonal.	McKay 1990
Forested environment, perhaps thick bush.	McKay 1991
Wet, well vegetated, lake shoreline.	Rayner 1987

Moist vegetated conditions.	Rayner and Waters 1989
Moist vegetated environment.	Rayner and Waters 1990
Warm temperate moist.	Rayner et al. 1997
Well forested humid conditions.	Waters 1989a
Forest moist, environments.	Waters 1989b
Moist vegetated environment.	Waters 1990

Seasonal rainfall is suggested by the varve-like layering in the fine-grained fossiliferous sediments McKay (1990), and by the temperate conditions in the palaeoenvironmental reconstructions in McKay and Rayner 1986; McKay 1990, 1991; Rayner and McKay 1986, Rayner 1987; and Rayner et al. 1991, 1997). During the Turonian, ODM would have been located about 34°S, the same latitude as Cape Town in South Africa today, further supporting a temperate environment (McKay 1991). On the other hand, findings suggest that the Cretaceous was a warmer, more humid time, with tropical conditions, extending North and South of their present boundaries (Huber et al. 2018).

9.6 Depositional environment

The taphonomy analysis presented in this study, although hampered by a small sample size, suggests that the staphylinids, which were all preserved in fine grained pink-red-brown lacustrine mudstone blocks, were probably preserved in a shallow, oxidising, nearshore environment. They were probably moving around the lake edge, and unexpectedly became trapped in the lake. There is also evidence that they were flying, since the elytra are preserved slightly open.

The hind wings are very delicate, and would have decomposed faster than the rest of the body. It appears as though they were not scavenged, since the fossil insects described in this study, and the majority of the those in the taphonomical analysis of this study were preserved almost completely. However, the presence of ¾ body, wings, legs, antennae, elytra, thorax and abdomen suggests that there would have been predators and scavengers roaming in and around the lake. Further studies of this nature, focusing on collecting a larger sample size are required to clarify this hypothesis, and consolidate the findings reported in this study.

9.7 Limitations of this study and future recommendations

This study started as a general study of Coleoptera, with the hope to find a group that has well-enough-preserved diagnostic features, and with fine structures, to warrant further study. It is for this reason that the Staphylinidae were chosen. It is hoped that the descriptions made in this study, with further input from staphylinid specialists, will make a contribution to our understanding of staphylinid evolution. During the study, it was recognised that a strong knowledge of the global staphylinid fauna is essential for studies such as this.

The study was significantly hampered by technology. The binocular microscope (Olympus SZX7) at the ESI (Johannesburg, South Africa) struggled to clearly show the different characters on the specimens, particularly the diagnostic features. The digital microscope (DSX 110) at SANBI (Pretoria, South Africa) with the vertical stacking feature proved much better. However, it struggled to stitch multiple images together, resulting in blurred margins at the image boundaries, and also resulted in non-uniform lighting on some of the photographs.

However, these specimens do preserve a huge amount of information, which may become more visible when better technology is used. The Olympus DSX 110 is considered to be the ‘state of the art’ in South Africa at the moment, but maybe there are better techniques that will reveal more features in the future. Historically, descriptions of compression fossils have been very superficial (e.g., Ryvkin 1988; and Puthz 2010). However, more recently, descriptions have become far more detailed. Examples of such studies were undertaken by Solodovnikov et al. (2013) and Liu et al. (2021). Despite the challenges, the study has attempted to meet those high standards.

In addition, we would like to complete this study by creating a morphological character matrix for each specimen, to conduct a phylogenetic analysis. It is uncertain, however, whether or not enough characters will be preserved for this exercise to be possible. Finally, there are staphylinids that have been figured in publications (e.g., McCarthy and Rubidge 2005; and Rayner and McKay 1986), but these specimens have not been located, and it is a priority to do so in the future. Furthermore, although some of them are already being achieved, it is important to point out that Betz et al. (2018) suggested a few things to do in the future, and they included the following: (1) reviewing the work of early palaeontologists, (2) incorporating fossils into phylogenetic analyses, (3) exploring new or neglected deposits, (4) bridging the gap between palaeontologists and neontologists, and (5) producing generic revisions for all staphylinoid subfamilies.

9.8 Conclusions

Other than *Apticax* Schomann and Solodovnikov, 2012 from the Crato/Santana Formation of Brazil (Schomann and Solodovnikov 2012), *Afristenus orapensis*, *Cretatachinus orapensis* and *Afrinophilina orapensis* are the only staphylinids described from the southern hemisphere, particularly from the African continent. They include a new genus of Steninae, and the earliest occurrence of Paederinae belonging to the subtribe Pinophilina. The specimen (*Afrinophilina orapensis*) has interesting similarities with *Mesostaphylinus* that was redescribed by Solodovnikov et al. (2013). Confident placement of the Tachyporinae is hampered by its similarity to the most speciose and variable Aleocharinae, which heavily rely on genitalia, and are currently in need of taxonomic and systematic revision. It is likely that a staphylinid specialist with greater knowledge of the world fauna may be able to contribute to their more accurate placement, as would creating a morphological matrix, and phylogenetic analysis, especially for the paederine, which is particularly well-preserved. These compression and impression fossils preserve many details, which may become visible with better technology. The interpretation of some diagnostic features was rather difficult, due to distortions, and suggests that a better understanding of how rock fossils are altered during fossilisation is a necessity. These fossil have a valuable story to tell, given that they sample a different palaeoenvironment from the more commonly found amber fossils. Finally, all the fossils described in this study reinforce the current model of ODM as a wet, well-vegetated environment. The similarity of all the fossils with all the modern forms of staphylinids implies a slower rate of evolution (perhaps even morphological stasis), when compared to tetrapods, which have, in general, changed dramatically over the same time period. These findings suggests a punctuated equilibrium pattern of evolution, as reported in McKay (1990) and Waters (1990).

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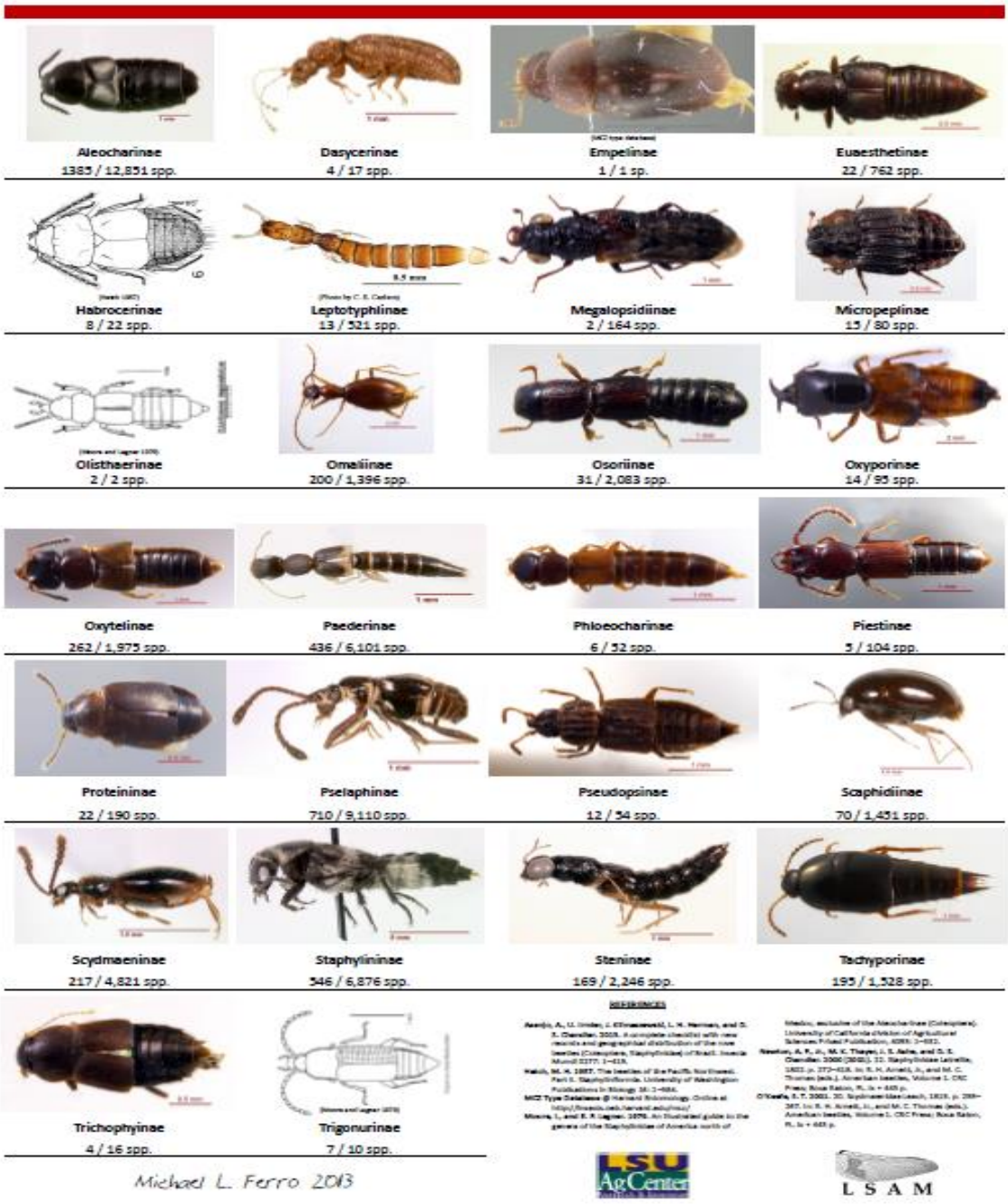
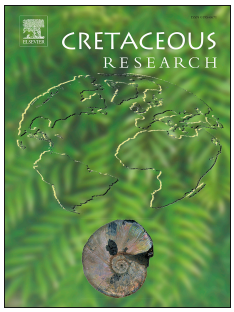
Appendix



Appendix 1: A depiction of 26 subfamilies of the Staphylinidae. Taken from Ferro (2013).

Journal Pre-proof



Afristenus orapensis: a new genus and species of Steninae (Coleoptera: Staphylinidae) with “harpoon-like” mouthparts from the Upper Cretaceous lacustrine deposits at Orapa Diamond Mine, Botswana.

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Afristenus orapensis: a new genus and species of Steninae (Coleoptera: Staphylinidae) with "harpoon-like" mouthparts from the Upper Cretaceous lacustrine deposits at Orapa Diamond Mine, Botswana.

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Abstract.

A new genus and species of rove beetle, *Afristenus orapensis* gen. et sp. n., is described and figured based on one well-preserved compression fossil from the African, Upper Cretaceous fossil insect deposit, Orapa Diamond Mine in north-east Botswana. *Afristenus* is definitively placed in the extant subfamily Steninae based on its harpoon-like labial feeding apparatus. *Afristenus* differs from all other Steninae by head and neck behind the eyes being broader than eyes that cover two-thirds of the side of the head and transverse prothorax, which is distinctly broader than the head. The new specimen from Orapa Diamond Mine possibly has unique antennae with a compact 5-segmented-club, and a stem composed of elongated antennal segments. This is the first stenine compression fossil with a preserved labial feeding mechanism. It confirms the widespread distribution of stenines with this feeding apparatus in the mid-Cretaceous; supporting the hypothesis that this feeding mechanism was part of the

ground plan of the Steninae, and the Lower Cretaceous, but probably not Jurassic origin for the group. It also demonstrates morphological conservatism dating back to the Cretaceous.

Keywords: Rove beetle, Labial feeding mechanism, 5-segmented antennal club, Africa, southern Hemisphere.

1 Introduction.

The hyper-diverse rove beetles or Staphylinidae Latreille, 1802 is the largest animal family with 62 453 species (Lü et al., 2020), and within the Staphylinidae, the subfamily Steninae MacLeay, 1825 contains the most speciose staphylinid genus *Stenus* Latreille, 1797, which is also the second largest animal genus, with approximately 3 000 species (Betz et al., 2018). Although the higher order systematics of the Staphylinidae is continuously being revised, there is some consensus that the Steninae are monophyletic and the sister group of the Euaesthetinae (Clarke and Chatzimanolis, 2009; Clarke and Grebennikov, 2009; McKenna et al., 2015; Żyła et al., 2017), although Lü et al. (2020) could not find strong evidence for this relationship.

The Steninae comprise of only four genera. The two extant genera are *Stenus* (with > 3000 species), and *Dianous* (with > 300 species) (Betz et al., 2018; Clarke and Grebennikov, 2009; Cai et al. 2014; McKenna et al., 2015; Żyła et al., 2017). There are also two extinct genera; the monospecific *Eocenostenus fossilis* Cai et al. 2014 from the Eocene Alés-Monteils of France (Cai et al., 2014) and the Cretaceous genus *Festenus* with three species; viz. *F. gracilis* Żyła et al. 2017 and *F. robustus* Żyła et al. 2017 (Żyła et al., 2017), and one undescribed species (Cai et al., 2019) from Burmese amber, Myanmar.

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In addition to *Eocenostenus* and *Festenus*, there are 24 specimens ascribed to *Stenus* (Betz et al., 2018). Schlüter (1978) reported *Stenus inexpectatus* based on a single specimen from a Cretaceous Bezonnaise amber in France, and Ryvkin (1988) described a single rock fossil as *Stenus imputribulus* from a Cretaceous Obeschayushchiy amber in Russia. These specimens are figured in Puthz (2010). While the details are difficult to ascertain, both specimens have the typical habitus of *Stenus*, which includes a large pair of protruding eyes, a cylindrical prothorax, and a flattened or cylindrical abdomen. *Stenus inexpectatus* Schlüter 1978 (Puthz, 2010: fig. 18) has antennae with a 3-segmented club, originating from just behind the anterior margins of the eyes, but poor preservation makes a more accurate determination impossible.

Except for *Eocenostenus* Cai et al. 2014, which differs from other stenines because it combines a strongly transverse prothorax with anterolateral projections and anteriorly placed antennal insertions (Cai et al., 2014), all Cenozoic fossils belong to the genus *Stenus* (Żyła et al., 2017). Żyła et al. (2017) have, therefore, questioned whether *Eocenostenus* might not be a typical *Stenus* species that was deformed during fossilization. However, an alternative interpretation of the fossil suggests that this is rather not likely.

Nine of the Cenozoic *Stenus* fossils are known from Baltic amber (Alekseev, 2013). Some of them have typical features of the genus, such as the characteristic elongated mouthparts (Żyła et al., 2017). The remaining *Stenus* species are from Eocene, Oligocene, Miocene, and Pleistocene rock fossils (Smith and Marcot, 2015; Żyła et al., 2017).

The Steninae have been studied since the 19th century. Their biology is relatively well-known, and has recently been reviewed by Betz et al. (2018), who, along with Żyła et al. (2017), and Cai et al. (2019), have described one of the unique features of the taxa, namely their "harpoon-like" mouthparts, in some detail. The genus *Stenus* has a unique elongated labium, tipped by an adhesive-covered cushions, called paraglossae, which can be protruded to trap and retrieve small invertebrate prey animals such as Collembola (i.e., springtails) within a few milliseconds. The complex mechanism of the rapid protrusion of the labium relies upon an increase in haemolymph pressure, co-ordinated with the contraction and relaxation of various muscles (Betz et al., 2018).

Aside from the genus *Stenus*, these "harpoon-like" mouthparts have also been identified in the undescribed Australian species (Clarke and Grebennikov, 2009), and in fossil specimens of the genus *Festenus* (Cai et al., 2019). The "harpoon-like" mouthparts are also common in *Stenus* described from Cenozoic amber (Żyła et al., 2017). On the other hand, the mouthparts appear to have been secondarily lost from the paraphyletic genus *Dianous*, which arose from within the genus *Stenus* (Koerner et al., 2013; Żyła et al., 2017). Also, the mouthparts possibly evolved more than once in the Steninae (Żyła et al., 2017). Given the discovery of the specialized mouthparts in *Festenus*, it seems most likely that they are part of the ground plan of the Steninae, and that perhaps the loss in *Dianous* is secondary (Cai et al., 2019). Moreover, it should be noted, that the degree of development of the mouthparts is variable across the subfamily, and even *Dianous* can slightly protrude its labium, but does not have the sticky-pad paraglossae like *Stenus* (Żyła et al., 2017).

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Another intriguing characteristic, which may be a synapomorphy of the subfamily (Clarke and Grebennikov, 2009), are the secretions of the larger set of their paired pygidial glands, that empty into the rectum and which, in addition to a defensive and anti-microbial function, can be squirted onto the water surface, where they spread rapidly, propelling the stenines forward at high speed on their hydrophobic tarsi. This is termed skimming behaviour (Betz et al., 2018; Lang et al., 2012).

Clarke and Grebennikov (2009) identified 23 adult and larval synapomorphies for the family Staphylinidae. Of these, they selected 12 features (6 adult and 6 larval) as being most significant because they have the highest consistency index. The 6 adult features are: antennae inserted into the frons between the eyes, although the genera *Festenus, Eocenostenus,* and one specimen of the unidentified Australian species have the antennae inserted at the front of the eyes (Żyła et al., 2017); apodemes arising from interantennal pits; labial palps situated close together at the anterior margin of labrum; pronotal marginal carina meeting pronotosternal suture laterally, although in *Festenus,* the pronotal marginal carina does not meet the pronotosternal suture (Żyła et al., 2017); apex of mesoventral intercoxal process abuts metaventral intercoxal process, although in *Festenus* these two processes overlap; and paired pygidial glands opening into the rectum.

It is not surprising that some of the most consistent synapomorphies are associated with the specialized “harpoon-like” labial feeding apparatus. For example, Żyła et al. (2017) listed several of these characteristics: adhesive cushions of labium present, with labial palps almost contiguous and nearer to medial apex than sides of labium; lateral palpomere rests on mentum divided by medial longitudinal carina; and prementum modified, elongated into eversible rod-

like structure. Given that these are traditionally accepted as properties of *Stenus*, and are now known to be properties of *Festenus* (Betz et al., 2018; Żyła et al., 2017), and the undescribed Australian genus (Clarke and Grebennikov, 2009), it can be confidently stated that they are synapomorphies for basal Steninae, and that most, except for the "almost contiguous labial palps", have been secondarily lost in *Dianous* (Cai et al., 2019; Żyła et al., 2017).

The habitat preference of the Steninae have been reviewed by Betz et al. (2018). They are associated with damp environments ranging from waterside, moist tree litter, forest canopy, foliage, grass clumps, moss, dung, ant nests and mammal middens. A few are found in dry habitats; and on the other extreme, *Dianous* can be found in the water spray associated with rapids and cascades. *Stenus* is found on all continents, except for Antarctica. It is also absent in New Zealand. Although *Stenus* is relatively common in almost all the regions, there are only 29 species recorded in Australia. Meanwhile, *Dianous* is restricted to the Palaearctic, Nearctic and Oriental regions (Betz et al., 2018).

This paper describes the first fossil stenine from the Southern Hemisphere and the African continent. It is an Upper Cretaceous compression fossil from Orapa Diamond Mine, located in north-east Botswana, with well-preserved typical stenine "harpoon-like" labial mouthparts.

2 Materials and Methods.

The specimen described here was recovered from Orapa Diamond Mine (Fig. 1), in the north-east of Botswana, about 240 km due west of Francistown (-21°03.870´S, 25°41.055´E). The fossiliferous rocks were deposited in a crater lake, which was formed by a double eruption of

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diamondiferous kimberlites that were subsequently uncovered by mining operations, and collected from eighteen sites within the mine, between 1983 and 1988 (McKay 1990, Fig. 2). Sedimentary rocks are currently considered as Upper Cretaceous, with an age range based on the decay of ^{238}U in zircons in the kimberlite, between 93.1 Ma (Davis, 1977) and 87.4 ± 5.7 and 92.4 ± 6.1 Ma (Haggerty et al., 1983). The sedimentary rocks are aged between 81.7 and 98.5 Ma, with a midpoint of 90.1 Ma. They are, therefore, probably Turonian, but possibly Cenomanian (on the upper side of the error range), or Campanian (on the lower side of the error range).

The specimen described here is a well-preserved compression fossil. The holotype is housed in the Herbarium of the Evolutionary Studies Institute (ESI), at the University of the Witwatersrand, Johannesburg, South Africa. Observation and photography were taken using a combination of an Olympus SZX7 binocular microscope (with Olympus U-TV0.36XC camera) and an Olympus DSX 110 digital microscope. Multiple images were stacked and measured using an Olympus Stream 2.4. All the images were prepared using Adobe Photoshop version 5.6.5.58 (Adobe Creative Cloud, University of the Witwatersrand, Johannesburg, South Africa). The specimen was examined under cross polars to clarify the outlines, and non-polarized light was used at various angles to show relief. Polarizing filters were attached to the swan necked lights (the objective lens of the microscope), and were rotated to polarize the light and remove reflection. The scale in all the photographs is 1 mm.

A single specimen of an adult fossil with carbonaceous material preserving dorsal and ventral structures is described. The antennae, eyes, legs, and mouthparts are only partially preserved in a large, triangular piece (205 x 115 x 45 mm) of brown lacustrine mudstone. Originally, the

specimen had the tip of the abdomen preserved (Fig. 3A and B). Due to the fragile nature of the mudstone, the tip was accidentally lost (Fig. 4A, top). The palaeofauna of the Orapa deposit is diverse, and aspects of it have been reviewed (Brothers and Rasnitsyn, 2003; McKay and Rayner, 1986; McKay, 1990; Rayner and McKay, 1986; Rayner, 1987; Rayner et al., 1991, 1997).

All taxonomic acts established in the present work have been registered in ZooBank LSID, together with the electronic publication under ZooBank: urn:lsid:zoobank.org:pub:40333918-5B61-4F4C-93C4-BAD436A2D0D4.

3 Systematic Palaeontology.

Order: Coleoptera Linnaeus, 1758.

Series: Staphyliniformia Latreille, 1802

Superfamily Staphylinoidea Latreille, 1802

Family: Staphylinidae Latreille, 1802.

Subfamily: Steninae MacLeay, 1825.

Genus: ***Afristenus* gen. nov.**

Type species. *Afristenus orapensis* species. nov., by monotypy and present designation.

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183 *Etymology:* The generic epithet is a combination of "Afri", from Africa, and the generic name
184 *Stenus*.

185

186 *Composition*. Only type species *Afristenus orapensis* species. nov.

187

188 *Diagnosis.* With "harpoon-like" labial feeding apparatus, which is characteristic of the
189 Steninae. It can be distinguished from all the other Steninae by: the transverse pronotum, which
190 is distinctly broader than the head (although this character could be caused by the distortion of
191 the specimen), eyes covering two-thirds of the side of the head, and antennae with a 5-
192 segmented-club originating on frons between the eyes.

193

194 ***Afristenus orapensis*** species. nov. (Figs. 3A and B; 4A and B, top; 4A and B, bottom; and 5)

195

196 *Etymology:* The epiphet of the new species is a combination of Orap- from 'Orapa', the deposit
197 where the specimen described here was recovered, which is derived from Sesarwa (a San or
198 Bushmen language), and is named after a nearby cattle post, meaning 'the resting place of
199 lions' (https://www.debeersgroup.com/about-us/our-operations/our-mines/botswana), and also
200 see Brook (2019); and -ensis, a Latin suffix meaning 'from'.

201

202 *Material studied:* Holotype, adult, [specimen number BP/2/25912, Herbarium, Evolutionary
203 Studies Institute (ESI), University of the Witwatersrand, Johannesburg, South Africa].

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205 *Type locality and horizon:* Botswana, Orapa Diamond Mine, lacustrine deposit, Upper
206 Cretaceous (Cenomanian–Campanian, possibly Turonian) (Davis, 1977; Haggerty et al., 1983).

207

208 *Diagnosis:* As for genus ***Afristenus* gen. nov.**.

209

210 *Description:* Body small measuring 2.33 mm in length (from tip of mandibles to tip of
211 abdomen) (Fig. 3A and B).

212

213 Head (Figs. 3A and B; 4A and B, top; 4A and B, bottom; and 5) angled to right, prognathous,
214 longer than wide, 0.33 mm long (from tip of mandible to anterior margin of pronotum) and
215 0.30 mm wide at the posterior of eyes. Eyes large, slightly protruding, cover two-thirds of side
216 of head, with pair of dark internal ring structures, possibly representing entrance of optic nerve
217 into head capsule (Fig. 4A, top). Distinct ridge runs across head between posterior margins of
218 eyes, presumably representing negative of nuchal depression found in some Steninae, lacks
219 nuchal constriction behind eyes, head and neck behind the eyes broader than eyes (Figs. 3A
220 and B; 4A and B, top; and 4A and B, bottom). Antenna with 5-segmented club (Figs. 4A and
221 B, bottom) and stem of indistinct elongated antennomeres (Figs. 4A, top; and 4A and B,
222 bottom). Antennal stem cannot be misinterpreted as lateral margin of rectangular pronotum,
223 pronotum distinctly broader than head. Antennal insertions on frons between eyes (Figs. 4A,
224 top; and 4A, bottom). Protruding labium with connecting tube (mentum), elongated prementum
225 and paired paraglossae (Figs. 3A and B; 4B, top; and 4A and B, bottom). Two segments of
226 maxillary palps indistinctly preserved on left hand side, possibly one segment of right maxilla
227 or right mandible. Labium distinctly longer than maxillary palps, similarly to extant stenines
228 (Fig. 6). Mandibles indistinct (Figs. 3A and B; 4A and B, top; and 4A and B, bottom).

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Pronotum (Figs. 3A and B; 4A, top; and 4A and B, bottom) transverse, rectangular (although this character could be caused by the distortion of the specimen), finely punctate, possibly representing a compressed tube, wider than long (0,43 mm long, 0.55 mm wide) with rectangular anterior and posterior margins, lateral margins slightly curved (greatest width medially), anteriorly distinctly wider than head. Left elytron well preserved with a circular clear area which may be a pigment spot, right elytron indistinct. Elytron (Figs. 3A and B; 4A, top; and 4A and B, bottom) rectangular, broader distally than at base, coarsely punctate, longer than wide (0.60 mm long, from posterior margin of pronotum to posterior margin of elytron, 0.30 mm wide at base and 0.37 mm wide distally), together distinctly wider than abdomen, slightly wider than pronotum, lacks epipleural ridge and fold. Legs poorly preserved, procoxae not preserved, mesa and metacoxae indistinct, legs on left side of body represented by indistinct meso-femur and compact tarsus, difficult to distinguish individual segments, legs on the right side of body represented by three pairs of femora and tibia (Figs. 3A and B; 4A, top; and 4A and B, bottom).

Abdomen (Figs. 3A and B; and 4A, top) apparently flat, flexible, and gradually tapering posteriorly (1.22 mm long, 0.34 mm wide at base and 0.41 mm wide distally), with six visible abdominal segments, distinctly narrower than elytra; widest around segments III-V, first four visible abdominal segments with paratergites, segment VIII at tip of abdomen with V-shaped imagination posteriorly, possibly representing male sternite.

4 Discussion.

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Remarkably, for a fossil embedded in rock, the specimen preserves the delicate "harpoon-like" extended labial mouthparts, which are characteristic (if variably developed), of all the Steninae except *Dianous*, where it appears to have been secondarily lost (Koerner et al., 2013; Żyła et al., 2017). It is distinctly longer than maxillary palps, similarly to extant stenines (Fig. 5). On the other hand, the characters which separate the specimen from other stenines are more difficult to interpret. The transverse prothorax is also a characteristic of *Eocenostenus* (Cai et al., 2014). However, it has been suggested that the feature may have been produced by distortion during fossilization (Żyła et al., 2017), although an alternative interpretation of the fossil suggests that this is unlikely. Moreover, a similar distortion has not been reported from any other typical *Stenus* rock fossils (Żyła et al., 2017).

Rasnitsyn and Quicke (2002) mentioned that fossil insects are often distorted in unpredictable ways during fossilization. However, in the case of the fossil insect described here (*Afristenus orapensis* gen. nov., species. nov.), the head and abdomen appear to be of relative normal proportions. Thus, if the prothorax was distorted (and therefore not rectangular), it would have had to occur selectively. The ground work on carabid beetles (McKay, 1990) and weevils (Kuschel et al., 1994) from the same deposit has shown that they are relatively undistorted.

The specimen also lacks a laterally constricted neck, another characteristic apparently shared by *Eocenostenus*. However, in the latter specimen, the head appears almost retracted into the prothorax, possibly making it difficult to view the neck. Characteristics such as the presence or absence of a distinct neck or nuchal constriction are often commonly used in adult staphylinid taxonomy/systematics, dating back to Moore (1964). Moreover, the character is widely used at a higher taxonomic/systematic level than genus. However, the posterior portion

of the head of the fossil (which is broader than the eyes) is apparently unique. Nonetheless, *Stenus gypsi* in Puthz (2010: fig. 22) may have a similar neck, though it is more typically narrower than the eyes.

The antenna with a 5-segmented-club, and an elongated stem, which originates more-or-less distinctly on the frons between the eyes, is clearly visible on the right-hand side of the specimen. The compound eyes are also interesting, because they have a faint, but distinct outline running up the side of the head, and within each eye, there is a circle with a dark ring around it. These are clearly associated with the eyes, and may be openings in the head capsule, where the optic nerve enters.

Apart from one species from Crato Formation, which is a Lower Cretaceous deposit in Brazil, *Afristenus* is the first fossil staphylinid beetle described from the southern Hemisphere and African continent. With a median age of 90.1 Ma (Davis, 1977; Haggerty, 1983), the Orapa deposits are slightly younger than the other Cretaceous stenines from Burmese amber (Cai et al., 2019; Żyła et al., 2017), French Bezonnaise resin (Schlüter, 1978), and the East Russian, Obeschayushchiy locality (Ryvkin 1988), which are all Cenomanian in age (Kopylov 2010; Rasnitsyn et al., 2016; Shi et al., 2012). The presence of *Afristenus* preserved with its elongated protrusible labium supports (Figs. 3A and B; 4B, top; 4A and B, bottom) the evidence provided by *Festenus* (Cai et al., 2019), and possibly the other two *Stenus* fossils (which presumably had the same mouthparts), that these specialized “harpoon-like” mouthparts with paraglossae (Fig. 5) had evolved by the mid-Cretaceous.

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299 At the time, the Steninae were present in widely distributed localities, spanning approximately
300 60° N and 35 ° S (Fig. 6), suggesting a much earlier, common point of origin. This is supported
301 by the occurrence of the crown group Euaesthetinae in the Early Cretaceous (125-135 Ma)
302 Lebanese amber of Lebanon (Lefebvre et al., 2005). Given that euaesthatines and stenines are
303 monophyletic and sister clades (Clarke and Chatzimanolis, 2009; Clarke and Grebennikov,
304 2009; Grebennikov and Newton, 2009; Liu et al. 2021; McKenna et al., 2015; Żyła et al., 2017),
305 it is likely that the stenines would have been present at the same time. Since no staphylinine
306 group fossils are known from the Jurassic (Liu et al. 2021), and that the first unambiguous
307 staphylinines belonging to the Oxyporinae, Paederinae, Staphylininae and Euaesthetinae (Liu
308 et al. 2021), in addition to the Lebanese amber euaesthetine (Lefebvre et al., 2005), are known
309 from the 125 Ma Lower Cretaceous Yixian Formation (Liu et al. 2021), it is likely that the
310 Steninae may have also appeared around this time.

311

312 However, it is equally probable that they appeared even earlier than the proposed date of 125-
313 135 Ma, but not as early as the Jurassic (145-200 Ma), because they are deeply nested within
314 the staphylinines in most phylogenies (Clarke and Chatzimanolis, 2009; Clarke and
315 Grebennikov, 2009; Grebennikov and Newton, 2009; Liu et al. 2021; Lü et al., 2020; McKenna
316 et al., 2015; Żyła et al., 2017), and no staphylinines are known from the Jurassic (e.g., Liu et
317 al. 2021). Support for this conjecture comes from molecular clock data analyses, which suggest
318 that the Staphylinidae originated in the Triassic/Jurassic boundary, and diversified in the
319 Jurassic/Cretaceous boundary (McKenna et al. 2015; Toussaint et al. 2016; Zhang et al., 2018;
320 Lü et al., 2020; Cai et al., 2022). To be more specific, Lü et al. (2020) estimated that the
321 Steninae diverged 122.22 Ma.

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The paleoenvironment at Orapa has been reconstructed as a lake within a well-vegetated crater (McKay and Rayner, 1986; McKay, 1990; Rayner and McKay, 1986, Rayner, 1987; Rayner et al., 1991, 1997; Brothers and Rasnitsyn, 2003), thus resembling the damp, vegetated habitat preferred by many extant stenines (Betz et al., 2018). Interestingly, no Collembola (i.e., springtails, which are known to be soil and litter-dwelling organisms that prefer wet or damp surroundings) have been described from Orapa Diamond Mine thus far (Brothers and Rasnitsyn, 2003). This suggest that the stenines would have been feeding on something else.

5 Conclusions.

The presence of stenine fossils with their characteristic "harpoon-like" mouthparts at localities as far apart as Orapa, France, Russia and Myanmar (Cai et al., 2014, 2019; Żyła et al., 2017) during the Upper Cretaceous suggests that stenines must have arisen at a far earlier time; possibly at about the same time, or even earlier than the appearance of their sister group (the euaesthatines), in Lebanon amber during the Lower Cretaceous (Lefebvre et al., 2005). However, it is unlikely that they originated in the Jurassic, since the Steninae are nested within the non-monophyletic staphylinine group, whose presence have not been recorded in the Jurassic (Liu et al., 2021). The stenine from Orapa has several features which differentiate it from other stenines that have been described between Cretaceous and Holocene ages. However, the interpretation of some of those diagnostic features is rather difficult, due to a possible distortion of the fossil, and suggests that researchers need a better understanding of how rock fossils are altered during fossilization. This will assist with a better interpretation of these fossils, and other fossils preserved in rock deposits, which have a valuable story to tell, given that they sample a different palaeoenvironment from the more commonly-found amber fossils. Finally, *Afristenus orapensis* gen. nov., species. nov. appears to have occupied damp or moist

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environments, ranging from waterside or moist tree litter, like many extant stenines (Betz et al., 2018), suggesting constancy in habitat preference (accompanied by morphological stasis) for this group over a long evolutionary time.

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7 Dedication.

SM would like to dedicate this publication to his late supervisor, Dr I.J. McKay. This work would not have been possible without his valuable contribution. Moreover, SM would like to dedicate this to his late great-grandmother, Ms. B.J. Mnguni, late grandfather Mr. C. Nqwili and late father, Mr. T. Peteni.

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8 Competing interests.

The authors declare no competing or financial interests.

9 Author contributions.

SM and IJM - Conceptualization; SM and IJM - Data curation; SM and IJM - Formal analysis; SM and IJM- Funding acquisition; SM and IJM - Investigation; SM and IJM - Methodology; SM and IJM - Project administration; SM and IJM - Resources; IJM - Software; IJM and SB - Supervision; IJM and SB - Validation; SM and IJM - Visualization; SM - Roles/Writing - original draft; SM, IJM and SB - Writing - review and editing.

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11 Data availability.

Datasets related to this article will be shared with the audience upon request.

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525 **13 Figure Captions.**

526 **Figure 1.** Location of Orapa Diamond Mine in Botswana. Taken from Mashabila (2019, page
527 9).

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Figure 2. View of the Orapa Diamond Mine in Botswana in 2018 with fossiliferous sedimentary rocks removed (Photo by I.J. McKay).

Figure 3. Whole body of *Afristenus orapensis* gen. nov., species. nov. holotype, BP./2/25912, Orapa Diamond Mine. A. Polarized light, scale bar = 1mm. Arrow is pointing towards the protruding "harpoon-like" feeding apparatus, the labium, with its paired paraglossae. B. Polarized light drawing, scale bar = 1mm.

Figure 4. Top: Body parts of *Afristenus orapensis* gen. nov., species. nov. holotype, BP./2/25912, under different light conditions. A. Whole body, non-polarized light, scale bar = 1 mm; B. Mouthparts, polarized light, scale bar = 0.5 mm. Abbreviations: ab = antennal base, ac = antennal club, as = antennal stem, ct = connecting tube, le = left eye, lf = left femur, lp = labial palp, lrs = left ring structure of eye, man = mandible, mc = meso coxae, mp = maxillary palp, pg = paraglossae, ps = pigment spot on elytra, re = right eye, rrs = right ring structure of eye, and prm = prementum. Bottom: Body parts of *Afristenus orapensis* gen. nov., species nov. holotype, BP/2/25912, under different light conditions. A. Polarized light, scale bar = 0.5 mm. B. Non-polarized light, scale bar = 0.5 mm. Abbreviations: ab = antennal base, ac = antennal club, as = antennal stem, ct = connecting tube, lp = labial palp, man = mandible, pg = paraglossae, ps = pigment spot on elytra, mt = metatarsus and prm = prementum.

Figure 5. Extant species of *Stenus*, with protrusible and extended feeding apparatus, the labium, with its paired paraglossae (Photo by IJ McKay).

Figure 6. Global palaeogeographic reconstruction of the mid-Cretaceous (100 Ma) showing the localities where the fossil Steninae have been recovered. The map in Mollweide projection was sourced from Scotese (2021). The position of the localities was adapted from Rasnitsyn and Quicke (2002). Abbreviations: Be = Bezonnaise amber, France; Bu = Burmese amber, Myanmar; Or = Orapa, Botswana; Ob = Obeschayushchiy locality, Russia.

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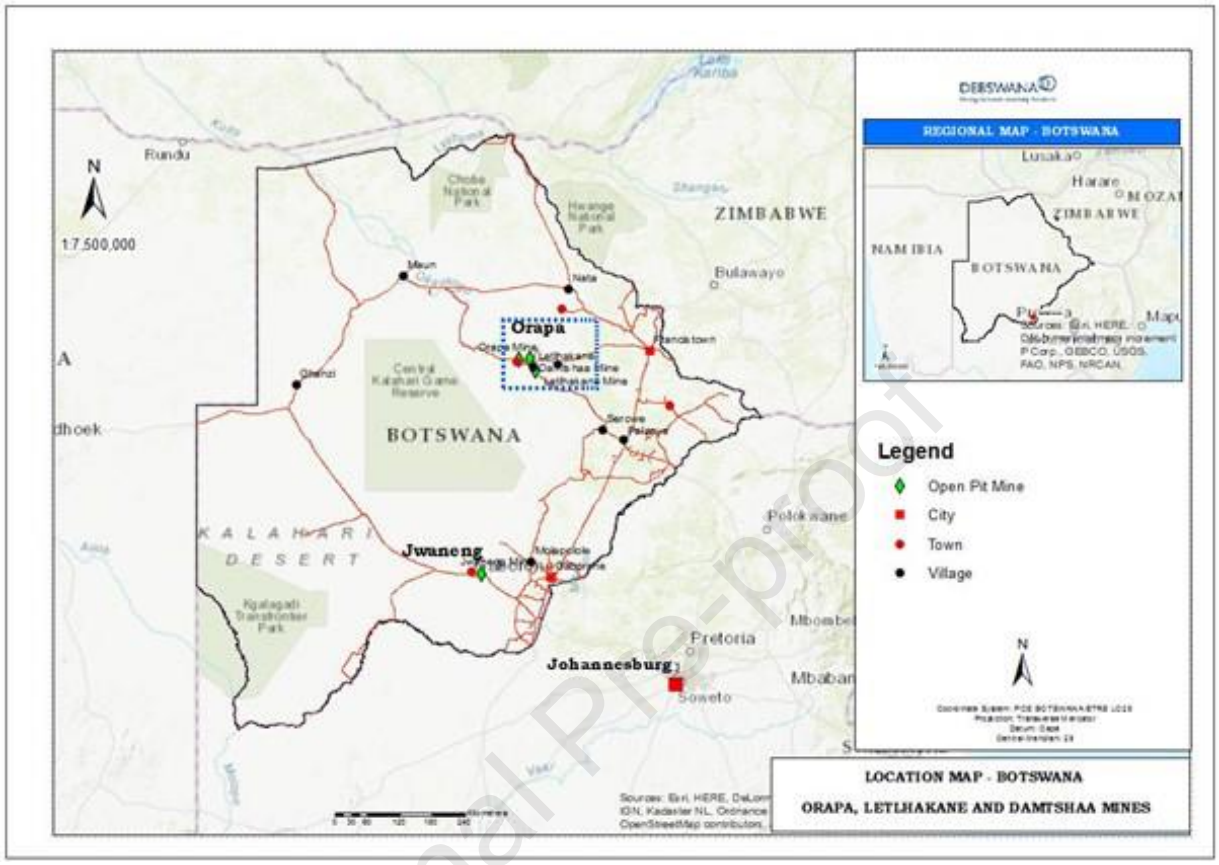
Figure 1.



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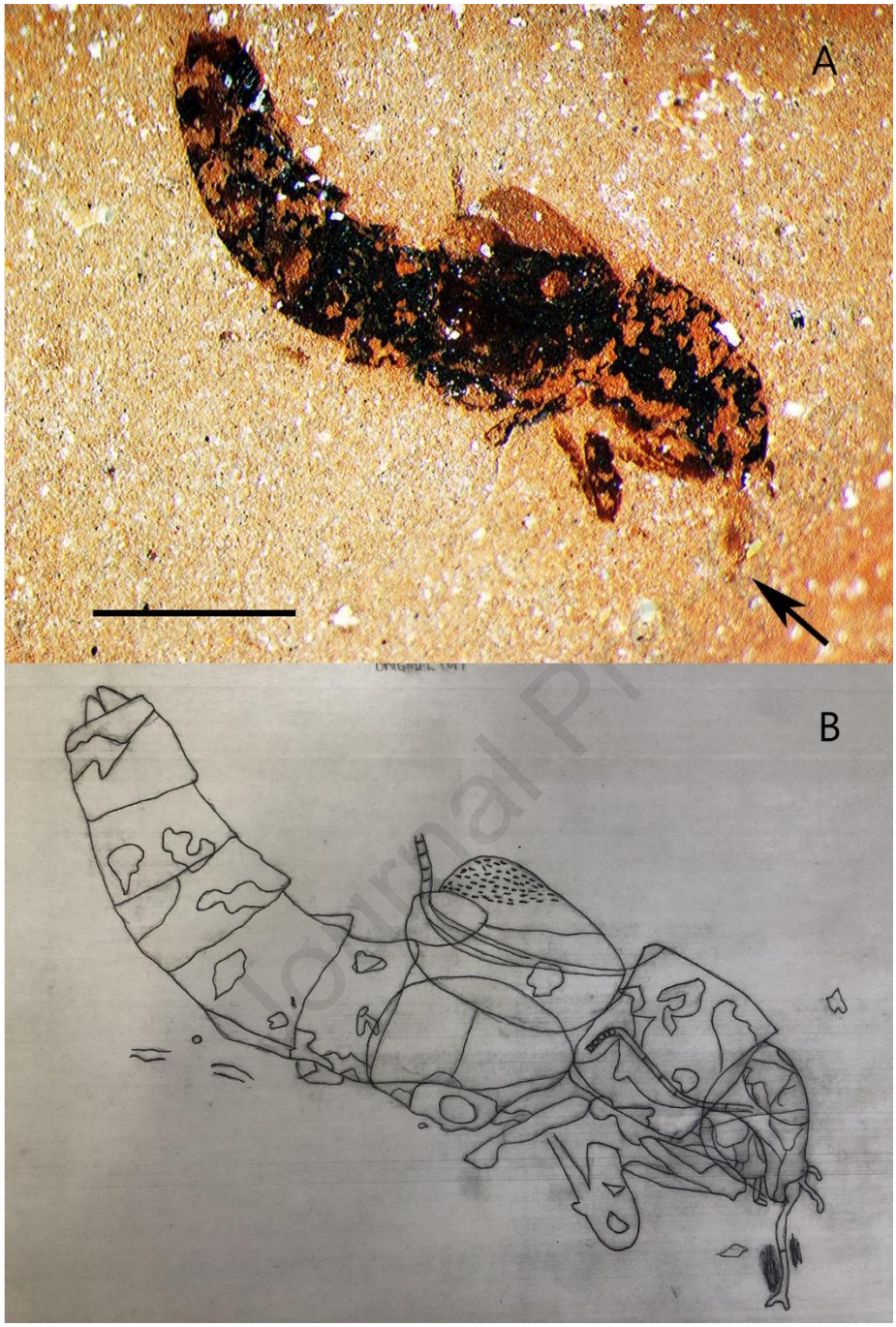

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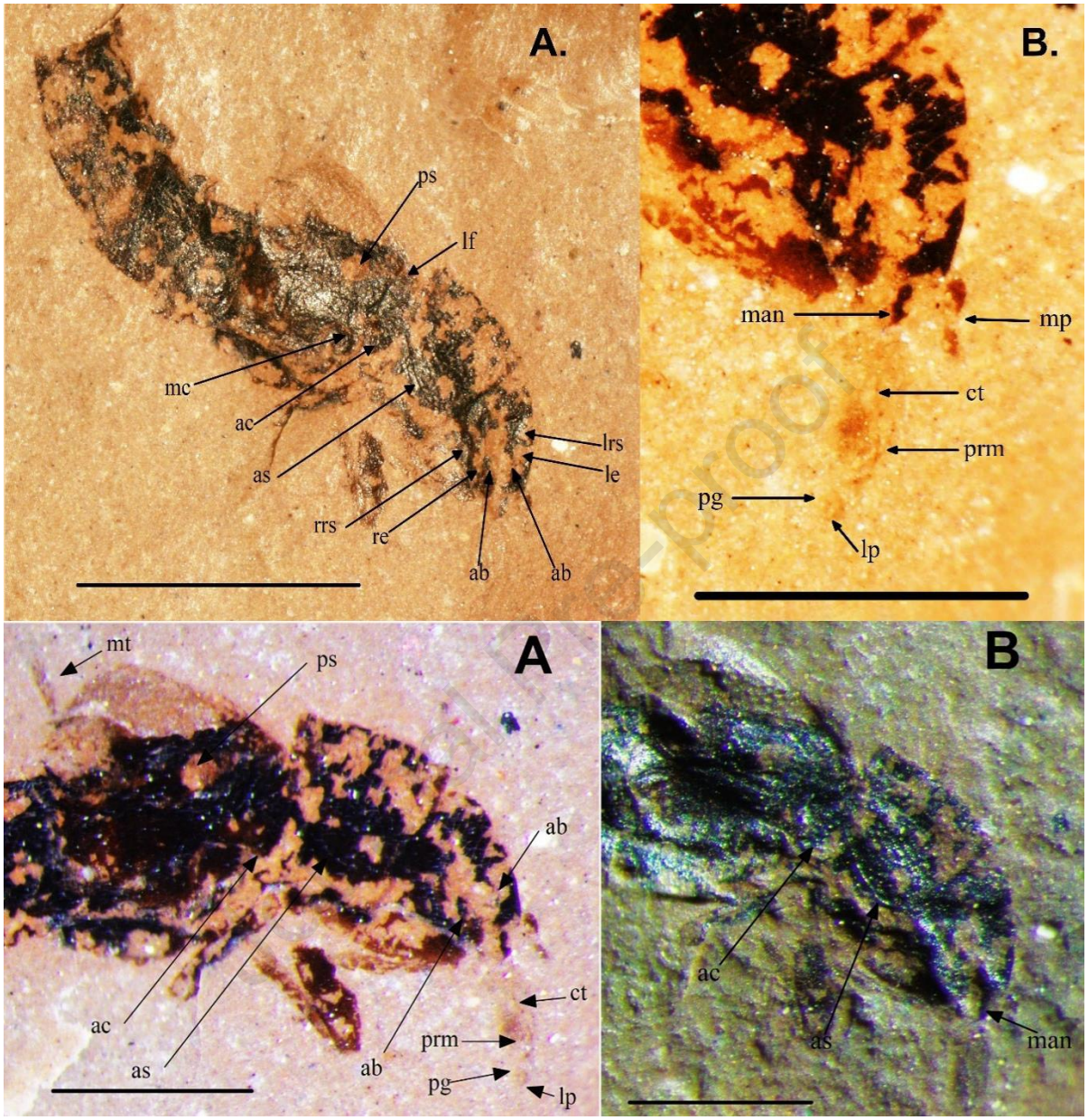

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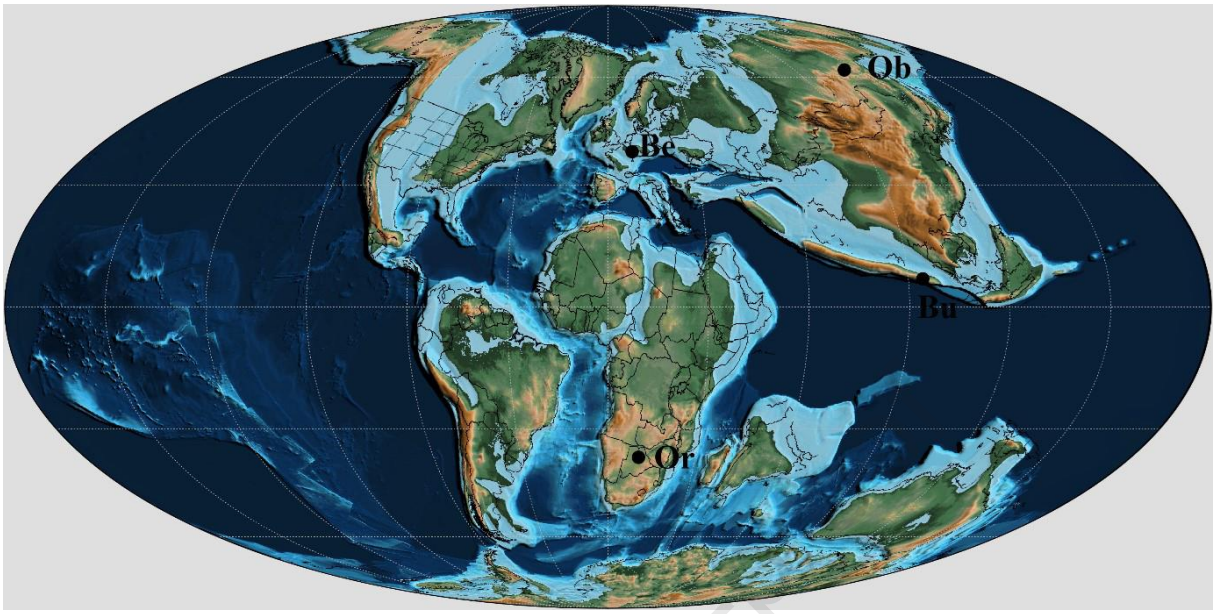

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Author contributions.

SM & IJM - Conceptualization; SM & IJM - Data curation; SM & IJM - Formal analysis; SM & IJM- Funding acquisition; SM & IJM - Investigation; SM & IJM - Methodology; SM & IJM - Project administration; SM & IJM - Resources; IJM - Software; IJM & SB - Supervision; IJM & SB - Validation; SM & IJM - Visualization; SM - Roles/Writing - original draft; SM, IJM & SB - Writing - review & editing.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Laboratory-Based Mate Choices of Two *Eccritotarsus* Species (Hemiptera: Miridae) Used as Biological Control Agents of Water Hyacinth, *Pontederia (Eichhornia) crassipes* (Pontederiaceae), in South Africa[1]

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Abstract Many biological control agents have been released to manage the water hyacinth, *Pontederia (Eichhornia) crassipes* (C. Martius) Solms-Laubach (Pontederiaceae). They include two *Eccritotarsus* sources from Brazil and Peru. Distinguishing features between the two were found, necessitating their redescription. The Brazil source remained *Eccritotarsus catarinensis* (Carvalho), whereas the Peru source is *Eccritotarsus eichhorniae* Henry. Our objectives in this laboratory study were to assess the mate choice in the two species and, thus, determine which species performs better than the other. Mate choice in the form of no-choice, bichoice, and multichoice tests were conducted within and between species in a 3:1, 2:1, and 1:1 sex ratios. The *E. catarinensis* pair had short copula latency but long copula duration in multichoice experiments, whereas the *E. eichhorniae* pair had short copula latency but long copula duration in no-choice experiments. In no-choice and bichoice experiments, *E. eichhorniae*♀ and *E. eichhorniae*♂ outcompeted *E. catarinensis*♀ and *E. catarinensis*♂. In multichoice experiments, *E. catarinensis*♂ outcompeted *E. eichhorniae*♂, whereas *E. eichhorniae*♀ outcompeted *E. catarinensis*♀. Despite being shown to be more fecund, *E. eichhorniae* is likely not to outcompete *E. catarinensis* where the two species co-occur and that *E. catarinensis*♂ and *E. eichhorniae*♀ will outcompete their counterparts. Previous research indicated that when *E. catarinensis*♂ and *E. eichhorniae*♀ are crossed, they produce few offspring, underscoring the need to confirm if the two species will coexist, compete, or displace each other in their natural habitats. Our findings further support that prezygotic reproductive isolation mechanisms led to the speciation of these species.

Key Words biological control, water hyacinth, mate choice, prezygotic reproductive isolation mechanisms, copula latency and duration

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Biological control as the most suitable method for controlling floating aquatic weeds has proven very successful in South Africa (Coetzee et al. 2011). For example, red water fern (*Azolla filiculoides* Lamarck), water lettuce (*Pistia stratiotes* L.), and salvinia (*Salvinia molesta* Mitchell) currently have minimal effects on aquatic ecosystems (Hill and Coetzee 2008). In some aquatic systems, water hyacinth, *Pontederia (Eichhornia) crassipes* (Martius) Solms-Laubach (Pontederiaceae), is under complete control (Hill and Coetzee 2017). However, the success rate of water hyacinth is considered to be substantial as it tends to be limited under eutrophic conditions and in cooler areas of the country that receive winter frost (Coetzee et al. 2007a). This has necessitated the release of additional biological control agents. The success or failure of many programs depends on climatic compatibility, habitat condition, number of agents released, plant phenology, predation, and parasitism.

Water hyacinth is now the most problematic aquatic weed in the world (Coetzee et al. 2009). It is recognized as a noxious weed by the U.S. Department of Agriculture, a class 1 noxious weed in Australia, a prohibited weed in New Zealand, a declared invader in Botswana, and a category 1b weed in South Africa (Coetzee et al. 2017). In many countries, the transportation and exchange of water hyacinth are prohibited. Land users and water authorities are required to control infestations in an appropriate manner (Coetzee et al. 2017). Biological control agents released against this aquatic weed in South Africa include 10 arthropods and 4 pathogens (Mnguni 2019a). Biological control agents responsible for the substantial control of water hyacinth in the country are two weevils, two mirids, a moth, and a mite (Klein 2011). In addition, there are other imported biological control agents, including a planthopper, a grasshopper, and a moth (Coetzee et al. 2011).

Two mirids, namely, *Eccritotarsus* spp. (Hemiptera: Miridae), sourced from Brazil and Peru are morphologically similar, but Paterson et al. (2016) and Henry (2017) found slight differences between them. They measure 2–3 mm in length and are primarily black in color. They feed on the palisade parenchyma of water hyacinth (Hill et al. 1999). Both nymphs and adults are able to move quickly (S.M. pers. obs.). Their feeding stunts the growth rate of water hyacinth by causing chlorosis, which leads to reduced photosynthesis and overall biomass of the weed (Coetzee et al. 2007b). The mirids can survive on chlorotic leaves for several weeks under laboratory conditions (Mnguni 2019a), and they can live for up to 50 d as adults under natural conditions (Hill et al. 1999).

These biological control agents were collected in their native range in South America from two geographically isolated populations that are separated by a distance of over 3,800 km (Taylor et al. 2011). *Eccritotarsus catarinensis* (Carvhalho) was originally collected from Florianopolis (Santa Catarina) in Brazil in 1994, whereas *Eccritotarsus eichhorniae* Henry was collected from the upper Amazonian basin (near Iquitos in Yarapa River) in Peru in 1999 (Taylor et al. 2011). Prior to their release in South Africa, the Brazil source was subjected to a severe genetic bottleneck event. Therefore, the intention of the second collection from Brazil was to increase the genetic diversity and the efficacy of *E. catarinensis* in over 30 release sites (Paterson et al. 2016). Subsequent releases were conducted, but the establishment of the Peru source is yet to be confirmed (Winston et al. 2014). It was thought that an interbreeding of these two populations would increase the chances of establishment in colder regions (Mnguni 2019b). It was assumed

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that the resulting population from these two species would more easily overcome unforeseen catastrophic events; yet an estimated 44% of all weed biological control agent releases fail to result in establishment (Griffith et al. 2019, Porter et al. 2019).

Temperature affects the biology and behavior of all organisms (Porter et al. 2019). As such, acclimation studies have received more attention recently. The Brazil source is more cold tolerant as it is from a cooler climate. Brazil has a higher latitude of 27°S and offers a subtropical climate with a maximum temperature of 28.2°C, whereas Peru has a lower latitude of 5°S and offers a tropical climate with a maximum temperature of 33°C (Ismail and Brooks 2016). In addition, the Peru source has a higher fecundity and developmental rate above 27°C (Griffith et al. 2019). Prior to their respective releases, both of these sources were subjected to host-specificity tests (Paterson et al. 2016). This was done to screen them and effectively ensure that they do not feed on nontarget plants. This process involved subjecting the insects to water hyacinth and alternate host plant species that belong to the family Pontederiaceae. Hill et al. (1999) reported that only members of Pontederiaceae support any development of the insects. According to Paterson et al. (2019), 4 test plant species from the family Pontederiaceae and 62 other plant species were tested. Both sources from Brazil and Peru were shown to be host specific. This finding meant that they were suitable for release in South Africa since they were shown not to cause any damage to nontarget hosts.

Taylor et al. (2011), who assessed their genetics, found a 5.2% haplotype sequence divergence and 29 fixed differences between the 2 sources, which meant that they were cryptic species. Paterson et al. (2016) found reproductive incompatibilities between them. Ismail and Brooks (2016) found slight differences in their temperature tolerances. Soon after that, Henry (2017) separated the cryptic species by using observed differences in their antennae, scent glands, and genitalia. The redescription of these sources meant that the Brazilian source remained *E. catarinensis* (Carvalho), whereas the Peru source is now *E. eichhorniae* Henry. Furthermore, Ismail and Brooks (2018) found morphological differences and assessed the male mating preferences, and Mnguni (2019b) found a mating incompatibility because *E. eichhorniae*♀ and *E. catarinensis*♂ preferred to mate with their respective conspecifics only. Therefore, this paper assessed laboratory-based mate choices between the two *Eccritotarsus* species under room temperature to investigate field-based interactions between females and males, within and between species. This work was done to investigate which species performs better under laboratory conditions.

Materials and Methods

Insect culture. The two *Eccritotarsus* sources were kept in separate laboratories at Rhodes University in Makhanda (formerly known as Grahamstown), South Africa, 1 km away from each other. *Eccritotarsus catarinensis* (EC) was maintained at the Biological Control Quarantine Facility, and *Eccritotarsus eichhorniae* (EE) was housed at the Waainek Mass Rearing Facility. They were kept at 25± 1°C and 65 ± 5% relative humidity for many generations over 10 yr, under 12:12-h light:dark conditions. Cultures were purposely kept separate from each other to avoid interbreeding. All experiments reported in this study were done in the Centre for

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Biological Control Laboratory at Rhodes University under 23 ± 5°C and 45 ± 20% relative humidity conditions. All 30 replicates for each interbreeding experiment were observed simultaneously for 3 h.

Interbreeding experiments. No-choice, bichoice, and multichoice experiments were conducted. Thirty replicates were conducted for each experiment. All no-choice experiment replicates had pairs as follows: EC♀×EC♂, EE♀×EE♂, EC♀×EE♂, and EE♀×EC♂. All bichoice experiment replicates had tridents as follows: EC♀×EE♀×EC♂, EC♀×EE♀×EE♂, EC♂×EE♂×EC♀, and EC♂×EE♂×EE♀. All multichoice experiment replicates had tetrads as follows: EC♀×EE♀×EC♂×EE♂. Copula latency and copula duration were measured in minutes. Copula duration was recorded as the difference between the start and end of a mating encounter. Copula latency was recorded as the difference between the time the experiment started until the time insects started mating. Mating in this study is defined as a male climbing and staying in contact with a female, forming a V-shape.

The parameters that were measured in the three interbreeding experiments were compared to determine a favorable interbreeding experiment, in which a particular pair, trident, and tetrad outperformed others. Measures also were taken to determine which particular sex performed better than the other in all the three interbreeding experiments. This was done to assess competition between females and males of these two sympatric *Eccritotarsus* species. All the assessments were done within and between species.

Statistical analysis. Statistica software version 13 (Statistica, 2015) was used for all statistical analyses. Data for copula latency and copula duration were checked for normality for all interbreeding experiments. Both copula latency and copula duration did not meet the normality assumptions, and thus, generalized linear modeling using a log linear link ($P < 0.05$) and Kruskal–Wallis test were used to analyze the data, depending on the assumptions. Tukey's honestly significant post hoc and multiple comparisons of mean ranks for all groups tests were then used to test for significant differences between the treatments. The data for the copula latency and copula duration only involved pairs, tridents, and tetrads that successfully mated. Further details of the methods employed are as per Mnguni (2019b).

Results

Copula latency comparisons.

- EC♀×EC♂. When the three interbreeding experiments were compared against each other, overall (generally), this pair had a significantly shorter copula latency in bichoice and the multichoice experiments and had a significantly longer copula latency in no-choice experiments (Wald χ^2 [2] = 128.640, $P < 0.001$). The results in the no-choice experiments were significantly different from those of the bichoice and the multichoice experiments (Fig. 1).
- EE♀×EE♂. When the three interbreeding experiments were compared against each other, overall (generally), this pair showed an opposite trend to the EC♀×EC♂ pair, as it had a significantly shorter copula latency in no-

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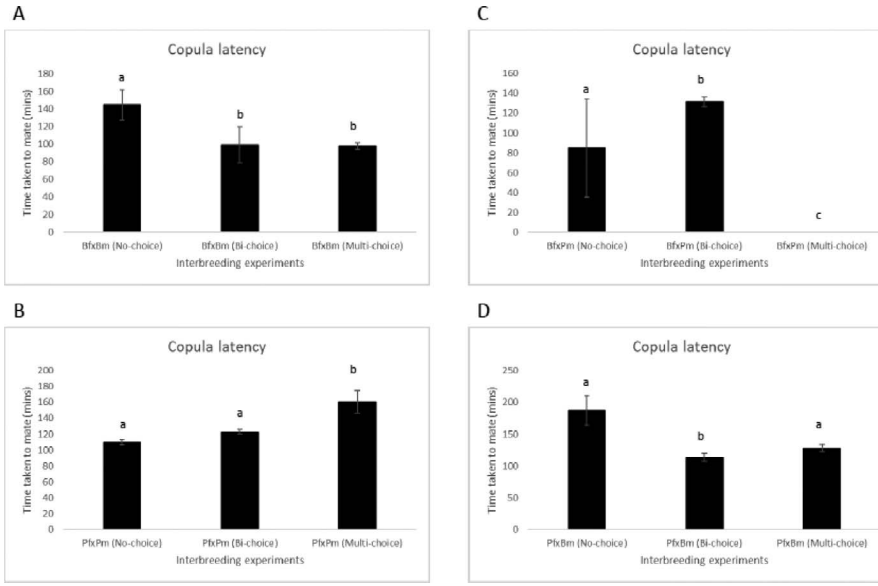

Fig. 1. (a) The *E. catarinensis*$_{\text{female}}$ × *E. catarinensis*$_{\text{male}}$ copula latency (min) in no-choice, bichoice, and multichoice experiments, analyzed using generalized linear model (log-linear link) (Wald χ^2 [2] = 128.640, $P < 0.001$). Error bars = standard error; means followed by the same letter are not significantly different ($P > 0.05$). (b) The *E. eichhorniae*$_{\text{female}}$ × *E. eichhorniae*$_{\text{male}}$ copula latency (min) in no-choice, bichoice, and multichoice experiments, analyzed using generalized linear model (log-linear link) (Wald χ^2 [2] = 173.050, $P < 0.001$). Error bars = standard error; means followed by the same letter are not significantly different ($P > 0.05$). (c) The *E. catarinensis*$_{\text{female}}$ × *E. eichhorniae*$_{\text{male}}$ copula latency (min) in no-choice, bichoice, and multichoice experiments using generalized linear model (log-linear link) (Wald χ^2 [2] = 12E11, $P < 0.001$). Error bars = standard error; means followed by the same letter are not significantly different ($P > 0.05$). (d) The *E. eichhorniae*$_{\text{female}}$ × *E. catarinensis*$_{\text{male}}$ copula latency (min) in no-choice, bichoice, and multichoice experiments using generalized linear model (log-linear link) (Wald χ^2 [2] = 34E11, $P < 0.001$). Error bars = standard error; means followed by the same letter are not significantly different ($P > 0.05$).

choice and bichoice experiments and had a significantly longer copula latency in multichoice experiments (Wald χ^2 [2] = 173.050, $P < 0.001$). The results in the multichoice experiments were significantly different from those of the no-choice and the bichoice experiments (Fig. 1).

- EC♀×EE♂. When the three interbreeding experiments were compared against each other, this pair had a significantly longer copula latency in no-

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choice and bichoice experiments but had a significantly shorter copula latency in the multichoice experiment (Wald χ^2 [2] = 12E11, $P = 0.001$). The results in the three interbreeding experiments were significantly different from each other (Fig. 1).

- EE♀×EC♂. When the three interbreeding experiments were compared against each other, this pair had a significantly longer copula latency in no-choice and multichoice experiments and had a significantly shorter copula latency in bichoice experiments (Wald χ^2 [2] = 34E11, $P < 0.001$). The results in the bichoice experiments were significantly different from those of the no-choice and multichoice experiments (Fig. 1).

Copula duration comparisons.

- EC♀×EC♂. When the three interbreeding experiments were compared against each other, this pair had a significantly shorter copula duration in no-choice and bichoice experiments and had a significantly longer copula duration in multichoice experiments (Wald χ^2 [2] = 493.260, $P < 0.001$). The results in the multichoice experiments were significantly different from those of the no-choice and the bichoice experiments (Fig. 2).
- EE♀×EE♂. When the three interbreeding experiments were compared against each other, this pair had a significantly shorter copula duration in bichoice and multichoice experiments and had a significantly longer copula duration in no-choice experiments (Wald χ^2 [2] = 95.563, $P < 0.001$). The results in no-choice experiments were significantly different from those of the bichoice and multichoice experiments (Fig. 2).
- EC♀×EE♂. When the three interbreeding experiments were compared against each other, this pair had a significantly longer copula duration in no-choice and bichoice experiments and had a significantly shorter copula duration in multichoice experiments (Wald χ^2 [2] = 906E8, $P < 0.001$). The results in the multichoice experiments were significantly different from those of no-choice and bichoice experiments (Fig. 2).
- EE♀×EC♂. When the three interbreeding experiments were compared against each other, this pair had a significantly longer copula duration in no-choice and multichoice experiments and had a significantly shorter copula duration in bichoice experiments (Wald χ^2 [2] = 39E10, $P < 0.001$). The results in bichoice experiments were significantly different from those of no-choice and multichoice experiments (Fig. 2).

Sex comparisons using copula incidences.

- No-choice experiment. Although not significant, concerning females, EE♀ had higher copula incidences than EC♀ (Fig. 3). Likewise, concerning males, EE♂ had higher copula incidences than EC♂ ($H_{3,\ 8} = 0.70$; df = 3; $P = 0.87$).
- Bichoice experiment. Similarly, or consistent with the no-choice experiments reported above, concerning females, EE♀ continued to have higher copula incidences than EC♀ (Fig. 3). Likewise, concerning males, EE♂ continued to have higher copula incidences than EC♂ ($H_{3,\ 12} = 3.19$; df = 3; $P = 0.36$).

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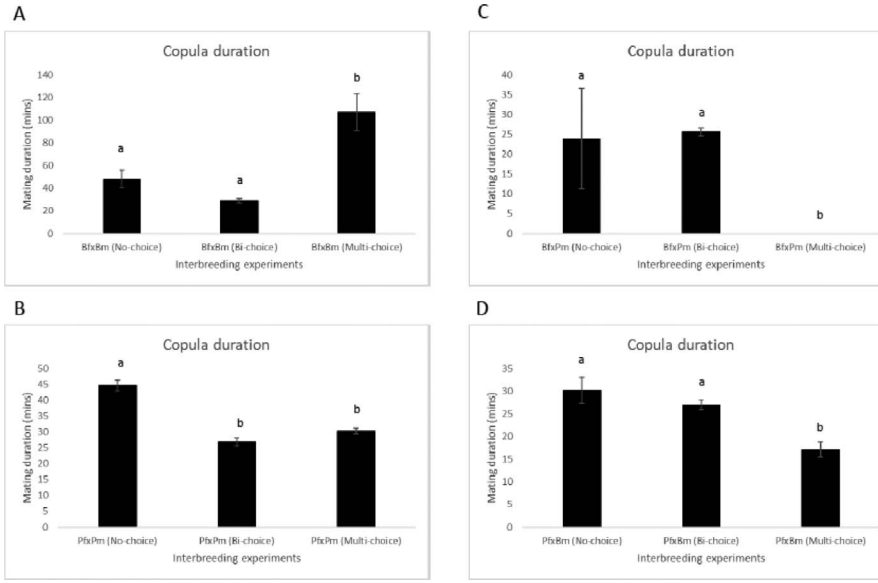

Fig. 2. (a) The *E. catarinensis*$_{\text{female}}$ × *E. catarinensis*$_{\text{male}}$ average total copula duration (min) in no-choice, bichoice, and multichoice experiments, analyzed using generalized linear model (log-linear link) (Wald χ^2 [2] = 493.260, $P < 0.001$). Error bars = standard error; means followed by the same letter are not significantly different ($P > 0.05$). (b) The *E. eichhorniae*$_{\text{female}}$ × *E. eichhorniae*$_{\text{male}}$ average total copula duration (min) in no-choice, bichoice, and multichoice experiments, analyzed using generalized linear model (log-linear link) (Wald χ^2 [2] = 95.563, $P < 0.001$). Error bars = standard error; means followed by the same letter are not significantly different ($P > 0.05$). (c) The *E. catarinensis*$_{\text{female}}$ × *E. eichhorniae*$_{\text{male}}$ average total copula duration (min) in no-choice, bichoice, and multichoice experiments, analyzed using generalized linear model (log-linear link) (Wald χ^2 [2] = 906E8, $P < 0.001$). Error bars = standard error; means followed by the same letter are not significantly different ($P > 0.05$). (d) The *E. eichhorniae*$_{\text{female}}$ × *E. catarinensis*$_{\text{male}}$ average total copula duration (min) in no-choice, bichoice, and multichoice experiments using generalized linear model (log-linear link) (Wald χ^2 [2] = 39E10, $P < 0.001$). Error bars = standard error; means followed by the same letter are not significantly different ($P > 0.05$).

- Multichoice experiment. Consistent with no-choice and bichoice experiments reported above, concerning females, EE♀ continued to have higher copula incidences than EC♀ (Fig. 3). However, concerning males, surprisingly (as this was occurring for the first time), EC♂ had higher copula incidences than EE♂ ($H_{3,\ 8} = 0.70$; df = 3; $P = 0.87$).

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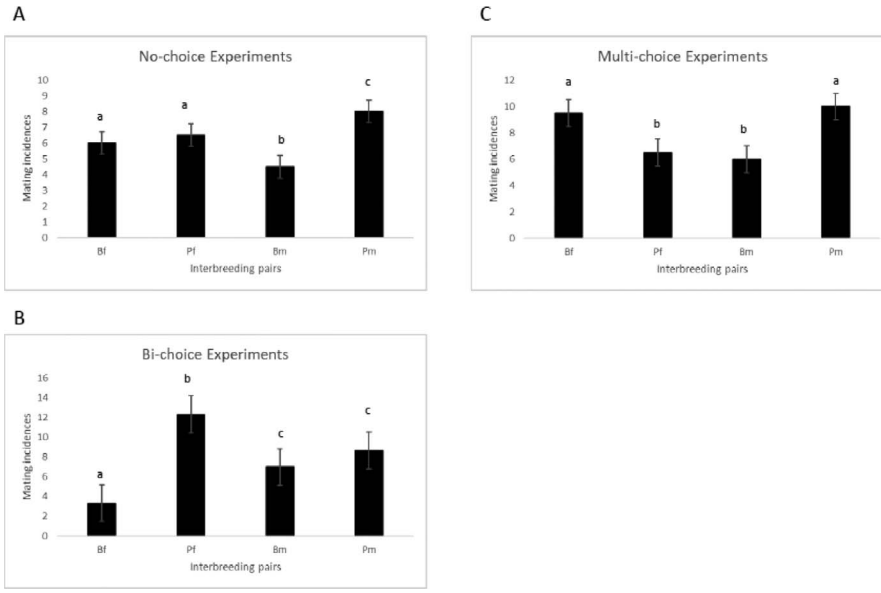

Fig. 3. Female (ECf and EEf) and male (ECm and EEm) copula incidence presence-absence comparisons in no-choice ($H_{3,8} = 0.70$; df = 3; $P = 0.87$) (A), bichoice ($H_{3,12} = 3.19$; df = 3; $P = 0.36$) (B), and multichoice ($H_{3,8} = 0.70$ df = 3 $P = 0.87$) (C) interbreeding experiments compared using a Kruskal-Wallis Test. The final values were achieved by cross-referencing copula incidences within and between species. EC, *E. catarinensis*; EE, *E. eichhorniae*; f, female; m, male. Thirty replicates were done for each interbreeding experiment. Error bars = standard error; means followed by the same letter are not significantly different ($P > 0.05$).

Discussion

Overall, EC♀×EC♂ had a short copula latency but long copula duration in the multichoice experiments, whereas EE♀×EE♂ had a short copula latency but long copula duration in the no-choice experiments. In addition to that result, sex comparisons suggest that in no-choice and bichoice experiments, EE♀ and EE♂ outcompete EC♀ and EC♂ in terms of copula incidences. However, in multichoice experiments, EC♂ suddenly outcompete EE♂ while EE♀ continue to outcompete EC♀ in terms of copula incidences. Notably, in a previous study that used interbreeding experiments, EE♀ and EC♂ were reported to have a mating incompatibility in a form of only mating with their respective conspecific in Mnguni (2019b). Furthermore, the same pair (EE♀ and EC♂) was reported to have a reproductive incompatibility in a form of not producing any offspring in Paterson et al. (2016). Recently, this pair was reported to have the same chemical emissions in Mnguni and Heshula (2021). Their opposite sexes (EC♀ and EE♂) produced very few offspring, but they were shown to be nonviable in Paterson et al. (2016). The

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same pair (EC♀ and EE♂) was shown to have the same chemical emissions, as well as those emissions that their conspecific and the same sex of the other species lack in Mnguni and Heshula (2021). Perhaps the little or no gene flow observed was caused by the large geographic distance that separates the two species.

This study has shown that *E. catarinensis* individuals recognize and mate with each other for a very long time in multichoice experiments, whereas *E. eichhorniae* individuals recognize and mate with each other for a very long time in no-choice experiments. These findings suggests that the mate recognition of the two species differs slightly depending on the conditions of the choice experiment. Multichoice experiments have also shown that *E. catarinensis*♂ and *E. eichhorniae*♀ outcompete their counterparts under laboratory conditions. Because multichoice experiments were done to resemble an ecological setting that is similar to natural conditions in the field, it is likely that *E. catarinensis* will outcompete *E. eichhorniae* under the field conditions. *Eccritotarsus catarinensis* is reported to be less fecund, but it appears that being more fecund only comes into play in the absence of competition, as demonstrated in no-choice experiments. This study supports the findings of Mnguni (2019b) that suggest that mate recognition is important in these *Eccritotarsus* species. This is because Mnguni (2019b) showed that when *E. eichhorniae*♀ and *E. catarinensis*♂ have a choice, they only mate with their respective conspecifics. Interestingly, the same *E. eichhorniae*♀ and *E. catarinensis*♂ pairs are shown to outcompete their counterparts in this study. However, they do not produce any offspring when crossed.

This study further suggests that *E. eichhorniae* (more proactive or less discerning) outcompetes *E. catarinensis* (less proactive or more discerning) in the absence of competition. It is likely that being more discerning results in limited copula incidences under the laboratory conditions, which might not be favorable for *E. catarinensis* under their natural conditions in the field, except in the presence of competition that was demonstrated in the multichoice experiments. Taylor et al. (2011) reported that *E. catarinensis* has less genetic variability and diversity than *E. eichhorniae* (represented by the average within-population Jaccard similarity index), and the authors suggested it would result in fewer copula incidences, as demonstrated by this study. The lack of differences in premating behavioral gestures of the two species, which involved rubbing legs against each other, straightening the antennae, and straightening the wings, suggests that they may not be key in their mate recognition (Mnguni 2019a).

There are very few studies that have assessed mating behavior in mirids. Franco et al. (2011) discussed the importance of a copula duration and how males maximize the transfer of sperm in a monandrous species, *Macrolophus pygmaeus* (Heteroptera: Miridae), and a polyandrous species, *Nesidiocoris tenuis* (Heteroptera: Miridae). The authors found that females of the monandrous species were shy in accepting a male partner, had short copula durations, and received only a small volume of ejaculate; whereas females of the polyandrous species readily accepted any mating partner, had long copula durations, and received a large volume of ejaculate. In the two *Eccritotarsus* species, the volume of ejaculate has not been measured to date, but Paterson et al. (2016), together with Ismail and Brooks (2018), reported that *E. eichhorniae* has more fecundity than *E. catarinensis,* despite having longer copulation in no-choice experiments, in which competition is absent. However, the study reports that the females have longer copulatory

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duration in different choice experiments. For example, *E. catarinensis* had the shortest copula latency but longest copula duration in the multichoice experiment that had a 3:1 sex ratio, whereas *E. eichhorniae* had the shortest copula latency but the longest copula duration in the no-choice experiment that had a 1:1 sex ratio. Therefore, these findings mean that these two *Eccritotarsus* species outcompeted each other in different choice experiments under laboratory conditions.

These results suggest that *E. catarinensis* is more responsive in the presence of competition (where mate recognition is supposedly harder), whereas *E. eichhorniae* is more responsive in the absence of competition (where mate recognition is supposedly easier). This study concurs with Paterson et al. (2016) who predicted that *E. catarinensis* is a better competitor than *E. eichhorniae*. However, this result is likely to be temperature dependent. Thermal studies have recently reported that temperatures below or above the thermal thresholds have the potential to increase the mortality of individuals and/or reduce the performances of the surviving individuals (Griffith et al. 2019). If the two species were to be found together under natural conditions, *E. catarinensis* is likely to mate very quickly but for a very long time, possibly to ensure that females do not see the need to mate again (Franco et al. 2011). This combination would disadvantage other males belonging to the same or another species from copulating with the same female. Because hatching rate and nymphal survival rates decrease with increasing temperature (Ismail and Brooks 2016), in cooler or subtropical regions, *E. catarinensis* is likely to outperform *E. eichhorniae*, and *E. eichhorniae* is likely to do well in hotter or tropical regions instead. Going forward, it would be useful to measure the volume of ejaculate in males of the two species and correlate it with copulatory durations.

It was important to assess competition between the two *Eccritotarsus* species. These results might have implications for the biological control of water hyacinth in South Africa and anywhere else where these insects have been released because the two species will most likely not interbreed with each other and will, therefore, not mix any genetic information with each other as initially intended. More importantly, no viable offspring are produced when the two species are crossed with each other. Thus, the two species will not increase the genetic diversity and variability in the country as it was originally intended (Taylor et al. 2011). Instead, it might place the two species in competition with one another. One species may even be displaced by the other, and this displacement may be temperature dependent; hence, the use of climatically suitable agents is necessary to ensure the success of a control method (Ismail and Brooks 2016, Paterson et al. 2019).

Most notably, *E. eichhorniae* produces more nymphs, has greater genetic diversity and variability (Taylor et al. 2011), and has greater fecundity than *E. catarinensis* (Paterson et al. 2016). These characteristics may be advantageous under natural conditions in the field. Furthermore, Paterson et al. (2019) recently reported that *E. eichhorniae* is more damaging and removes more chlorophyll from water hyacinth leaves than *E. catarinensis* under certain climatic conditions. It also has been recently documented that *E. catarinensis* survives at lower temperatures and has a lower thermal requirement for development than *E. eichhorniae* (Paterson et al. 2019). However, it is possible that maintaining the genetic diversity and variability ensures that *E. catarinensis* individuals can easily recognize each other under natural conditions in the field and will probably reduce their fitness costs since very little energy will need to be invested in mate recognition. We speculate

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that this could possibly allow *E. catarinensis* to focus more on copulation, growth, and development (Gröning and Hochkirch 2008). Nevertheless, up to this point, the prezygotic reproductive isolation mechanisms that are responsible for maintaining speciation between the two species have not been identified.

It appears as though assessment tests may have to be repeated. An additional biological control agent could lead to better control of water hyacinth, particularly in hotter regions, where the new biological control agent could possibly thrive (Ismail and Brooks 2016). An additional biological control agent requires thorough assessments, in an attempt to understand its biology, distribution, and impacts in the country. Additionally, host-specificity tests have already been reconducted by Paterson et al. (2019) to ensure that the new biological control agent still does not feed on nontarget or alternate host plants. Such findings can potentially assist in developing a more effective biological control program for water hyacinth in South Africa. Most importantly, reassessments of the feeding habits of the two *Eccritotarsus* species were performed to ensure that appropriate tests were done in the past. They are still considered safe for release in the country without any detrimental consequences.

Weyl and Hill (2012) discussed how interactions involving two or more species of herbivorous insects are key population abundance and dynamics regulators, as well as how they have been demonstrated to be spatial segregation and distribution drivers. The work was done by investigating interactions between three biological control agents of water hyacinth, namely, *E. catarinensis* (Carvalho), *Neochetina bruchi* Hustache, and *N. eichhorniae* Warner, to measure the impact that pairwise combinations have on performance. They further highlighted the importance of fully understanding the ecology of an insect by using insect–insect interactions and plant–insect interactions. The same authors discussed how the two approaches can have implications for determining the success or failure of biological control programs. Mnguni (2019b) advised that the interactions between the two species and how each species performs at varying temperatures will play an important role in the biological control program against water hyacinth in South Africa and elsewhere in the world where the agents have been released. Ismail et al. (2021) have already attempted to address these aspects.

The postrelease assessments of *E. catarinensis* and *E. eichhorniae* in South Africa are now a necessity in all the sites to determine their prevalence and effectiveness against the weed. Male fitness cost in the two *Eccritotarsus* species also needs to be evaluated because it may have dire consequences in the control of water hyacinth in many regions where both species are present (Mallet 2005). *Eccritotarsus catarinensis* has been successfully established in over 30 out of 33 sites where the biological control agent has been released (Coetzee et al. 2007b), but now the next step is to confirm whether it is *E. catarinensis* or *E. eichhorniae*, or both species. Then the following step would be to understand why a species is present or absent in a particular region. Going forward, knowing why a species is successful or why it is failing in a particular region will be very important. Understanding why *E. catarinensis*♂ outcompetes *E. eichhorniae*♂ only in the multichoice experiment is now also equally warranted. The new findings can be compared with those of other locations outside South Africa where both these *Eccritotarsus* species have been released.

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Reproductively isolated species have been previously confirmed using cross-breeding experiments (e.g., Xu et al. 2010; Liu et al. 2012). Most importantly, moving forward, morphologically similar species need to be reassessed before they threaten the safety record of biological control. Griffith et al. (2019) have stressed the need to exploit the thermal plasticity and the microevolutionary changes of all the biological control agents to maximize their impact on all invasive alien weeds. Nevertheless, it is important to always be cognizant of the fact that the extrapolation of laboratory-based results into natural conditions in the field needs to be done with caution, although the studies may be designed to resemble each other.

In conclusion, our results show that *E. catarinensis* mates for a very long time in multichoice experiments, whereas *E. eichhorniae* mates for a very long time in no-choice experiments. Females and males of *E. eichhorniae* mate more in no-choice and bichoice experiments. Females of *E. eichhorniae* and males of *E. catarinensis* mate more in multichoice experiments. It is not yet clear whether the two sympatric *Eccritotarsus* species will co-occur, compete, or displace one another in natural conditions in the field. The probable mating competition between the two species may be temperature dependent. In terms of climatic suitability, it is highly possible that *E. catarinensis* will flourish in cooler regions and *E. eichhorniae* will flourish in hotter regions. Cold hardening in the laboratory can be used prior to release to condition the agents to survive or cope better in winter frosts. However, what is clear so far is that there are prezygotic reproductive isolation barriers present in these two geographically isolated *Eccritotarsus* species. Speciation has occurred, but the speciation has resulted in very little morphological change. The changes that have resulted in prezygotic reproductive isolation mechanisms in the two species have been uncovered by physical, behavioral, or chemical studies. More laboratory and field studies are needed to confirm whether the two sympatric *Eccritotarsus* species will coexist, compete, or displace each another under natural conditions in the country, as well as anywhere else in the world where they have been released to control water hyacinth.

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Chemical Emission of Two *Eccritotarsus* Species (Hemiptera: Miridae) Released as Biological Control Agents of Water Hyacinth[1]

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Abstract In the family Miridae (Hemiptera), females and males attract each other by means of sex pheromones. Among insects, these pheromones are characterized by a variety of chemical structures, including saturated and unsaturated, long- and short-chain esters, as well as unsaturated ketoaldehydes. The aim of this study was to assess the chemical emissions in *Eccritotarsus catarinensis* (Carvalho) and *Eccritotarsus eichhorniae* Henry to determine their similarity and their possible role in reproductive isolation mechanisms that led to speciation. Chemicals emitted by adults inserted in air-entrainment chambers were collected in absorbent tubes and were analyzed using gas chromatography–mass spectrometry (GC-MS). Results from the GC-MS library indicate that *E. catarinensis* females and *E. eichhorniae* males have chemical emissions that their conspecific and the same sex of the other species lack. Also, *E. catarinensis* males lack benzenebutanoic that the other sexes have, while *E. eichhorniae* males have 1,2,3,4-tetrahydro-6-(phenyl methyl) that other sexes lack. Further analysis using statistical approaches (e.g., cluster analysis, multidimensional scaling plot, and principal component ordination) indicated that cross-breeding pairs have similar chemical emissions in that *E. eichhorniae* females had similar chemical emissions to those of *E. catarinensis* males, while *E. catarinensis* females had similar chemical emissions to those of *E. eichhorniae* males. These unique differences in chemical emissions could be caused by the recently identified differences in the metathoracic scent glands and the antennae of the two *Eccritotarsus* species, and they may serve as a basis in explaining the interbreeding and mating incompatibilities reported in these two *Eccritotarsus* species.

Key Words *Eccritotarsus*, chemical emissions, metathoracic scent gland, antennae

The metathoracic scent glands (MSG) of mirids (Hemiptera: Miridae) produce defensive chemical compounds emitted when insects are disturbed or provoked (Zhang and Aldrich 2003b). In addition to defensive compounds, the MSG also release sexual, aggregation, alarm, and dispersal pheromones that function intraspecifically (e.g., Aldrich et al. 1991, Aldrich 1994, 1996, McBrien and Millar

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1999, Millar et al. 1997, Millar and Rice 1998, Smith et al. 1991, Wardle et al. 2003, Zhang and Aldrich 2003b); but they also can be used by various other insects interspecifically as kairomones (e.g., Aldrich and Barros 1995, Eisner et al. 1991, Zhang and Aldrich 2003b). Additionally, Millar et al. (1997) found that chemical compounds also can be emitted from the thorax. Pheromones in Miridae include simple esters and monoterpenes; linear, monocyclic and multicyclic sesquiterpenoids; and novel acetogenins (Millar 2005), while typical defensive compounds include short-chain alcohols, aldehydes, esters, alkenals, alkanes, monoterpenes, and aromatic alcohols and aldehydes (Millar 2005), although these can be interchangeable.

This study investigated chemical emissions of two sympatric species: *Eccritotarsus catarinensis* (Carvalho) and *Eccritotarsus eichhorniae* Henry (Hemiptera: Miridae). These herbivorous bugs were once thought to be morphologically identical. Both measure 2–3 mm and are primarily black with darkly marked, but mostly transparent, wings (Stanley and Julien 1999). Both are leaf-sap–sucking biological control agents of a problematic invasive weed known as water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) (Hill et al. 1999). The nymphs and adults of these species are mobile and feed gregariously underneath water hyacinth leaves, causing chlorosis that leads to an eventual leaf death because of excessive acquisition of chlorophyll from the palisade parenchyma (Hill et al. 1999). Feeding stunts the growth rate of the weed by reducing photosynthesis, and that eventually leads to a reduction in overall biomass of the weed (Coetzee et al. 2007). Their development is about 23 d, with 15 of those in 4 to 5 nymphal instars, after which the adult lifespan is approximately 50 d (Hill et al. 1999). These insects can live for several weeks in suitable laboratory conditions and can persist even when the leaves are subjected to chlorosis (Mnguni 2019a).

Eccritotarsus catarinensis was imported from Florianopolis (Santa Catarina), Brazil in 1994. This collection was subjected to host-specificity testing after which the insect was released throughout South Africa in 1996 (Taylor et al. 2011). The Brazilian collection was subjected to a severe genetic bottleneck event after importation into quarantine, and it was later shown to be unable to cope with cold winter temperatures, so resampling was subsequently conducted in South America (Paterson et al. 2016). The recently described *E. eichhorniae* was then imported from Yarapa River near Iquitos, Peru, in 1999, and was introduced in South Africa in 2007, after having gone through host-specificity testing that deemed it suitable for release (Paterson et al. 2016). Initially, it was thought that these were two introductions of the same species. A few years later, an interbreeding of the two populations of the same species occurred. The redescription of *E. eichhorniae* was justified by findings reported in the genetic analysis conducted by Taylor et al. (2011) as well as the subsequent behavioral experiments reported in Paterson et al. (2016), which are briefly discussed below. Therefore, Henry (2017) rightfully redescribed the two species into separate *E. catarinensis* (Carvalho) and *E. eichhorniae* Henry.

Taylor et al. (2011) found a significant 5.2% haplotype sequence divergence, infertile hybrids, and 29 fixed differences. Paterson et al. (2016) found an interbreeding incompatibility since *E. eichhorniae* females × *E. catarinensis* males did not produce any offspring while *E. catarinensis* females × *E. eichhorniae* males produced nonviable offspring. Ismail and Brooks (2016) found differences in fitness

traits caused by differences in thermal limits, while Henry (2017) found slight morphological differences in the genitalia, MSG, and antennae that are known to store, emit, and receive chemicals. Ismail and Brooks (2018) found morphological differences and assessed male mating preferences while Mnguni (2019b) found a mating incompatibility since *E. eichhorniae* females and *E. catarinensis* males preferred to mate with their respective conspecifics only. Nevertheless, the most relevant finding for this chemical emission assessment work is that *E. eichhorniae* has a shorter antennal segment II than *E. catarinensis* (Henry 2017).

However, at the time, the intention of the second collection of the same agent from a different population was to increase genetic diversity and variability in the hope that this would increase the efficacy of the biocontrol agent in the country (Paterson et al. 2016). It also was thought that interbreeding and, therefore, hybridization of these two populations, would increase the chances of establishment in colder regions in South Africa. The underlying assumption was that the resulting population from these two interbreeding populations would yield a more resistant, resilient, efficient, and effective biological control agent that would easily overcome unforeseen catastrophic events (Mnguni 2019b, Paterson et al. 2016, Taylor et al. 2011).

The differences in the MSG and the antennae of these two mirids could be a key factor concerning their chemical emissions. They may enable or disable one species from detecting a chemical emission from a bouquet of pheromones released in an ecosystem. They may also change the functional groups of some of the chemical emissions, causing these two species to have one or more different chemical emissions. They may also cause a unique emission that is shared by the two species to be emitted in different ratios, proportions, or relative abundances. Therefore, the aim of the study was to assess the chemical emissions in the two species to investigate whether or not they are identical, and if they can explain the behavioral patterns reported in the mating studies that have been briefly discussed above. We tested the hypothesis that the chemical emissions of the two *Eccritotarsus* species are different. Also, knowing the chemical emissions of the two species is the first step towards identifying major emissions, which could possibly be sex pheromones. Furthermore, this first step will help in identifying possible sexual, aggregation, kairomones, alarm, and dispersal pheromones.

Materials and Methods

Insect culture. The two species of *Eccritotarsus* were kept in the Department of Zoology and Entomology at Rhodes University in Grahamstown. *Eccritotarsus catarinensis* was maintained at the Biological Control Quarantine Facility, while *E. eichhorniae* was housed at the Waainek Mass Rearing Facility, approximately 1 km away from the quarantine facility. Both species were maintained at 25 $\pm$ 1°C and 65 $\pm$ 5% relative humidity (RH) for many generations over 10 yr, under 12:12-h light:dark conditions. The cultures were kept separate from each other to avoid interbreeding between the two species. The invasive alien plant, water hyacinth, was provided as food and was constantly changed to consistently provide optimum nutrition for the mirids.

Insect collection and maintenance. Mirids were collected as third- to fifth-instar nymphs from the respective *E. catarinensis* and *E. eichhorniae* cultures using an aspirator. Forty petri dishes (90 mm, Munktell, Lasec, Ahlstrom, Fisher Scientific, Waltham, PA) lined with filter paper, with one nymph in each, were prepared for both species. Water hyacinth leaves were placed into the petri dishes. Humidity in the petri dishes was maintained by adding distilled water using a 3-ml Pasteur pipette. Petri dishes were then inserted into plastic wrappers to increase humidity and placed under a 12:12-h light:dark fluorescent growing light conditions. The experiment was conducted at 23 ± 5°C and 45 ± 20% RH.

When nymphs had developed into adults, the insects were individually removed from the petri dish using a small paintbrush and viewed either ventrally or laterally to observe their genitalia. The genitalia identification followed Hill et al. (1999). Nymphs were placed individually to ensure that all adults used for the experiment had not mated. Each petri dish was labelled with the species and sex of the individual contained within. When sufficient numbers of adult insects were available, air-entrainment chamber experiments were conducted.

Air-entrainment chambers. The entire air-entrainment system was sterilized by thoroughly washing using distilled water and ethanol. All components were then dried in an oven at 30°C for 12 h. Emissions by adults were collected by placing insects in air-entrainment chambers and using Tenax sorbent tubes (SKC Inc., Eighty Four, PA) to trap the emissions. The test organisms were females and males from both *Eccritotarsus* species. Throughout the experiment, air flowed through Teflon tubes (ClearAir Engineering Inc., Chicago, IL). A pump (Sonic Silent Power 108, Sonic Aquarium Air Pump, Zhenhua Electric, Zhejiang, China) was used to generate air throughout the system. Charcoal-filtered air passed through 500 ml of distilled water. The air then passed through the air-entrainment chamber, which had openings on either side. Air entering and existing the air-entrainment chambers was adjusted at 35 ± 5 L/h using a flowmeter. Insects were then placed inside the air-entrainment chamber. A Tenax sorbent tube was placed in the opening, where air was exiting, to trap all the insect emissions that had been placed inside the chamber.

When emissions were trapped from test organisms into the Tenax tube, the Tenax tube was quickly sealed and stored in a refrigerator at −20°C. Five replicates were conducted concurrently for both female and males of *E. catarinensis* and *E. eichhorniae* species. Each replicate had 10 insects placed in the air-entrainment chamber. All replicates ran for 12 h. All these experiments were conducted from 6 a.m. to 5 p.m. Only 2- to 3-d-old adults were used throughout the experiment. In *Eccritotarsus*, adults need only 2 d to be considered to be sexually mature (Ismail and Brooks 2016).

Chromatographic separation. Tenax tubes used in the volatile chamber experiment were transported to the Central Analytical Facility (CAF) at Stellenbosch University. Analysis of volatile compounds was performed on a Thermo Scientific TRACE^{TM} 1300 gas chromatograph coupled to a TSQ 8000 Mass Spectrometer detector. Empty Tenax tubes were analyzed as controls to distinguish between contaminants and insect emissions. Peaks 9.06, 10.80, 12.19, 13.41, and 17.94 were shown to be siloxanes (breakthrough material from the Tenax), and these were treated as contaminants. As such, these peaks had to be ignored when interpreting chemical emissions of insects. The analysis and the separation of the

chemicals was performed on a nonpolar ZB-MultiResidue-1 instrument (30 m, 0.25-mm inner diameter, 0.25-μm film thickness; part number 7HG-G016-11). The initial oven temperature was adjusted to 40°C, held for 1 min, and finally adjusted to 250°C at 15°C/min, and held for 5 min. The injector temperature was maintained at 250°C. The injection was split-less, and the carrier gas was helium. The transfer line temperature was held at 300°C. The ionization source temperature was set at 250°C.

Statistical analysis. The widely used gas chromatography–mass spectrometry (GC-MS) data were received from the Stellenbosch University CAF as chromatogram graphs. The graphs were received with slightly different retention times as it was well established that peaks were only being recorded after 5 min (Lucky Mokoena, Stellenbosch Univ., pers. comm.).

All the peaks were given with relative abundances that ranged between 0 and 100. As such, all the measures were equated to percentages for females and males of both species. Names of the peaks, adopted from the GC-MS library, are listed in Table 1. Presence and absence analyses were conducted using the chromatograms (Table 2). Since five replicates were conducted for both females and males of both species, each peak had five different measurements. These measurements were summed to calculate the mean and the standard error (SE) for all the peaks (Table 3).

Data from both *Eccritotarsus* species (mean ± SE) were log transformed using log $(X + 1)$ to fit the assumption of homogeneity. Generalized linear modelling was used to test for significant differences among the treatments using Statistica software (version 13). The log-transformed data also were subjected to cluster analysis (CA), multidimensional scaling (MDS), and principal component ordination (PCO) graphs using PRIMER 6.1.8 and PERMANOVA+.

Results

In both species of *Eccritotarsus,* females and males share chemical compounds: propionic acid; 5-t-butyl-1,2,3-trimethylbenzene; 1-methyl-1,3-propanediyl; n-ethyl-2-methyl; 1,2,3,4-tetrahydro-1-phenyl; and n-ethyl-4-methyl (Fig. 1; Table 3). There is no chemical compound unique to either *E. catarinensis* or *E. eichhorniae* females. Males from *E. catarinensis* lack benzenebutanoic acid that other sexes have, while males from *E. eichhorniae* have 1,2,3,4-tetrahydro-6-(phenyl methyl) that other sexes lack.

When comparing within species, *E. catarinensis* females have chemical compounds benzenebutanoic acid; phenol (4-cyclohexyl); 1-methyl-1-propene-1,3-diyl; and 1,1,3-trimethyl-3-phenyl that *E. catarinensis* males lack. On the other hand, *E. eichhorniae* females have chemical compounds phenol (4-cyclohexyl); 1-methyl-1-propene-1,3-diyl; 1,2,3,4-tetrahydro-6-(phenyl methyl); and 2-pentene-1,5-diyl that *E. eichhorniae* males lack.

When comparing the species, *E. eichhorniae* males have 1,2,3,4-tetrahydro-6-(phenyl methyl) that *E. catarinensis* females lack, while *E .eichhorniae* females have benzenebutanoic acid that *E. catarinensis* males lack. When comparing the sexes between species, *E. catarinensis* males have chemical compounds phenol (4-cyclohexyl); 1-methyl-1-propene-1,3-diyl; and 2-pentene-1,5-diyl that *E. eichhor-*

Table 1. Chemical emissions of *Eccritotarsus* spp. (Hemiptera: Miridae) as denoted by the retention time (in minutes) of both females and males, for both *E. catarinensis* (Carvalho) and *E. eichhorniae* (Henry) in the gas chromatographs.

Retention Time (min)	Chemical Compound Name
9.06; 10.80; 12.19; 13.41 & 17.94	Siloxanes (breakthrough material from the Tenax); ignored
12.89	2-Phenylpropenal
14.89	Benzenebutanoic acid (methyl ester) (ç-methyl-)
15.65–15.66	3-(5,6,7,8-Tetrahydro-1-naphtyl)-propionic acid
16.58	5-t-Butyl-1,2,3-trimethylbenzene
16.66	3-(5,6,7,8-Tetrahydro-1-naphtyl)-propionic acid
17.00	Phenol (2-cyclohexyl)
17.01–17.01	Phenol (4-cyclohexyl)
17.05–17.06	Benzene: 1,1′-(1-methyl-1,3-propanediyl)-bis-
17.12–17.12	Benzene: 1,1′-(1-methyl-1-propene-1,3-diyl)-bis-
17.15–17.16	Naphthalene: 1,2,3,4-tetrahydro-6-(phenyl methyl)-
17.28–17.28	1H Indene: 2,3-dihydro (1,1,3-trimethyl-3-phenyl)
17.55	Benzene: 1,1′-(2-pentene-1,5-diyl)-bis-
17.89–17.90	Benzenesulfonamide (n-ethyl-2-methyl)
17.96–17.97	Sclareoloxide
18.19–18.20	Naphthalene: 1,2,3,4-tetrahydro-1-phenyl
18.37	Benzenesulfonamide (n-ethyl-4-methyl)

niae females lack, while *E. eichhorniae* males have chemical compounds benzenebutanoic acid; phenol (4-cyclohexyl); 1-methyl-1-propene-1,3-diyl; 1,2,3,4-tetrahydro-6-(phenyl methyl); and 1,1,3-trimethyl-3-phenyl that *E. catarinensis* males lack.

In summary, GC-MS results suggest that *E. catarinensis* females and *E. eichhorniae* males have chemical emissions that their conspecifics and the same sex of the other species lack. These differences could be caused by the differences in the MSG, thorax, and antennae of the insects that were reported by Henry (2017). More importantly, when this pair was crossed in an interbreeding experiment, they produced very few offspring (Paterson et al. 2016). Furthermore, these results may explain why *E. echhorniae* males and *E. catarinensis* females preferred to mate only with their respective conspecifics (Mnguni 2019b).

Table 2. Gas chromatography–mass spectrometry (GCMS) presence-absence of *Eccritotarsus* spp. (Hemiptera: Miridae) chemical emissions. The left column represents five replicates of each sex for both *Eccritotarsus* species. Each replicate had 10 females and 10 males placed in separate air entrainment collecting chamber. The top column represents the observed peaks based on retention times (mins). The presence of the peak was represented by (I) while the absence was represented by (0). Symbols: EC stands for *E. catarinensis*, EE for *E. eichhorniae*, f for females, and m for males ($n = 5$).

Retention Time (min) of Peaks Denoting Chemical Compounds of Mirids Using GCMS Analysis Excluding Siloxanes																
Sex	**12.89**	**14.89**	**15.66**	**16.58**	**16.66**	**17.00**	**17.01**	**17.06**	**17.12**	**17.16**	**17.28**	**17.55**	**17.89**	**17.97**	**18.20**	**18.37**
ECf1	0	I	I	I	I	0	I	I	I	0	I	I	I	I	I	I
ECf2	0	I	I	I	I	0	I	I	I	0	I	I	I	I	I	I
ECf3	0	I	I	I	I	0	0	I	I	0	I	I	I	I	I	I
ECf4	0	I	I	I	I	0	I	I	I	0	I	I	I	I	I	I
ECf5	0	I	I	I	I	0	I	I	I	0	I	I	I	I	I	I
ECm1	0	0	I	I	I	0	0	I	0	0	0	0	I	0	I	I
ECm2	0	0	I	I	I	0	0	I	0	0	0	I	I	I	I	I
ECm3	0	0	I	I	I	0	0	I	0	0	0	I	I	0	I	I
ECm4	0	0	I	I	I	0	0	I	0	0	0	0	I	I	I	I
ECm5	0	0	I	I	I	0	0	I	0	0	0	I	I	I	I	I
EEf1	0	I	I	I	I	0	0	I	0	0	0	0	I	I	I	I
EEf2	0	I	I	I	I	0	0	I	0	0	I	0	I	I	I	I
EEf3	0	I	I	I	I	0	0	I	0	0	I	0	I	I	I	I
EEf4	0	I	I	I	I	0	0	I	0	0	0	0	I	I	I	I

Table 2. Continued.

Retention Time (min) of Peaks Denoting Chemical Compounds of Mirids Using GCMS Analysis Excluding Siloxanes																
Sex	**12.89**	**14.89**	**15.66**	**16.58**	**16.66**	**17.00**	**17.01**	**17.06**	**17.12**	**17.16**	**17.28**	**17.55**	**17.89**	**17.97**	**18.20**	**18.37**
EEf5	0	I	I	I	I	0	0	I	0	0	0	0	I	I	I	I
EEm1	0	I	I	I	I	0	I	I	I	I	I	I	I	I	I	I
EEm2	0	I	I	I	I	0	I	I	I	I	I	I	I	I	I	I
EEm3	0	I	I	I	I	0	I	I	I	I	I	I	I	I	I	I
EEm4	0	I	I	I	I	0	I	I	I	I	I	I	I	I	I	I
EEm5	0	I	I	I	I	0	I	I	I	I	I	I	I	I	I	I

Table 3. Relative abundances (mean ± SE) of the gas chromatography–mass spectrometry spectra found in the metathoracic scent glands of the two *Eccritotarsus* species.

Peaks[a]	*E. catarinensis* Females	*E. catarinensis* Males	*E. eichhorniae* Females	*E. eichhorniae* Males
12.89	0	0	0	0
14.89	2.4 ± 1.8	0	2	2
15.66	30.4 ± 1.24	22.4 ± 0.82	20.4 ± 0.82	17.2 ± 1.43
16.58	12.8 ± 0.66	28.4 ± 0.44	26.8 ± 0.92	20.4 ± 2.23
16.66	50.8 ± 2.40	99.6 ± 0.18	98.8 ± 0.36	100
17.00	0	0	0	0
17.01	3.2 ± 0.22	0	0	6 ± 0.40
17.06	10.4 ± 0.95	7.6 ± 0.33	5.6 ± 0.18	11.2 ± 0.54
17.12	3.2 ± 0.36	0	0	3.6 ± 0.33
17.16	0	0	0	2
17.28	3.6 ± 0.33	0	2	2
17.55	7.6 ± 0.52	6.8 ± 0.22	0	4.4 ± 0.18
17.89	5.6 ± 0.18	4.4 ± 0.18	4.4 ± 0.18	3.6 ± 0.18
17.97	14 ± 1.65	10.4 ± 0.18	8.4 ± 0.33	7.2 ± 0.36
18.20	36 ± 2.61	27.6 ± 0.72	22.4 ± 0.52	10.8 ± 0.96
18.37	29.6 ± 2.25	36.8 ± 1.34	34 ± 1.44	23.6 ± 1.78

[a] 12.89 = 2-phenylpropenal; 14.89 = benzenebutanoic acid (methyl ester) (ç-methyl-), lacked by *E. catarinensis* males; 15.66 = propionic acid; 16.58 = 5-t-butyl-1,2,3-trimethylbenzene; 16.66 = propionic acid; 17.00 = phenol (2-cyclohexyl); 17.01 = phenol (4-cyclohexyl); 17.06 = 1-methyl-1,3-propanediyl; 17.12 = 1-methyl-1-propene-1,3-diyl; 17.16 = 1,2,3,4-tetrahydro-6-(phenyl methyl), exists only in *E. eichhorniae* males; 17.28 = 1,1,3-trimethyl-3-phenyl; 17.55 = (2-pentene-1,5-diyl; 17.90 = n-ethyl-2-methyl; 17.97 = sclareoloxide; 18.20 = 1,2,3,4-tetrahydro-1-phenyl and 18.37 = n-ethyl-4-methyl. ($\chi^2 = 2.20$, df = 3, $P = 0.532$).

The cluster analysis suggests *E. eichhorniae* males and *E. catarinensis* females have similar chemical emissions inasmuch as *E.catarinensis* males and *E.eichhorniae* females also have similar chemical emissions based on relative abundances. The MDS plot and the PCO also suggest that *E. eichhorniae* females and *E. catarinensis* males have similar chemical emissions that have very little variability inasmuch as *E. catarinensis* females and *E. eichhorniae* males have similar chemical emissions that have very high variability. Also, *E. catarinensis* males and *E. eichhorniae* males, as well as *E. catarinensis* females and *E. eichhorniae* females are at opposite ends of the graph from each other and, therefore, have significantly different chemical emission variations (Fig. 2).

Discussion

Similar to Yasuda and Higuchi (2012), the chemical emissions reported in our study involving the two sympatric species of *Eccritotarsus* helps us better understand the patterns reported in previously discussed genetic, interbreeding, and mating studies. More importantly, the chemical emissions support the observed minimal mating incidences between the two species that have been reported in Mnguni (2019b). They also support the interbreeding incompatibility reported in Paterson et al. (2016). The unique chemical emission differences revealing that *E. catarinensis* females and *E. eichhorniae* males have chemical emissions that their respective conspecifics and the same sex of the other species lack could be important. A few statistical techniques used in this study in a form of a cluster analysis, MDS plot, and PCO have further supported the observation that the cross-breeding pairs, *E. catarinensis* females and *E. eichhorniae* males have similar chemical emissions inasmuch as *E. eichhorniae* females and *E. catarinensis* males also have similar chemical emissions.

The fact that *E. catarinensis* males lack benzenebutanoic acid that other individuals have, while *E. eichhorniae* males have 1,2,3,4-tetrahydro-6-(phenyl methyl) that other individuals lack could also prove to be significant, especially if males attract females in *Eccritotarsus*. The presence or absence of a unique chemical emission could be the barrier that exists between the two species, and they may explain the interbreeding and mating incompatibilities that have somewhat exposed the presence of prezygotic reproductive isolation mechanisms. Nevertheless, the differences in chemical emissions between the two species still need further investigation.

These unique differences could be key in explaining the interbreeding and mating incompatibilities reported in Paterson et al. (2016) and Mnguni (2019b). These differences may have been caused by the subtle morphological differences in the MSG, thorax, and antennae of these two species of *Eccritotarsus* as reported by Henry (2017). The results suggest that, although the chemical emissions of the two species are very similar, there are some consistent differences present between the species, particularly between the sexes, and these could be of evolutionary importance. The quantity and quality of chemical emission assessments as key determinants in reproductive isolation were necessary in *Eccritotarsus* as suggested by Groot et al. (1999), Millar et al. (1997), Millar and Rice (1998), Zhang and Aldrich (2003a), and Yang et al. (2015). Furthermore, it is possible that emissions present in small amounts also could have an important role in the attraction and mate recognition of one sex towards the other. Therefore, an antennal stimulation assessment of these two species is warranted for further study.

In the Miridae, research has shown that it is common for females and males to have very similar or identical chemical emissions within and between species, but the reason for that is not clear yet (Zhang et al. 2015). Some authors have argued that the same emissions that females use to attract males are instead used by males of the same species as defense pheromones, allomones, or by individuals from other species as kairomones (Millar et al. 1997). Although there are slight differences in chemical emissions produced by the two species, the similarities and differences of the functions served by the major compounds remain unknown.

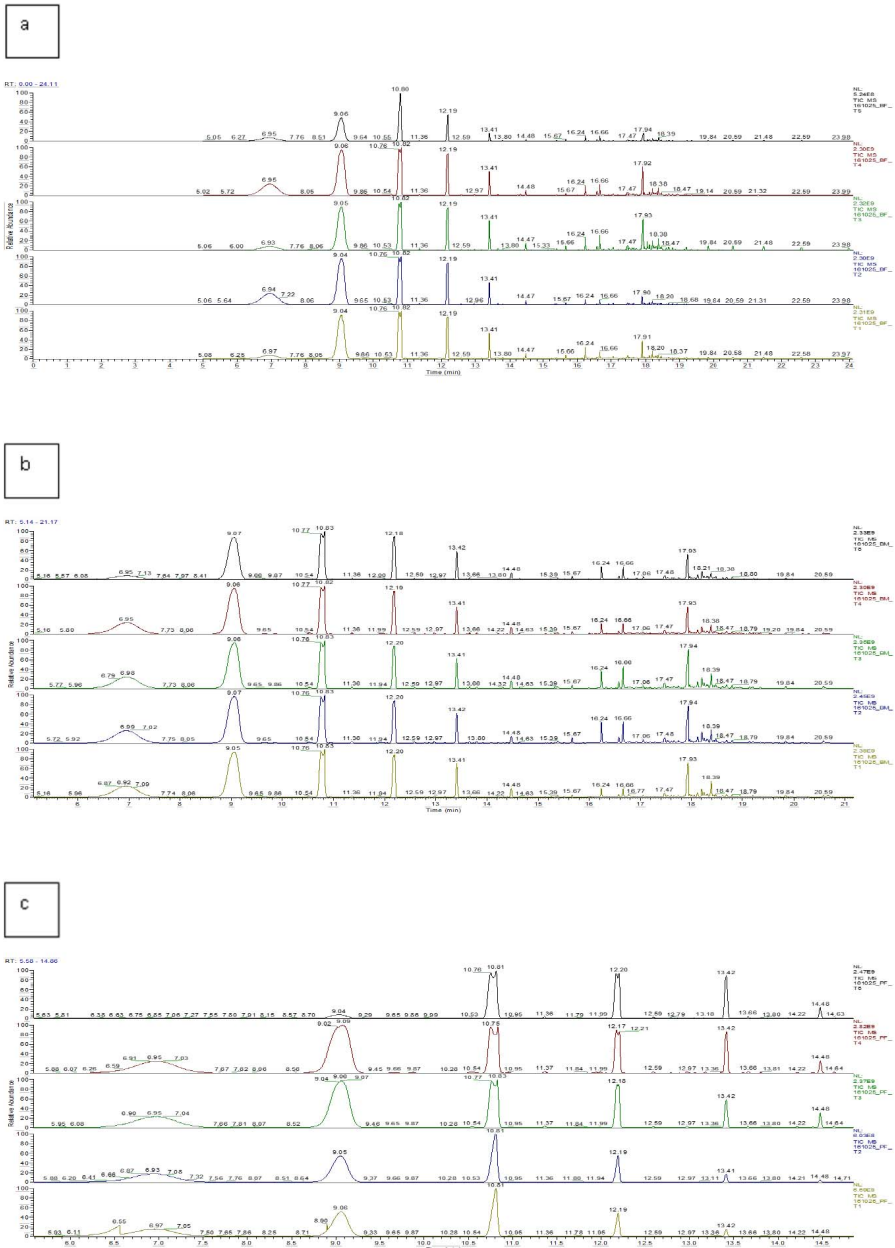

Fig. 1. Gas chromatographs of *Eccritotarsus*: (a) *E. catarinensis* females, (b) *E. catarinensis* males, (c) *E. eichhorniae* females, (d) *E. eichhorniae* males, and (e) single replicates of females and males from both species. The five replicates for both sexes of both species are color-coded as follows: T1 (gold), T2 (navy), T3 (green), T4 (red), and T5 (black); the single replicate is color-coded in this way: *E. catarinensis* females (blue), *E. catarinensis* males (green), *E. eichhorniae* females (red), and *E. eichhorniae* males (black).

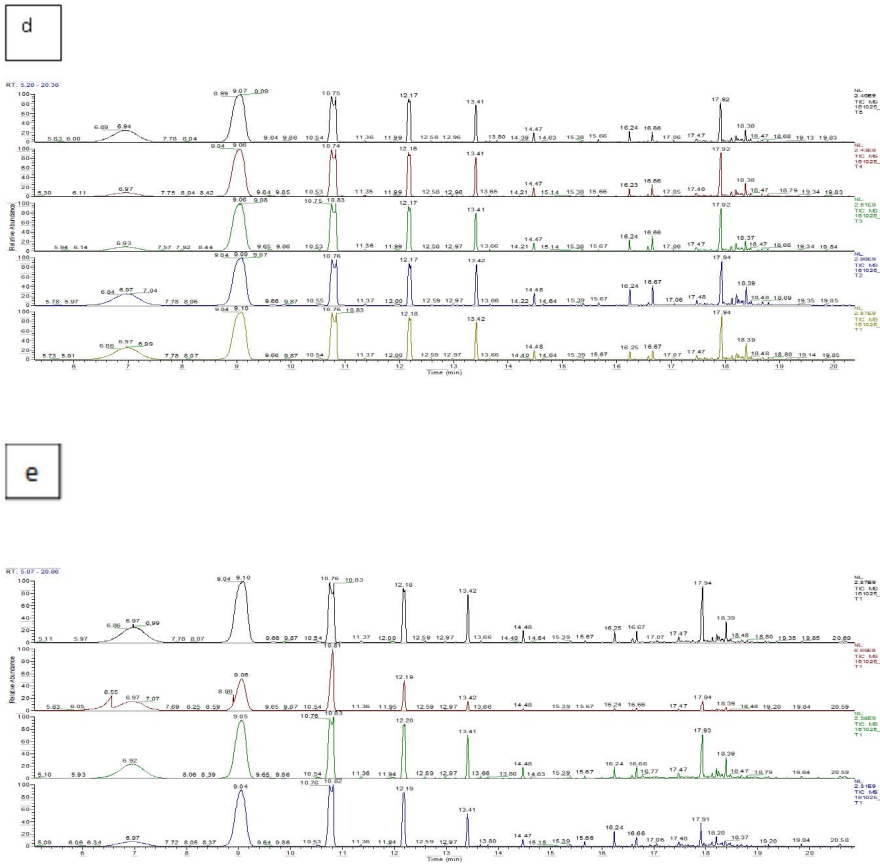

Fig. 1. Continued.

Establishing which sex attracts the other sex in *Eccritotarsus* will be important going forward and is warranted.

Furthermore, establishing the role played by the chemical emissions reported here, individually, binary, and in multiple combinations, would drive towards establishing the major chemical emissions in the two species. From then, it would be ideal to use those major emissions to try to pinpoint specific sex pheromones. Interaction studies will have to be conducted to assess whether one species will affect the fitness of the other species positively, negatively, or neutrally. More pheromone communication investigations are needed to make a better distinction between these two species assessed in this study, and one way could be through an antennal stimulation. Zhang and Aldrich (2008) have further suggested that assessing the specificity of receptor neurons involving the behaviorally and physiologically active chemical compounds using a single-cell recording technique could prove useful.

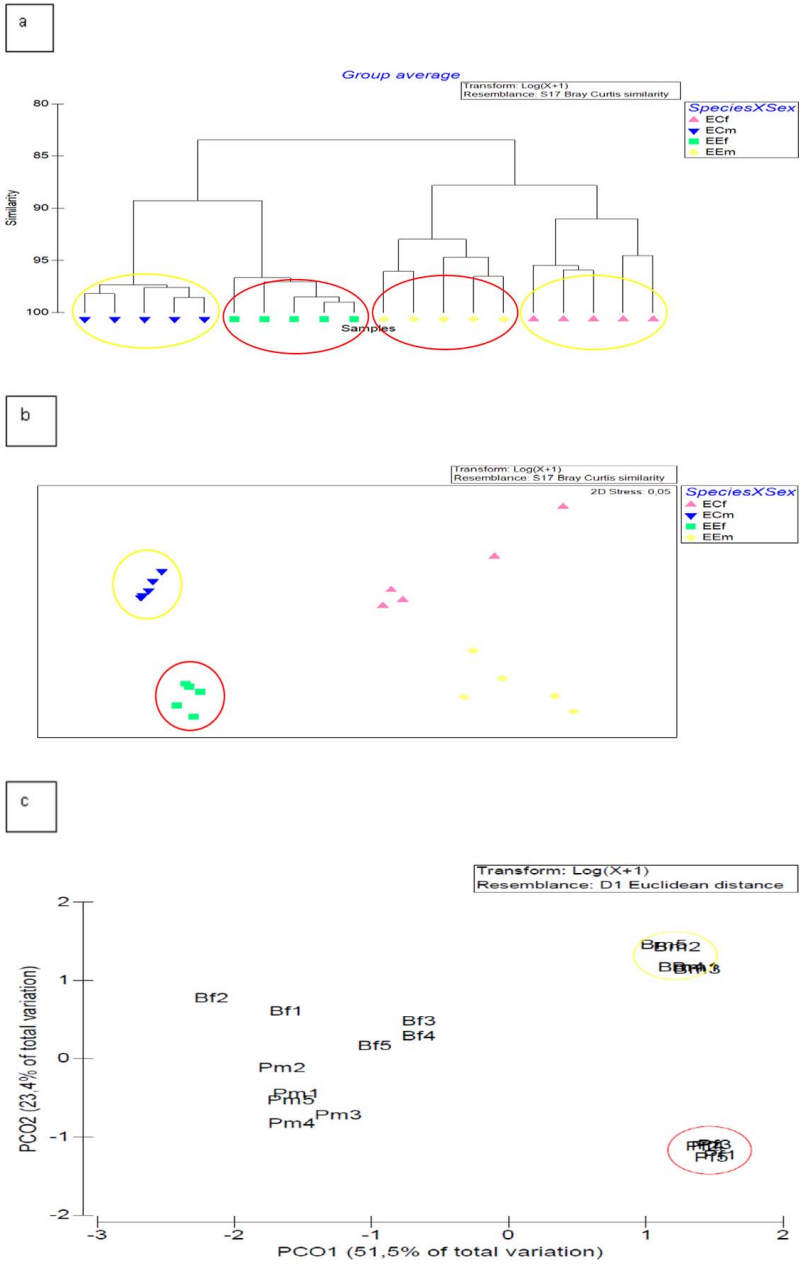

Fig. 2. Graphs showing (a) cluster analysis (CA), (b) multidimensional scaling (MDS) scatterplot (2D stress: 0.06) and (c) principal component ordination (PCO1, 51.5% and PCO2, 23.4% of total variation) using log-transformed (mean ± SE) chemical compound compositions of two *Eccritotarsus* species. EC and B indicate *E. catarinensis*, EE and P

Other aspects worth considering are the sensilla on the antennae, as well as the neurons and chemoreceptors that pick up pheromone blends, as they have also been shown to play a key role in chemical communication (Teal and Tumlinson 1992). Most importantly, investigating how the MSG regulates the production of pheromones between the *Eccritotarsus* species could also give an indication as to what might have caused the reproductive isolation and eventual speciation caused by sexual selection mechanisms. This could prove to be key since the compartmentalized MSG produces many forms of pheromones that serve different functions and could be intertwined, making it difficult to be able to tease apart sex pheromones from other pheromones produced and released for other purposes (Zhang and Aldrich 2008).

The chemical emission assessment of the two species of *Eccritotarsus* consist of saturated or unsaturated esters, as well as unsaturated ketoaldehyde, known to be consistent throughout the Miridae (Yang et al. 2015). However, it will be worthwhile to tweak the functional group of the chemical emissions reported here because a change in the functional group may cause species to react very differently to a pheromone (Schwarz et al. 1990). Schwarz et al. (1990) highlighted the importance of functional groups, to an extent that they may be a key determinant of whether one sex succeeds in attracting the opposite sex or not. Functional groups could also give an indication of the mechanisms that resulted in speciation.

In conclusion, the chemical emissions of *Eccritotarsus* spp. (Hemiptera: Miridae) has shown a sex difference involving the *E. catarinensis* females and the *E. eichhorniae* males, which could be a key mechanism that led to reproductive isolation and the eventual speciation between the two species. The two species share several chemical emissions, but *E. catarinensis* females and *E. eichhorniae* males have chemical emissions that are unique to them, but their conspecifics and the same sex of the other species lack those same emissions. There is also a difference observed in males since *E. catarinensis* males lack an emission that other sexes have, while *E. eichhorniae* males have an emission that other sexes lack. These results may be explaining the variation in mating preferences reported in Ismail and Brooks (2018) and Mnguni (2019b), and the crossbreeding results reported in Paterson et al. (2016). Chemical emissions confirm the presence of prezygotic reproductive isolation mechanisms that possibly led to speciation in the two sympatric *Eccritotarsus* species.

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←

indicate *E. eichhorniae*, f indicates female, and m indicates male. The *E. catarinensis* females were represented by pink, *E. eichhorniae* males were represented by green, *E. catarinensis* females were represented by blue, and *E. eichhorniae* males were represented by yellow ($n = 5$).

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RESEARCH ARTICLE

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Mating preferences of two species of *Eccritotarsus* (Hemiptera: Miridae)

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ABSTRACT

Eccritotarsus spp. are biological control agents that have been sourced from Brazil and Peru to control water hyacinth in South Africa. These agents have been released in over 30 sites and were thought to be the same species. The sequencing of mitochondrial DNA and interbreeding experiments have since confirmed the cryptic species to be separate species. The Brazilian population remains *E. catarinensis* while the Peruvian population is now *E. eichhorniae*. This paper assessed the mating behaviour of both species, to investigate behavioural traits that have resulted in reproductive isolation, which could have led to speciation. Mating choices in a form of no-choice, bi-choice and multi-choice tests were conducted within and between species in a 3:1, 2:1 and 1:1 sex ratio. The *E. eichhorniae* pair had more single and multiple copula incidences, higher average total copula duration and higher copula latency while the *E.catarinensis*♀×*E.eichhorniae*♂ pair had less single and multiple copula incidences, lower average total copula duration and lower copula latency. When the *E.eichhorniae*♀ and *E.catarinensis*♂ were given their respective choices, they only mated with conspecifics. However, when *E.eichhorniae*♀ are crossed with *E.catarinensis*♂ they do not produce offspring. These results suggest that interbreeding will be limited in South Africa.

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Introduction

The herbivorous mirid, *Eccritotarsus catarinensis* (Carvalho) [Hemiptera: Miridae] measures 2–3 mm, is primarily black with darkly marked but mostly transparent wings (Stanley & Julien, 1999), and is a leaf sap-sucking, biocontrol agent of water hyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach [Pontederiaceae]) (Hill, Cilliers, & Neser, 1999). Both nymphs and adults feed gregariously underneath water hyacinth leaves, causing chlorosis, and eventual leaf death because of excessive acquisition of chlorophyll from the palisade parenchyma (Hill et al., 1999). Feeding reduces photosynthesis and overall biomass (Coetzee, Byrne, & Hill, 2007). Its development is about 23 days,

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with 15 being used for undergoing four to five nymphal instars, after which the adult lifespan is approximately 50 days (Hill et al., 1999). The insect can live for several weeks in the laboratory even on chlorotic leaves.

The mirid was originally collected from Florianopolis (Santa Catarina) in Brazil in 1992 (Hill et al., 1999). During host specificity testing in quarantine in South Africa, the population suffered a massive bottleneck due to an air-conditioning malfunction (Hill, personal communication). Only one gravid female survived and subsequent host specificity testing and releases were the progeny of this single female (Taylor, Downie, & Paterson, 2011). Host specificity testing showed that it was suitably host-specific for release in South Africa, so it was released in 1996 and populations established at more than 30 sites in the country (Taylor et al., 2011). However, successful control was variable and limited, possibly due to climatic incompatibility and the lack of genetic diversity within the agent population (Taylor et al., 2011).

A new population of *E. catarinensis* was collected from the upper Amazonian basin (near Iquitos in Yarapa River), in Peru in 1999 (Taylor et al., 2011), a site more than 3500 km from the Brazilian population source (Paterson et al., 2016). Brazil has a higher latitude of 27°S, offers a subtropical climate with a maximum temperature of 28.2°C; whereas Peru has a lower latitude of 5°S, offers a tropical climate with a maximum temperature of 33°C (Ismail & Brooks, 2016). Brazil includes the same range of latitudes that Peru does, and extends further south and north. The intention of this second collection was to increase genetic diversity and the efficacy of *E. catarinensis* in release sites (Paterson et al., 2016). It was also thought that an interbreeding of these two populations would increase the chances of establishment in colder regions. The underlying assumption was that the resulting population from these two interbreeding populations would also more easily overcome unforeseen catastrophic events like population crashes in the future (Paterson et al., 2016), so the population was released in 2007. Four years later, Taylor et al. (2011) assessed the genetic variability of these two populations.

Research has shown that combining behavioural, biochemical and genetic experiments may broaden our understanding of speciation mechanisms (Andersson & Simmons, 2006). This paper assessed the mating behaviour of *E. catarinensis* (Carvalho) and *E. eichhorniae* (Henry), to investigate the hypothesis that there are behavioural traits that have resulted in reproductive isolation, which then led to speciation. The objective was to investigate whether females and males of each species can distinguish between partners from either species, and mate with partners from the opposite species. By assessing both sexes, this work expands on Ismail and Brooks (2018) who showed that *E. eichhorniae* males only mated with their conspecifics.

Materials and methods

Insect culture

Eccritotarsus spp. populations were kept at Rhodes University Department of Zoology and Entomology in Grahamstown. *Eccritotarsus catarinensis* (EC) was maintained at the Biological Control Quarantine Facility, while *Eccritotarsus eichhorniae* (EE) was housed at the Waainek Mass Rearing Facility. These are approximately 1 km apart. Both species were

kept at 25 ± 1°C and 65 ± 5% relative humidity for many generations over 10 years, under 12:12 light vs. dark conditions. Cultures were kept separate from each other to avoid interbreeding between the two species. Both separated species were exposed to the same conditions: enclosed in buckets filled with water hyacinth plants growing in water under ideal nutrient regimes.

The mirids were collected as third to fifth instar nymphs from the respective EC and EE cultures using an aspirator to perform behavioural observations in the form of no-choice, bi-choice and multi-choice interbreeding experiments. Forty Petri-dishes (90 mm, Munktell, Lasec, Ahlstrom) lined with filter paper, with one nymph in each were prepared, for both species. Water hyacinth leaves were placed into the Petri-dishes. Humidity in the Petri-dishes was maintained by adding distilled water using a 3 ml Pasteur pipette. Petri-dishes were then inserted into plastic wrappers to increase humidity, and these were placed under a 12:12 light vs. dark conditions fluorescent growing light. The experiment was conducted in the Rhodes University Biological Control Laboratory, Life Sciences Building, Barratt Square Complex, Rhodes University in Grahamstown, under 23 ± 5°C and 45 ± 20 relative humidity.

When nymphs had developed into adults, insects were individually taken out of the Petri-dish using a small paintbrush and were viewed either ventrally or laterally to observe their genitalia. The genitalia identification followed Hill et al. (1999). Nymphs were placed individually to ensure that all adults used for the respective interbreeding experiments had not mated. Each Petri-dish was labelled with the species and sex of the individual inside.

Behavioural-observations: interbreeding experiment

No-choice interbreeding experiments

Here I assessed the number of copula incidences, presence or absence of multiple copula incidences, duration of copula incidences as well as the copula latency. The behavioural cues assessed for both intra- (within species) and inter-population (between species) were in the form of pairs, triads and tetrads.

Each Petri-dish had half of water hyacinth leaf. The leaves were lightly sprinkled with distilled water. Putting a leaf in the Petri-dish and sprinkling water was very similar for all three breeding experiments. Four Petri-dishes were then inoculated with adults from both species. The combination for each trial was: ECf×ECm, EEf×EEm, ECf×EEm and EEf×ECm. Each replicate of four Petri-dishes was observed simultaneously for 3 h to assess behavioural patterns in the four interbreeding pairs (conducted between 06h00–18h00). The experiment was replicated 30 times. Treatments were used as independent variables, while the number of copula incidences, number of multiple copula incidences, average total copula duration and copula latency were used as dependent variables. Mating in this study was when a male was seen climbing onto the female and staying in contact for a few seconds, in which they would be joined together in copula forming a V-shape. The number of single and multiple copula incidences were counted. A multiple mating incident was when the male separated from the female for some time and then copulation between the same individuals occurred again. For all mating incidences, copula duration was recorded as the difference between the start and end of a mating encounter and this was counted in minutes while the

copula latency was also recorded in minutes, and was the difference from the time the experiment started until the time insects started mating.

Bi-choice and multi-choice interbreeding experiments

The 'bi-choice experiment' had ECf×EEf×ECm, ECf×EEf×EEm, ECm×EEm×ECf, ECm×EEm×EEf while the 'multi-choice experiment' had ECf×EEf×ECm×EEm. Coloured powders were consistently used for identification, blue for ECm, pink for ECf, yellow for EEm and green for EEf. These were also watched for 3 h to assess behavioural patterns in the four interbreeding triads and one interbreeding tetrad (conducted between 06h00–18h00). Both these experiments were also replicated 30 times. Parameters were measured similarly to the 'no-choice experiments' described above. This was done to assess whether competition is going to have an effect on copula incidences and copula duration.

Statistical analysis

Statistica Software version 13 (Statistica, 2015) was used for all statistical analyses for this study. Data for copula latency and average total copula duration were checked for normality for all interbreeding experiments. Both copula latency and average total copula duration did not meet the normality assumptions, thus Generalised Linear Modelling using a Log-Linear Link ($p < .05$) and Kruskal Wallis Test were used to analyse the data, depending on the assumptions. Tukey's HSD *post hoc* and the Multiple Comparisons of Mean Ranks For All Groups were then used to test for significant differences between the treatments. The data for the copula latency and average total copula duration only involved pairs, triads and tetrads that recorded copula incidences in all the interbreeding experiments. Chi-square Tests (50:50 distribution) were used to determine significant differences between single and multiple copula incidences. The tests have been analysed similarly to make interpretation relatively easy.

Results

No-choice interbreeding experiments

Number of copula incidences

Although not significantly higher at the 95% confidence limit (but significant at the 93.3% limit), the highest number of copula incidences was found within species (ECf×ECm & EEf×EEm) than between species (ECf×EEm & EEf×ECm) ($\chi^2 = 6.84$, df = 3, $p = .077$) (Figure 1). Although not significantly higher, the highest number of copula incidences was found in EEf×EEm pair while the lowest number of copula incidences was found in ECf×EEm pair. The ECf×EEm pair which had the lowest number of copula incidences had no multiple copula incidences recorded.

Copula latency

The highest copula latency was found in the EEf×ECm pair and this was significantly higher than other treatments (Wald χ^2 (2) = 74.645, $p < .001$). The ECf×EEm pair, which had the lowest average total copula duration had the lowest copula latency. Pairs that did not mate were excluded from the analysis (Figure 2).

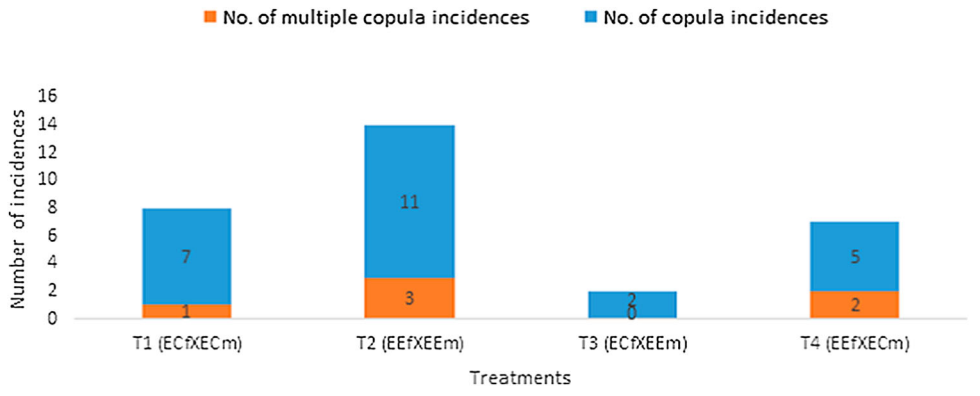

Figure 1. The number of single (in blue) and multiple (in orange) copula incidences of four breeding pairs of each interbreeding treatment under no-choice experiments. EC is *E. catarinensis*, EE is *E. eichhorniae*, f is female and m is male. Thirty replicates were done and used to calculate percentages, and each replicate had four different combinations ($n = 30$). There were significant differences in single ($\chi^2 = 6.84$, df = 3, $p = .077$) but not in multiple ($\chi^2 = 3.33$, df = 3, $p = .343$) copula incidences.

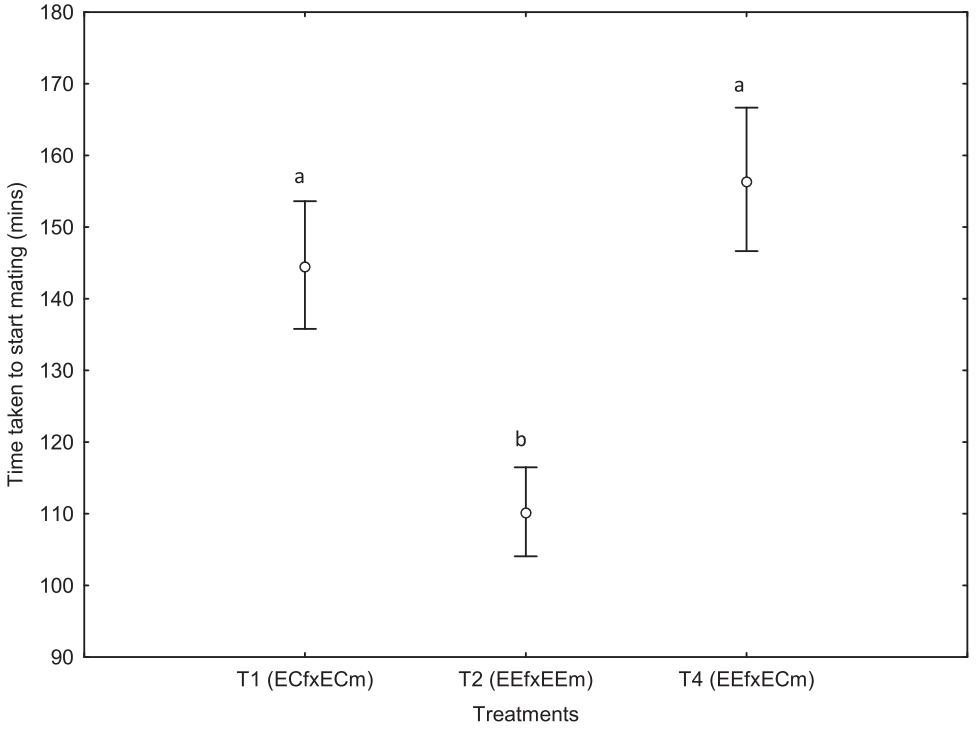

Figure 2. The average copula latency (in mins.) in no-choice experiment using Generalised Linear Model (Log-Linear Link) to test for significant differences. T3 (ECf×EEm) treatment was excluded from the analysis due to having 2 copula incidences. EC is *E. catarinensis*, EE is *E. eichhorniae*, f is female and m is male. Error bars = standard error, and means labelled with the same letter are not significantly different ($p > .05$), (Wald χ^2 (2) = 74.645, $p < 0.001$).

Average total copula duration

The highest average total copula duration was found in ECf×ECm pair and this was significantly higher than other treatments (Wald χ^2 (2) = 47.833, $p < .001$). The ECf×EEm pair had the lowest average total copula duration inasmuch as it also had the lowest single and multiple copula incidences, as well as the lowest copula latency (Figure 3). Pairs that did not mate were excluded from the analysis.

Bi-choice interbreeding experiments

ECf×EEf×ECm: This triad only recorded 2 copula incidences with no multiple copula incidences recorded. The ECm only mated with its conspecific, the ECf on both occasions. As a result, no copula incidences or multiple copula incidences were recorded between EEf×ECm. The high average total copula duration found in this triad in comparison to other triads between ECf×ECm (29 ± 2.12 min) could be because of very few copula incidences recorded overall, and may not be a true reflection of the average total copula duration for this pair. This figure proved to be the highest for all mating pairs found in all four treatments used in this experiment (Table 1).

ECf×EEf×EEm: This triad recorded 15 copula incidences with 3 multiple copula incidences in total. The EEm mated with both ECf and EEf but 13 out of 15 times, it

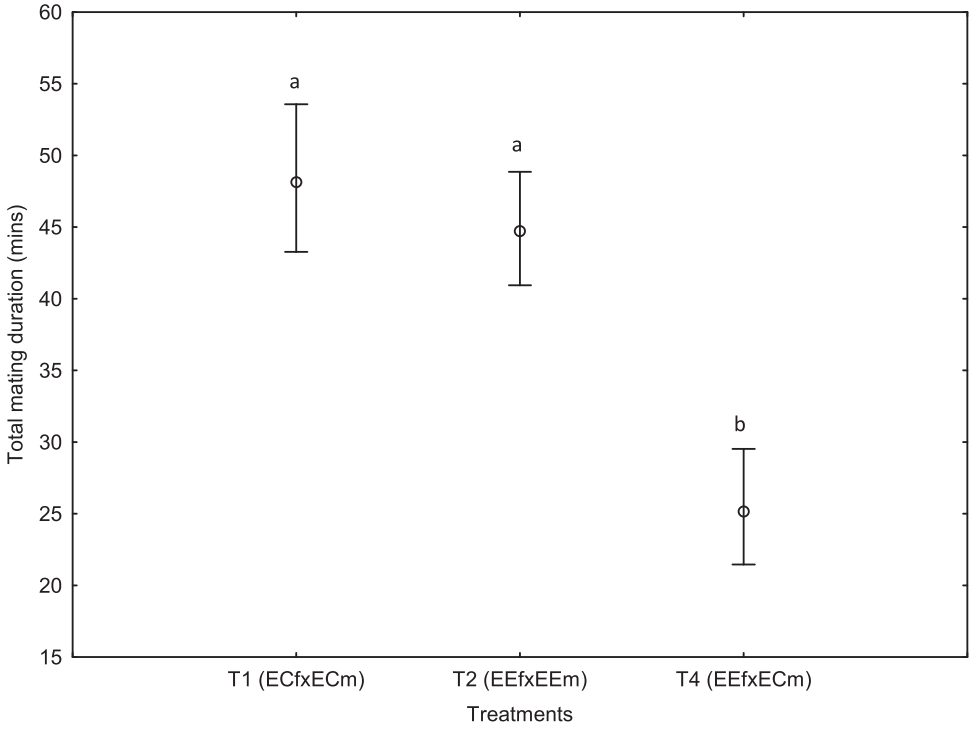

Figure 3. The average total copula duration (in mins.) in no-choice experiment using Generalised Linear Model (Log-Linear Link) for significant differences. T3 (ECf×EEm) treatment was excluded from the analysis due to having 2 copula incidences. EC is *E. catarinensis*, EE is *E. eichhorniae*, f is female and m is male. Error bars = standard error, and means labelled with the same letter are not significantly different ($p > .05$), (Wald χ^2 (2) = 47.833, $p < .001$).

Table 1. The average total copula duration including unmated triads (±SE) and the copula latency including unmated triads (±SE), involving 30 replicates of mated pairs of the mirid *Eccritotarsus* spp.

Trial	Mating pair	Single copula incidence	Multiple copula incidence	Average copula duration (mins)	Average copula latency (mins)
ECf×EEf×ECm	ECm×ECf	2	N/A	29 ± 2.12	99 ± 20.51
	ECm×EEf	0	0	0	0
ECf×EEf×EEm	EEm×EEf	13	3	27.15 ± 1.51	120.46 ± 3.68
	EEm×ECf	2	0	25 ± 10.61	137.5 ± 15.59
ECm×EEm×ECf *	ECf×ECm	8	1	28.75 ± 2.06	87.13 ± 7.37
	ECf×EEm	9	3	20.11 ± 1.28	156 ± 6.20
ECm×EEm×EEf	EEf×EEm	13	5	23.92 ± 1.06	108.46 ± 6.21
	EEf×ECm	0	0	0	0

Notes: (Hemiptera: Miridae) observed for a period of three hours. ECf = *E.catarinensis*♀, EEf = *E.eichhorniae*♀, ECm = *E.catarinensis*♂ and EEm = *E.eichhorniae*♂. The * next to *ECm×EEm×ECf* triad indicates the presence of a single replicate whereby a female mated with both males. Statistical analysis could not be done on these due to low or non-existent copula incidences in 4 of the 8 pairs.

mated with its conspecific, the EEf. Three of those 13 copula incidences recorded between EEf×EEm were multiple copula incidences and this EEm had no multiple copula incidences with the ECf. The EEm had a higher average total copula duration when it mated with its conspecific, the EEf (27.15 ± 1.51 min) compared to when it mated with the ECf (25 ± 10.61 min). It was not possible to do statistics involving the triads due to a low number of cross-copula incidences. Surprisingly, this EEm had a lower copula latency when it mated with its conspecific, the EEf (120 ± 46 min) compared to when it mated with the ECf (137.5 ± 15.59 min) (Table 1).

ECm×EEm×ECf: This triad recorded 17 copula incidences with 5 multiple copula incidences in total. The ECf mated with both ECm and EEm, but 8 out of 17 times it mated with its conspecific, the ECm while surprisingly, 9 out of 17 times it mated with EEm. Only in 1 of those 8 copula incidences recorded between ECf×ECm was a multiple copula incidence while 3 of those 9 copula incidences recorded between ECf×EEm pair were multiple copula incidences. The ECf had less multiple copula incidences with its conspecifics. One particular ECf was also seen mating with both ECm and EEm in this treatment in one replicate only. The ECf had a higher average total copula duration when it mated with its conspecific, the ECm (28.75 ± 2.06 min) compared to when it mated with the EEm (20.11 ± 1.28 min). Interestingly, ECf had a lower copula latency when they mated with their conspecifics, the ECm (87.13 ± 7.37 min) compared to when they mated with the EEm (156 ± 6.20 min) (Table 1).

ECm×EEm×EEf: This triad recorded 13 copula incidences with 5 multiple copula incidences recorded in total. The EEf only opted to mate with its conspecifics, the EEm in all 13 occasions. As a result, no copula incidences or multiple copula incidences were recorded between EEf×ECm. The relatively low average total copula duration found in this triad (in comparison to other intra-population pairs) between EEf×EEm pair (23.92 ± 1.06 min) is likely to be a better reflection due to a higher number of copula incidences recorded. This figure proved to be the lowest for all 8 possible mating pairs found in all four treatments used in this bi-choice interbreeding experiment (Table 1).

In summary, the highest average copula incidence and duration was recorded within species than between species. The EEm showcased a greater probability of recording copula incidences than ECm. The EEm mated more with conspecifics and also mated more relative to ECm when only exposed to the other species. The EEf and ECm opted to mate only with their respective conspecifics, and this could be key in explaining the reproductive isolation in these insects.

Multi-choice interbreeding experiments

Number of copula incidences

Significantly more copula incidences were observed within species (ECf×ECm & EEf×EEm) than between species (ECf×EEm & EEf×EEm) ($\chi^2 = 13.25$, df = 3, $p = .004$). The highest number of copula incidences was found in EEf×EEm and this was significantly higher than other treatments. The lowest number of copula incidences was found in ECf×EEm. The ECf×EEm pair which had no copula incidences also had no multiple copula incidences recorded (Figure 4).

Copula latency

The significantly highest copula latency was observed in the EEf×EEm pair (Wald χ^2 (2) = 187.850, $p < .001$). The ECf×EEm pair had no copula incidences (Figure 5). The pair that had no copula incidences was excluded in the analysis.

Average total copula duration

The significantly highest average total copula duration was observed within species (ECf×ECm & EEf×EEm) than between species (ECf×EEm & EEf×ECm) (Wald χ^2 (2) = 779.220, $p < .001$). The highest average total copula duration was observed in the ECf×ECm pair. The ECf×EEm pair had no average total copula duration as it had no

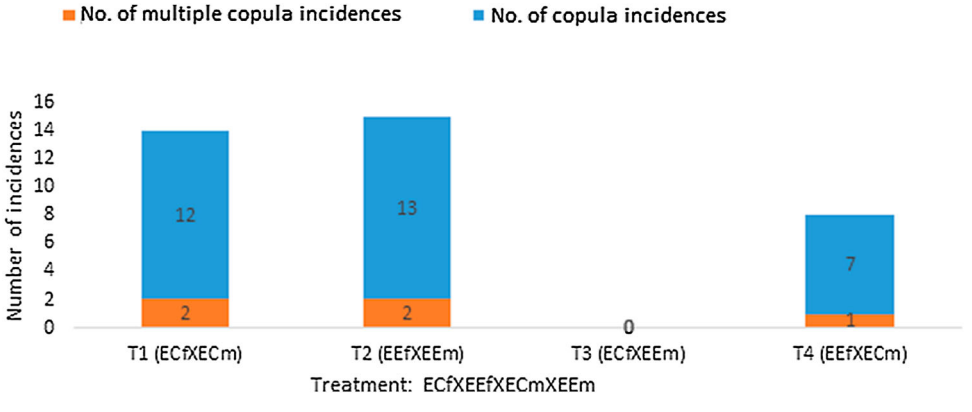

Figure 4. The number of single (in blue) and multiple (in orange) copula incidences of four possible breeding pairs involving both sexes of both populations as a single interbreeding treatment under multi-choice experiments. EC is *E. catarinensis*, EE is *E. eichhorniae*, f is female and m is male. Thirty replicates were done and used to calculate percentages, and each replicate had only one combination as shown above. There were significant differences in single ($\chi^2 = 13.25$, df = 3, $p = .004$) but not in multiple ($\chi^2 = 2.20$, df = 3, $p = .532$) copula incidences.

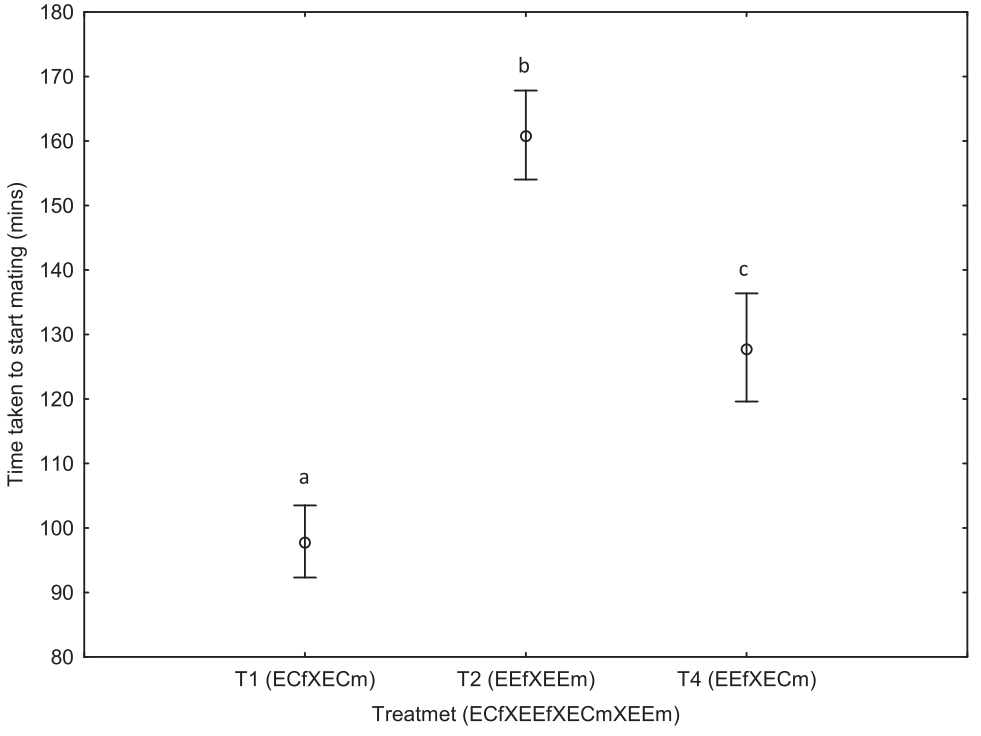

Figure 5. The copula latency (in mins.) in multi-choice experiment, analysed using Generalised Linear Model (Log-Linear Link) for significant differences. T3 (ECf×EEm) treatment was excluded from the analysis due to having no copula incidences. EC is *E. catarinensis*, EE is *E. eichhorniae*, f is female and m is male. Error bars = standard error, and means labelled with the same letter are not significantly different ($p > 0.05$), (Wald χ^2 (2) = 187.850, $p < .001$).

single and multiple copula incidences. The pair that had no copula incidences was excluded in the analysis (Figure 6).

Discussion

The findings of this paper can be linked to that of Taylor et al. (2011) who found 5.2% haplotype sequence divergence and 29 fixed differences, Paterson et al. (2016) who reported that EEf×ECm produced no offspring whatsoever while ECf×EEm produced few offspring and Ismail and Brooks (2018) who reported upon the lack of offspring from eggs resulting from forced hybridisation, and that EEm only mated with their conspecifics. Generally, the outcome of a copula incidence is the transfer of sperm, and that has been shown to have profound effects on female reproductive behaviour (Franco, Jauset, & Casta, 2011). Copula duration affects fecundity and fertility through the act of mating, presence of sperm and transfer of accessory substances (Gröning & Hochkirch, 2008), and a copula incidence is known to carry many ecological costs to females, and these may include general time and energy cost, increased predation rates, physical injury risk and pathogen infections (Watson, Arnqvist, & Stallman, 1998).

Behavioural observations of this study as copula incidences suggest that both *E. catarinensis* and *E. eichhorniae* females demonstrate the ability to choose suitable

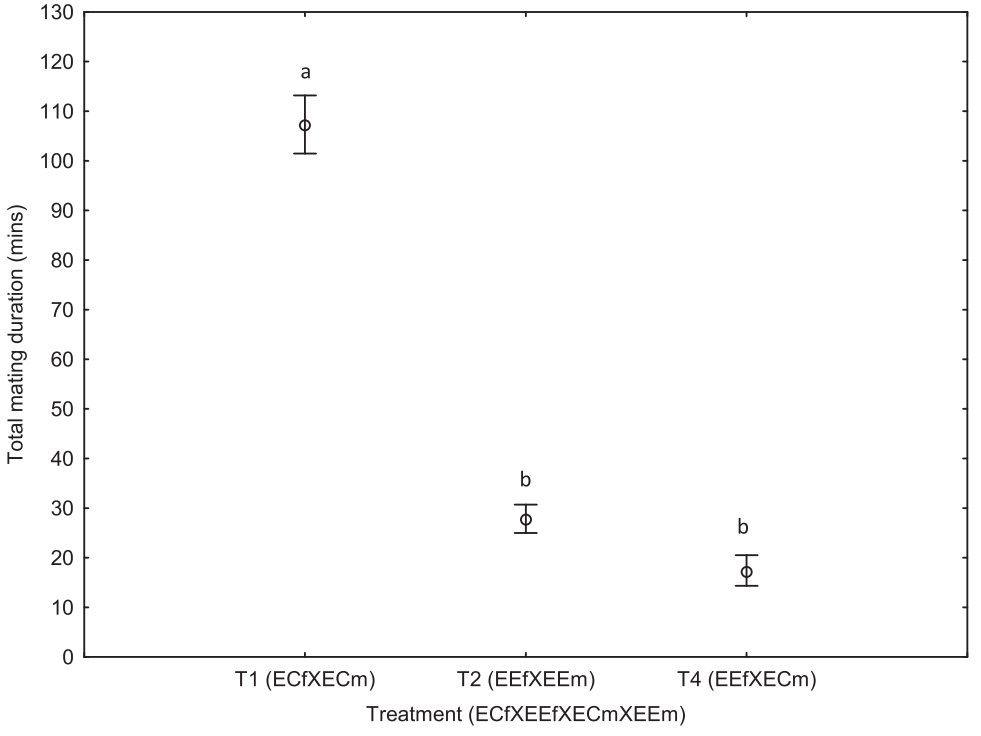

Figure 6. The average total copula duration (in mins.) in multi-choice experiment using Generalised Linear Model (Log-Linear Link) for significant differences. T3 (ECf×EEm) treatment was excluded from the analysis due to having no copula incidences. EC is *E. catarinensis*, EE is *E. eichhorniae*, f is female and m is male. Error bars = standard error, and means labelled with the same letter are not significantly different ($p > .05$), (Wald χ^2 (2) = 779.220, $p < .001$).

males because there were incidences where they rejected males by running away. Tregenza and Wedell (2000) further stipulate that males may be chosen because they have genes which will inevitably confer greater fitness (including mating success) on the female's offspring. This could somewhat explain why few copula incidences were recorded between species in these two species. Females choosing males based on the traits they possess is a well-known phenomenon as it has been reported that females tend to choose the best possible traits available to them as a mechanism that ensures their reproductive success over time (Mays & Hill, 2004).

Assessing the presence or absence of a mechanical precopulatory reproductive isolation barriers was also needed. As a result, Henry (2017) addressed this thorough examination of the male parameres in great detail and found differences in the genitalia, scent glands and antennae of these two species of *Eccritotarsus*. Those appendages are key in mate recognition. For instance, Higgie, Chenoweth, and Blows (2000) reported that high or low genetic diversity often has a role to play towards mate recognition, and this was achieved using experimental sympatry of *Drosophila birchii* (Diptera: Drosophilidae) and field sympatric and allopatric populations of *Drosophila serrate* (Diptera: Drosophilidae) species. They found that reproductive character

displacement (RCD) evolved within nine generations, indicating that there was strong selection on mate recognition. They then concluded that the substantial number of cases involving RCD in field populations in *Drosophila* spp. and in many other taxonomic groups suggests that natural selection on mate recognition may be a major component of the evolution in many species.

Furthermore, there is growing evidence that genetic incompatibility has a role to play in female mate choices, with mate preference potentially tweaking the evolutionary dynamics of sexual selection (Tregenza & Wedell, 2000), and that might be the case with these two species of *Eccritotarsus*. Tregenza and Wedell (2000) highlight that mate choice may be exercised by either sex, and because they invariably invest more in their gametes, females are generally choosier than males, and that females may choose mates on the basis of material benefits such as the quality of the male's territory or the size of a food gift she receives on mating.

The extended copulatory periods recorded in several mirids has often been linked with sperm competition mechanisms that exist between males, and it is a way in which males minimise their intra-sexual and inter-sexual competitions (Franco et al., 2011), while females are less likely to accept another male for copulation. Findings have shown that extended copulatory period results in more ejaculatory transfer of sperms by males, thereby causing the females not to see another reason to mate again, due to the sufficient number of sperms received in that one copulating incident (Franco et al., 2011). In *Nezara viridula* (Linnaeus), for example, an extended copulatory period has been attributed to reduced fertilisation success of other males (McLain, 1980); but in *Eccritotarsus*, one female was recorded copulating with both males from *E. catarinensis* and *E. eichhorniae* in choice experiments, although this was rare.

It is clear that very limited interbreeding is likely to occur in South Africa. Therefore, assessing the establishment of the *E. eichhorniae* in the country and comparing it to *E. catarinensis* is a necessity. *Eccritotarsus eichhorniae* seems to have a higher mating fitness than *E. catarinensis*, and is likely to out-compete *E. catarinensis* in the field, but this may be temperature-dependent. Choosing a species that is climatically suitable will be key in the success of these biological control agents. Differences in temperature as a result of latitude could be responsible for the differences in mating patterns reported in this study. This study somewhat supplements Taylor et al. (2011), Paterson et al. (2016) and Ismail & Brooks (2016, 2018) findings, and also supports Henry (2017) who described the Peruvian population as separate species. In summary, there are pre-copulatory isolation mechanisms in *E. catarinensis* and *E. eichhorniae*, and there are likely to be other isolation mechanisms indicated by female and male choices. Assuming morphologically similar or identical biocontrol agents are the same species may threaten the reliability of biological control programmes of invasive alien weeds in South Africa.

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RESEARCH ARTICLE

Adult diet does not compensate for impact of a poor larval diet on stress resistance in a tephritid fruit fly

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ABSTRACT

Adult holometabolous insects may derive metabolic resources from either larval or adult feeding, but little is known of whether adult diets can compensate for deficiencies in the larval diet in terms of stress resistance. We investigated how stress resistance is affected and compensated for by diet across life stages in the marula fruit fly *Ceratitis cosyra* (Diptera: Tephritidae). Larvae were fed diets containing either 8% torula yeast, the standard diet used to rear this species, or 1% yeast (low protein content similar to known host fruit). At emergence, adults from each larval diet were tested for initial mass, water content, body composition, and desiccation and starvation resistance or they were allocated to one of two adult diet treatments: sucrose only, or sucrose and yeast hydrolysate. The same assays were then repeated after 10 days of adult feeding. Development on a low protein larval diet led to lower body mass and improved desiccation and starvation resistance in newly emerged adults, even though adults from the high protein larval diet had the highest water content. Adult feeding decreased desiccation or starvation resistance, regardless of the diet provided. Irrespective of larval diet history, newly emerged, unfed adults had significantly higher dehydration tolerance than those that were fed. Lipid reserves played a role in starvation resistance. There was no evidence for metabolic water from stored nutrients extending desiccation resistance. Our findings show the possibility of a nutrient-poor larval environment leading to correlated improvement in adult performance, at least in the short term.

KEY WORDS: Body composition, Dehydration tolerance, Desiccation resistance, Diet, Starvation resistance, Water content

INTRODUCTION

Nutrition plays an essential role in enhancing insect resistance to environmental stress (Andersen et al., 2010; Djawdan et al., 1997; Sisodia and Singh, 2012). Animals obtain nutrients for somatic maintenance, growth and reproduction, but nutrient reserves also play a large role in tolerance of desiccation and starvation (Djawdan et al., 1998). Lipids represent the main form of energy storage in insects as triglycerides in the fat body (Arrese and Soulages, 2010) but also act as a source of water upon oxidation in some species (Djawdan et al., 1998; Kleynhans and Terblanche, 2009; Nicolson, 1980; Weldon et al., 2016). Glycogen can also serve as a source of metabolic water, with the additional benefit that water bound to glycogen also becomes available when the glycogen is metabolised (Djawdan et al., 1998; Gefen et al., 2006). A range of proteins is also associated with insect tolerance of dry conditions. For example, upregulation of heat shock protein expression protects water-stressed cells from changes in pH and solute concentrations by preventing protein aggregation and preserving enzyme activity (Benoit et al., 2010; Hayward et al., 2004).

Some insects may struggle to meet their minimum nutritional requirements, which can lead to nutrient deficiency or imbalance (Raubenheimer and Simpson, 1999). This detrimental effect hinders the fitness and everyday activities of insects (Andersen et al., 2010). This is largely due to the fact that insect body tissues require a specific amount and composition of nutrients to function adequately (Boggs, 2009), but dietary sources of nutrients, particularly plant-based diets, may have low nutrient content or unbalanced nutrient composition (Simpson and Raubenheimer, 2000). In response to nutritional stress, insects may extend their growth periods by changing their energy allocation to somatic maintenance (Boggs, 2009). This prolongs the time needed to reach maturity, reduces total body size and changes allometric relationships between body regions (Boggs and Niitepõld, 2016; Gefen et al., 2006). Alternatively, an insect may exhibit compensatory feeding and dietary selection (e.g. Abisgold and Simpson, 1987; Fanson et al., 2009; Maklakov et al., 2008; Malod et al., 2017) to achieve a nutrient intake that approaches an optimum defined by its evolved life history strategy. However, it has also been found that restriction of food intake (calories) can lead to 'hormetic' effects, whereby individuals experiencing a mild stress exhibit improved stress resistance and life extension (Le Bourg, 2009).

Larval nutrition determines how adult insects cope with various kinds of stress because the larval stage contributes to metabolic reserves in adults. When reared on a protein-rich larval medium, adult *Drosophila melanogaster* exhibited increased desiccation resistance and heat tolerance compared with those reared on a carbohydrate-enriched medium (Andersen et al., 2010). Larval development of *D. melanogaster* in a field diet of mostly decomposing apples led to lower critical thermal maximum, dry body mass and fat content, but higher starvation resistance, in comparison with development on a standard laboratory diet (Kristensen et al., 2016). Mexican fruit flies (*Anastrepha ludens*) reared on a carbohydrate-based larval diet exhibited longer larval and pupal development than those reared on a highly protein-biased diet (Pascacio-Villaf et al., 2016). However, pupae were larger, and adult desiccation and starvation resistance was improved when flies developed on the carbohydrate-biased larval diet. In some cases, feedback has been detected between environmental temperature and

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nutritional outcomes in developing insects (Coggan et al., 2011). Adult diet can also play a role in stress tolerance. For example, in *D. melanogaster*, dietary restriction on a low protein to carbohydrate ratio, which favors longevity over reproduction, also led to higher starvation resistance and associated elevated lipid deposition (Lee and Jang, 2014). Dietary restriction during the adult life stage in the oriental fruit fly (*Bactrocera dorsalis*) led to increased resistance to desiccation, starvation, and heat and cold shock, as well as improved longevity, but came at a cost of reduced fecundity (Chen et al., 2017). However, nutrient restriction did not have any consistent effects on thermal tolerance traits in the Mediterranean fruit fly (*Ceratitis capitata*) (Mitchell et al., 2017).

As mentioned above, compensatory feeding within an insect developmental stage to counteract the consequences of a poor diet is well documented, especially for adult stages (Nestel et al., 2016 and references therein). However, the capacity of holometabolous insects to compensate and correct for nutritional deficiencies in an earlier life stage has received much less attention (Nestel et al., 2016). In the mustard leaf beetle (*Phaedon cochleariae*), a larval diet of watercress led to slower development and reduced adult fecundity in comparison with beetles that developed on a highly nutritious cabbage diet (Müller and Müller, 2016). However, when beetles that had developed on watercress were switched to an adult diet of cabbage, their fecundity did not differ from those that had developed on cabbage, providing clear evidence for adult dietary compensation for reproductive output (Müller and Müller, 2016). When reared on a low quality larval diet, adult females of the map butterfly (*Araschnia levana*), were smaller but showed a significant preference for a nectar mimic with amino acids in comparison with females developed on a high quality larval diet (Mevi-Schütz and Erhardt, 2003). In contrast, adult feeding on a source of amino acids in another nymphalid butterfly, *Bicyclus anynana*, resulted in only limited reproductive compensation, in terms of increased egg size, for a brief period of larval starvation (Bauerfeind and Fischer, 2009). In two tephritid fruit fly species, *A. ludens* and the guava fruit fly *Anastrepha striata*, copulations achieved by males with access to an adult diet high in protein did not differ among males of different size, although size is affected by larval developmental environment (Aluja et al., 2008). Similar results were again obtained for *A. ludens* as well as the West Indian fruit fly (*Anastrepha obliqua*) (Aluja et al., 2009). Males with constant access to protein and sucrose, regardless of size, mated more often, had shorter copulations and induced longer refractory periods in females than males fed a low quality diet as adults (Aluja et al., 2009). These examples support the suggestion that larval nutritional conditions leading to deficiencies in adult stress tolerance traits can be compensated for by the adult diet.

The aim of this study was to determine the effect of larval and adult diets on desiccation resistance and starvation resistance of the marula fruit fly *Ceratitis cosyra* (Walker 1849) (Diptera: Tephritidae). By doing so, we could test for the potential for compensation for deficiencies in nutrition across life stages. Body nutrient reserves (total carbohydrates, glycogen, lipids and proteins) were determined to identify potential links between diet, nutrient storage, and desiccation and starvation resistance. We predicted that stress resistance would be improved by larval development in a nutrient-rich diet, leading to greater body reserves, but consumption of a nutrient-rich diet as an adult would compensate for poor stress resistance associated with a suboptimal larval diet. *C. cosyra* was chosen as the focus of this study due to availability of data on its adult nutritional intake targets and life history (Malod et al., 2017), its close phylogenetic relationship to other fruit fly species that have been the subject of water balance studies (Weldon et al., 2016, 2018, 2013) and because it exhibits relatively high desiccation resistance (Weldon et al., 2016). In addition, *C. cosyra* is an important quarantine pest across sub-Saharan Africa where it affects production of mangoes (Vayssières et al., 2009; White and Elson-Harris, 1992). An increased understanding of how the stress tolerance traits of *C. cosyra* vary with diet will contribute to predicting its distribution in a changing climate using physiological models that account for flows of energy and conservation of mass (Rosenblatt and Schmitz, 2016).

MATERIALS AND METHODS

Flies and colony maintenance

Flies were obtained from a *C. cosyra* culture from the Department of Zoology and Entomology, University of Pretoria, South Africa. The culture had been maintained for approximately 12 generations with 300 adults in each generation. Adults were kept in 5 liter clear plastic containers with one side replaced with an insect screen for ventilation. The lid of the plastic containers was replaced with a sleeve of white voile curtain fabric to permit access to the cage. Each cage was furnished with sugar and yeast hydrolysate (HG000BX6.500, Merck, Wadesville, South Africa) in separate Petri dishes. Each cage was also supplied with water-soaked cotton wool as a water source. When culture flies were more than 10 days old, oviposition substrates were provided into which females laid eggs. The oviposition substrates comprised 100 ml plastic cups containing water-soaked tissue paper and 3 ml guava concentrate (Halls; Tiger Consumer Brands Limited, Bryanston, South Africa). These were covered with a double layer of Parafilm (Bemis Flexible Packaging, Neenah, WI, USA) that was pierced multiple times with an insect pin. Females were given 2–3 h to lay eggs. Eggs were then washed off the Parafilm with distilled water into a glass dish where they were permitted to settle, then taken up with a 3 ml disposable plastic Pasteur pipette and again allowed to settle at the tip of the pipette. One drop of densely packed eggs, containing approximately 300 eggs, was transferred to each larval diet (see below). The cultures and all experiments described below were kept at a constant temperature of 24±1°C in a climate room with buffered relative humidity (40–60%) and a 12 h light:12 h dark cycle with a simulated dawn and dusk for the first and last hour of the light phase. Lights in the climate room turned on at 06:00 h (South Africa Standard Time; GMT+2) and turned off at 18:00 h.

Choice of larval diets

Before determining the effect of larval diet on adult stress resistance traits, it was first necessary to identify larval diets that contained high and low protein, but still permitted sufficient development and survival to obtain adults for testing. The standard larval diet used to rear *C. cosyra* comprises a blend of carrot powder, torula yeast and sodium benzoate as a preservative (Citrus Research International, Nelspruit, South Africa). This standard diet, plus a range of diets with different proportions of carrot powder (a source of carbohydrates and some amino acids) and torula yeast (a source of protein and other nutrients) were screened for development time, pupal production and adult eclosion. The diets included 8% (standard), 4%, 1% and 0% torula yeast by wet mass. As torula yeast content decreased, the quantity of carrot powder was increased. Protein content of known fruit hosts for *C. cosyra* is 0.5% for marula fruit (*Sclerocarya birrea*) (Wehmeyer, 1966) and 0.2% for mango (*Mangifera indica*) (Kansci et al., 2008). To 100 g of the dry mix of each diet, 250 ml of boiling water was added and combined to form a smooth paste. A quantity of 100 g of the wet mix of each diet was

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transferred to 100 ml plastic cups. There were 5 replicates for each diet.

After diets were prepared, *C. cosyra* eggs were obtained from the laboratory culture. Approximately 2700 eggs (three Petri dishes, each having three drops of eggs) were allowed to hatch on moist filter paper in covered Petri dishes. Using a dissecting microscope, 100 freshly hatched larvae were transferred with a moist, very fine paintbrush to each replicate of the four diets (i.e. a total of 2000 freshly hatched larvae). The inoculated diets were then placed on a 2 cm layer of dry sand in individual ventilated containers (with a volume of 2 liters). After 18 days, when all surviving larvae had completed development, migrated from the diets and entered the pupal stage, the sand from each container was sifted to count the number of pupae produced from each replicate of each diet. The pupae were returned to the ventilated containers and covered with 2 cm of dry sand. The containers were observed on a daily basis to record the date of first adult emergence and main emergence. The adults were left to starve until death; the number and sex of adults was then determined by counting them under a dissecting microscope.

Experimental protocol

Based on the results of the trials described above, the standard larval diet (8% torula yeast) and the larval diet containing 1% torula yeast were used for subsequent diet manipulations. These larval diets were referred to as 'high protein' and 'low protein', respectively. Approximately 2400 eggs were obtained from the laboratory culture, as described above. Rates of adult emergence from the pilot study were used to estimate the number of eggs needed to obtain the adults for planned desiccation and starvation resistance assays. A droplet containing approximately 300 eggs was inoculated into each of three 100 ml containers of high protein larval diet and of five 100 ml containers of low protein larval diet (to allow for the lower adult emergence of *C. cosyra* from the latter). These were placed on a 2 cm layer of dry sand in separate 2 liter plastic containers with ventilated lids. Larvae emerged out of the larval diets at the end of the third instar and entered the pupal stage. The sand was sifted after 18 days to obtain pupae. Pupae were then placed in an empty cage to emerge as adults. Upon emergence, the adults from each larval diet were allocated to assays for initial mass, water content, and desiccation and starvation resistance (described below) or transferred to one of two cages where they were allocated to one of two adult diet treatments: sucrose only, or sucrose and yeast hydrolysate (YH). Sucrose and YH were offered in separate Petri dishes so that flies could select their intake of each. YH is a rich source of amino acids, lipids and micronutrients (Taylor et al., 2013). Each cage was also supplied with water-soaked cotton wool as a water source. Flies provided with food as adults were assayed for initial mass, water content, and desiccation and starvation resistance 10 days after adult emergence. On emergence, females and males on each larval and adult diet were kept in the same cage. No oviposition substrates were provided for egg-laying by females.

Initial mass and water content

At 0 days and 10 days after adult emergence, 20 flies (10 females and 10 males) from each larval and adult diet treatment were placed in centrifuge tubes of known weight and weighed on a microbalance (to 0.001 mg; CPA2P, Sartorius AG, Germany). Initial mass of each fly was obtained by subtracting tube weight from tube+fly weight. These adult insects were frozen and stored at −20°C for several days. Flies were then freeze-dried using an automated freeze-drier (2KBTXL-75 Benchtop SLC Freeze Dryer, Virtis, Los Angeles, California, USA) for approximately 48 h before being weighed again on a microbalance to determine dry mass. Water content was determined by subtracting dry mass from body mass. Freeze-dried flies were stored at 4°C for later analyses of body composition.

Desiccation and starvation resistance

At 0 days and 10 days, 30 female and 30 male flies from each larval and adult diet treatment were placed into individual, numbered, 2 ml microcentrifuge tubes that had been pierced with 12 holes (diameter ∼1 mm) and then pre-weighed. Flies in tubes were then weighed (to 0.001 mg) and initial body mass of each fly was calculated by subtracting tube mass. Half of the tubes from each larval and adult diet treatment were then placed onto racks in airtight containers over anhydrous silica gel to maintain relative humidity below 5%. The other half were placed onto racks in airtight containers over distilled water to maintain relative humidity close to 100%. All airtight boxes were maintained at 25°C for the duration of the experiment. Desiccation and starvation resistance assays commenced at 10:30 h and all tubes were checked for mortality every 3 h by viewing them through the clear plastic top of the airtight containers. Dead flies were removed from the tubes and weighed to record body mass at death (to 0.001 mg). These were then placed in new, intact, labeled tubes for storage in a freezer at −20°C. Later, all flies were freeze-dried and weighed as described above to determine dry mass at death and body water at death (expressed as percentage of initial water content, this is referred to as dehydration tolerance; Gibbs et al., 1997). Higher values of water at death are interpreted as lower dehydration tolerance.

Measurement of whole-insect nutrient reserves

The methods used for the body composition analyses were adapted from Foray et al. (2012) to determine carbohydrate, glycogen, lipid and soluble protein content from the same individual. Five to seven freeze-dried flies were initially selected at random for each combination of larval diet, adult diet, sex and stress (desiccation or starvation). These were then randomly processed to avoid all individuals from the same treatments being assayed synchronously. Additional tests for carbohydrates only were also run in three to five additional randomly selected flies to verify low quantities that were detected in some groups. Precise sample sizes are available in the publicly available data file (http://hdl.handle.net/2263/66649). Each insect was individually homogenised in 180 µl lysis buffer using a microtube homogeniser (BeadBug™ 3 Position Bead Homogeniser, Benchmark Scientific, Sayreville, NJ, USA) for 30 s at the maximum setting. The lysis buffer consisted of 100 mmol l^{-1} potassium dihydrogen phosphate, 1 mmol l^{-1} DTT and 1 mmol l^{-1} EDTA (pH 7.4). After homogenisation, the samples were centrifuged at 14,000 ***g*** for 10 min.

For soluble protein determinations, 1.5 µl supernatant was transferred to a 96-well plate in triplicate. Total protein content was determined by the Bradford assay (Bradford, 1976) using Bradford Reagent (Sigma-Aldrich, St Louis, MO, USA) and serial dilutions of bovine serum albumin standard (Sigma-Aldrich) to establish a standard curve according to the manufacturer's instructions. Optical density was measured at 595 nm using a microplate reader (Eon™ High Performance Microplate Spectrophotometer, BioTek, Winooski, VT, USA).

To determine the carbohydrate and lipid content, 4.5 µl lysis buffer and 20 µl of 20% (w/v) sodium sulphate was added to the remaining homogenate (to reach a final volume of 200 µl). After adding 1500 µl of chloroform:methanol (1:2 v/v) to each sample, samples were vortexed vigorously and centrifuged at 14,000 ***g*** for 10 min. Three hundred microliters of the supernatant was aliquoted

for the lipid analysis and the pellet was kept for the determination of the glycogen content. The remainder of the supernatant was evaporated at room temperature overnight in a fumehood and made up to a final volume of 250 µl using chloroform:methanol (1:2 v/v). The anthrone method was employed to determine the water-soluble carbohydrate content of these samples. A glucose (Merck, Billerica, MA, USA) dilution range (0.1, 0.3, 0.5, 1.0, 2.5 and 5 mg ml^{-1}) was used as calibration standard. Two hundred microliters of standard or sample were pipetted into a 15 ml tube and 4.8 ml anthrone reagent (1.42 g l^{-1} anthrone dissolved in 70% sulphuric acid) added (Anthrone, ACS reagent 97% was obtained from Sigma-Aldrich). The reaction mixture was vortexed and incubated at 90°C in a water bath for 15 min before the optical density was measured at 625 nm using the microplate reader. Measurements were performed in duplicate using 250 µl reaction mixture.

The anthrone method was also used to determine the total glycogen content of each sample. The pellet was washed with 400 µl of 80% (v/v) methanol and centrifuged at 16,000 ***g*** for 5 min. The supernatant was discarded before adding 400 µl distilled water to the pellet. Subsequently, the samples were incubated at 70°C in a water bath for 5 min. Two hundred microliters of standard or sample were pipetted into a 2 ml Eppendorf tube and 1 ml anthrone reagent (1.42 g l^{-1} dissolved in 70% sulphuric acid) added. The reaction mixture was vortexed and incubated at 90°C in a water bath for 15 min. The reaction mixtures were cooled on ice and filtered using 0.22 µm, PVDF syringe filters (Millex®-GV, Merck, Billerica, MA, USA) before the optical density was measured at 625 nm using the microplate reader. Measurements were performed in triplicate using 200 µl reaction mixture. A glucose dilution range (0.05, 0.1, 0.25, 0.5, 0.75 and 1 mg ml^{-1}) was used as calibration standard.

The total amount of lipids in each insect was determined with the vanillin colorimetric assay. Glycerol trioleate (Sigma-Aldrich) dissolved in chloroform:methanol (1:2 v/v) was used as calibration standard (dilution range 0.1, 0.2, 1.0 and 2.0 µg $µl^{-1}$). One hundred microliters of standard or aliquoted supernatant was transferred to a 96-well microplate in triplicate. Following the complete evaporation of the samples at 90°C, the samples were incubated with 10 µl of 98% sulphuric acid at 90°C for 2 min. After cooling the samples on ice, 190 µl vanillin reagent (1.2 g l^{-1} vanillin dissolved in 68% orthophospohoric acid) were added to each sample (Vanillin, ReagentPlus® 99% was obtained from Sigma-Aldrich). The microplate was placed in the microplate reader and the shake function was used to mix the reaction mixtures before incubation for 15 min at room temperature and subsequent measurement of the optical density at 525 nm.

Data analysis

Analyses of variance were applied to data obtained from the preliminary study to select suitable larval diets. Development time (from egg inoculation to main adult emergence), number of pupae and adult emergence (expressed as a percentage of the number of pupae) were dependent variables, and larval diet torula yeast content (8%, 4%, 1% and 0% of wet mass) was the predictor variable. When we excluded the 0% torula yeast treatment from all analyses (after yielding 0 pupae and thus 0 adults), data met the assumptions of parametric tests. *Post hoc* multiple comparisons were performed using Tukey's honest significant difference tests to identify which diets differed from each other. A contingency analysis was used to determine if the sex ratio differed with larval diet.

Initial mass and water content of adults were related to larval diet, adult diet and sex using generalized linear models (GLZ) with normal distribution and identity link. Owing to the hierarchical nature of the experimental design, a nested model was used, with sex within adult diet [none (age: 0 days), sugar only and sugar+YH (age: 10 days)], and adult diet within larval diet (low yeast, high yeast). For the water content model, initial mass was included as a covariate to account for the effects of body size. Model residuals indicated that the data did not violate assumptions of constancy of variance and normality of errors. Model coefficients were inspected to identify simple effects. A significant effect of body mass was found on initial water content, so a series of ordinary least squares regressions were used to establish the linear relationship between initial mass and initial body water content for each combination of larval diet, adult diet and sex. The resulting regression equations (Table S1) were used to estimate initial body water content based on measurements of initial mass in later analyses.

Cox proportional hazards regression was used to determine the effects of larval diet, adult diet, stress, sex and initial mass of adult *C. cosyra* on time to death in desiccation and starvation resistance assays. Again, due to the hierarchical nature of the study design, a nested model was used, with sex within assay, assay within adult diet, adult diet within larval diet and initial mass as a covariate. Adjustment for ties was estimated using Breslow likelihood. Based on inspection of scaled Schoenfeld residuals over time, the data did not violate the assumption of proportionality of hazards. Model coefficients were inspected to identify simple effects. Model results were used to plot survivor functions.

The nested effects of larval diet, adult diet, stress, and sex on dehydration tolerance were determined using a GLZ with gamma distribution and log link function. Based on comparison of Akaike's information criterion (AIC), this model provided a better fit for the data than a normal distribution with identity or log link, and did not violate assumptions of constancy of variance and normality of errors. The dependent variable was body water content at death, which was related to estimated body water (from the regression equations in Table S1) by inclusion of the latter as a covariate in the model. Survival time during desiccation and starvation resistance assays were also included as a covariate in the model.

Dry mass, and whole insect carbohydrate, glycogen, lipid and protein content (expressed as µg mg^{-1} dry mass) were analysed using five separate GLZs with gamma distribution and log link function. In each model, the predictors were sex nested within stress, stress nested within adult diet, and adult diet nested within larval diet. In the model for dry mass, the interaction of initial mass with sex was also included in the model. Although it was often the case that the content of each nutrient was determined for a single fly, in some cases it was necessary to obtain carbohydrate content estimates from additional flies to verify very low levels detected in some treatments. For this reason, not all nutrients were measured in all flies, and it was consequently not possible to run a multivariate analysis of covariance model. In addition, the obtained data did not fit a normal distribution; based on AIC values, a gamma distribution with log link provided the best fit for the data.

All data analyses were performed using Statistica 64-bit v13 (Dell Inc., Tulsa, OK, USA).

RESULTS

Choice of larval diets

No pupae were obtained from the 0% torula yeast larval diet (Table 1). There was a significant difference between the number of pupae obtained from the remaining three larval diets ($F_{3,16}$=265.42, P< 0.0001). The number of pupae from the 4% and 8% torula yeast larval diets was significantly higher than from the 1% torula yeast diet. Larval diet also had a significant effect on adult emergence

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Table 1. Effect of larval diets varying in torula yeast content on mean (± 1 s.e.) larval survival, juvenile development time and adult emergence of *Ceratitis cosyra*

	Torula yeast content in larval diet			
Performance trait	0%	1%	4%	8%
Larval survival (%)	0	47.6±4.3^a	86.2±1.5^b	80.4±1.7^b
Juvenile development time (days)	0	32.6±0.2^a	28±0.0^b	27.6±0.2^b
Adult emergence (%)	0	76.9±7.7^a	90.4±1.2^b	91.0±3.1^b

Each replicate (n=5) began with 100 newly emerged larvae. Adult emergence is expressed as the percentage of pupae obtained from each diet that emerged as adults. Means for each performance trait with different superscript letters are significantly different (Tukey's HSD tests; P>0.05).

expressed as a percentage of adults emerged over pupae obtained from each diet ($F_{3,16}$=70. 39, P<0.0001). Adult emergence from the diets containing 4% and 8% torula yeast was significantly higher than from the 1% torula yeast larval diet. There was a significant effect of larval diet on development time ($F_{3,16}$=7374.60, P<0.0001), with the adult stage reached in a shorter period of time by individuals reared on 4% and 8% torula yeast diet in comparison with those reared on the 1% torula yeast diet. Larval diet had no significant effect on the sex ratio of *C. cosyra* (χ^2=3.13, d.f.=2, P=0.209), with the sex ratio being 1:1 in all diets where adults emerged. Based on these results, and because it is the standard diet used to rear *C. cosyra* in the laboratory, the 8% torula yeast larval diet was selected as the high protein larval diet, and the 1% torula yeast larval diet was selected as the low protein larval diet for stress resistance experiments.

Initial condition

Adult *C. cosyra* reared from a high yeast larval diet were significantly heavier (predicted mean±1 s.e.=9.24±0.12 mg) than those reared on a low yeast larval diet (7.92±0.12 mg) (GLZ: Wald χ^2=34.69, d.f.=1, P<0.001). Adult diet nested within larval diet also affected adult initial mass (GLZ: Wald χ^2=12.68, d.f.=4, P=0.013). That is, among flies fed a high yeast larval diet, those fed sugar+YH for 10 days after adult emergence were significantly heavier than those with no opportunity to feed as adults (0 days) or fed only sugar for 10 days (Fig. 1A). In contrast, among flies fed a low yeast larval diet, there was no significant difference in initial mass between the adult diet treatments. Within adult diet, sex had a significant effect on initial mass (GLZ: Wald χ^2=85.31, d.f.=3, P<0.001), with females consistently heavier than males within each adult diet treatment (0 days: female=9.08±0.21 mg, male=7.59±0.21 mg; 10 days sugar: female=9.14±0.21 mg, male=7.50±0.21 mg; 10 days sugar+YH: female=9.86±0.21 mg, male=8.29±0.21 mg).

Water content of adult *C. cosyra* significantly increased with every unit increase in initial mass (GLZ: Wald χ^2=1298.69, d.f.=1, P<0.001; parameter estimate: 0.682±0.019). Larval diet had a significant effect on water content (GLZ: Wald χ^2=5.433, d.f.=1, P=0.020). However, when accounting for initial mass (mean=8.57 mg) there was no overall difference in water content of adults reared on a high yeast (5.78±0.03 mg) or low yeast larval diet (5.78±0.03 mg). Among adults reared on a high yeast or low yeast larval diet, the nested effect of adult diet significantly predicted water content (GLZ: Wald χ^2=26.20, d.f.=4, P<0.001). This was apparent when adults were reared from a high yeast larval diet because water content of newly emerged adults was significantly higher than those fed for 10 days on sugar only or sugar+YH (Fig. 1B). In contrast, when reared on a low yeast larval diet, water content did not differ between adult diet treatments. The

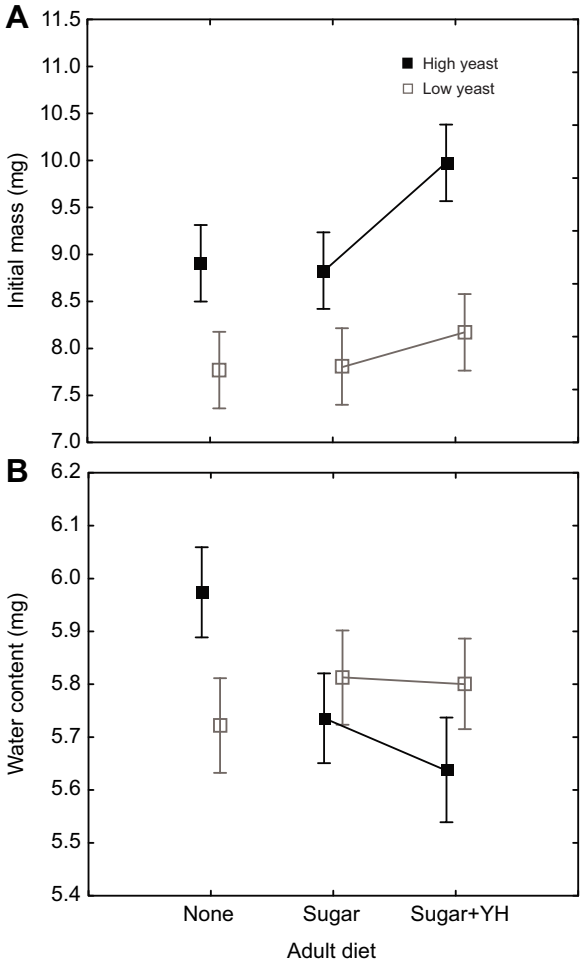

Fig. 1. Effect of adult diet nested within larval diet on body mass and water content of *Ceratitis cosyra*. Body mass (A) and water content (B) in flies from larvae fed a high yeast or low yeast diet. Flies receiving no adult diet were tested on the day of adult emergence from the puparium (none), whereas the two other adult diet treatments comprised flies given unrestricted access to sugar only (sugar) or sugar and yeast hydrolysate (sugar+YH), plus water, for 10 days following emergence. Mean water content was predicted from a generalised linear model with normal distribution and identity link, and initial mass at its mean (8.56 mg). For each combination of larval diet, adult diet and sex, n=10. Error bars indicate ±1 s.e.

effect of sex nested within adult diet had no significant effect on water content (GLZ: Wald χ^2=3.40, d.f.=3, P=0.335).

Desiccation and starvation resistance

Larval diet had a significant effect on mortality risk (Cox PHR: Wald χ^2=37.24, d.f.=1, P<0.001), as did adult diet nested within larval diet (Cox PHR: Wald χ^2=21.26, d.f.=2, P<0.001), and stress nested within adult diet (Cox PHR: Wald χ^2=109.19, d.f.=5, P<0.001). Adult *C. cosyra* mortality risk during desiccation and starvation resistance assays was significantly greater when flies had been reared on a high yeast larval diet (Fig. 2). Mortality risk during desiccation assays was 1.5–2.1 times greater (Fig. 2A,B) than during starvation resistance assays (Fig. 2C,D). In both assays, there was a highly significant decrease in mortality risk of newly emerged flies that had been reared on a low yeast larval diet when compared with those reared on a low yeast larval diet and fed as adults (Fig. 2B,D). Every unit increase in initial mass significantly decreased the likelihood of mortality (Cox PHR: Wald χ^2=19.00, d.f.=1, P<0.001;

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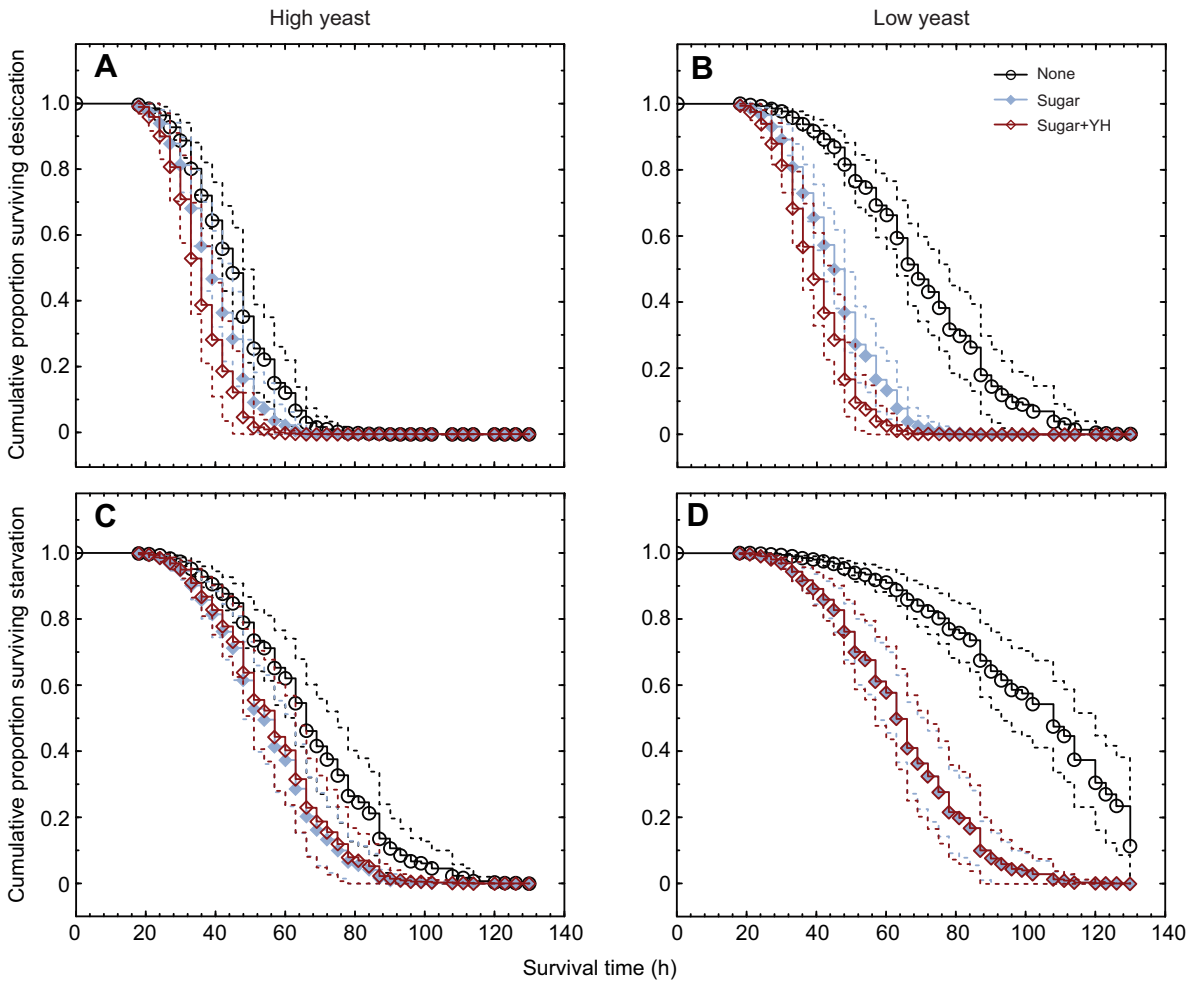

Fig. 2. Modeled survival functions for adult *C. cosyra* during desiccation and starvation resistance assays in relation to larval diet and adult diet. Survival during desiccation when reared on a high yeast (A) and low yeast (B) larval diet and survival during starvation when reared on a high yeast (C) and low yeast (D) larval diet. Adult diets as described in Fig. 1. Survival functions were estimated from a Cox proportional hazards model with initial mass held at its mean (8.56 mg). Dotted lines represent the upper and lower 95% confidence intervals for each survival function. Model plotted based on n=15 for each combination of larval diet, adult diet, sex and stress.

parameter estimate: −0.232±0.053; hazard ratio=0.793). Mortality risk of adults fed sugar or sugar+YH did not differ, irrespective of larval diet history. The effect of sex nested within stress did not significantly affect mortality risk (Cox PHR: Wald χ^2=1.31, d.f.=2, P=0.519).

Dehydration tolerance

Water content at death, a measure of dehydration tolerance (with higher water at death interpreted as lower dehydration tolerance), increased with predicted initial water content (GLZ: Wald χ^2=183.17, d.f.=1, P<0.001; parameter estimate: 0.134±0.010). Conversely, water content at death decreased slightly but significantly as fly longevity increased during stress resistance assays (GLZ: Wald χ^2=25.68, d.f.=1, P<0.001; parameter estimate: −0.002±0.000). Larval diet did not have a significant effect on water content at death (GLZ: Wald χ^2=0.83, d.f.=1, P=0.363). However, adult diet nested within larval diet did have a significant effect on water content at death (GLZ: Wald χ^2=29.56, d.f.=4, P<0.001), with newly emerged, unfed *C. cosyra* having significantly lower water content at death than those fed as adults (Fig. 3A). Within each adult diet, desiccated flies had lower water content at death than those that were starved (Fig. 3B; GLZ: Wald χ^2=125.78, d.f.=3, P<0.001). Females had significantly higher body water content at death than males following both the desiccation (female: 3.29±0.01 mg; male: 3.00±0.01 mg) and starvation resistance assays (female: 4.08±0.01 mg; male: 3.78±0.02 mg) (GLZ: Wald χ^2=183.17, d.f.=1, P<0.001).

Whole-insect nutrient reserves

Adult dry mass was significantly reduced when flies were reared on a low yeast larval diet (high yeast: 2.21±0.01 mg; low yeast: 2.17±0.01 mg; GLZ: Wald χ^2=21.00, d.f.=1, P<0.001), but this was largely due to adults fed for 10 days with sugar or sugar+YH being significantly lighter than newly emerged, unfed adults (Fig. 4A; GLZ: Wald χ^2=38.364, d.f.=4, P<0.001). When fed an adult diet of only sugar, dry mass at death did not differ among *C. cosyra* after desiccation or starvation, but when fed an adult diet of sugar+YH, dry mass of desiccated flies was significantly higher than those that had been starved (Fig. 4B; GLZ: Wald χ^2=61.29, d.f.=6, P<0.001). There was also an interaction of sex and initial mass within stress (GLZ: Wald χ^2=1530.74, d.f.=6, P<0.001). Female dry mass was greater than that of males (Fig. S1), but the relationship between initial mass and dry mass in unstressed flies had a steeper gradient (parameter estimate: female=0.106±0.006, male=0.106±0.007)

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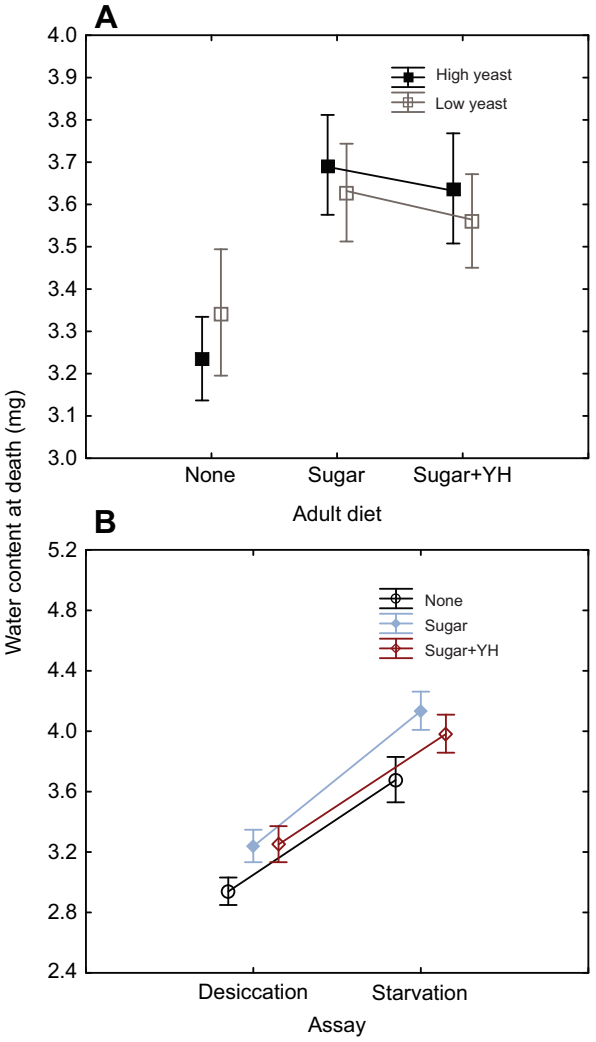

Fig. 3. Effect of adult diet or stress on water remaining at death in adult *C. cosyra*. (A) Water content in flies from larvae fed a high yeast or low yeast diet, which were then fed different adult diets. (B) Water content in flies fed different adult diets and exposed to either starvation or desiccation stress assays. Flies receiving no stress represent the condition of flies before being desiccated or starved to death. Adult diets as described in Fig. 1. Mean water content at death was predicted from a generalised linear model with Gaussian distribution and log link, and longevity (57.50 h) and estimated initial water content (5.76 mg) at their mean. For each combination of larval diet, adult diet, sex and stress, n=15. Error bars indicate ±1 s.e.

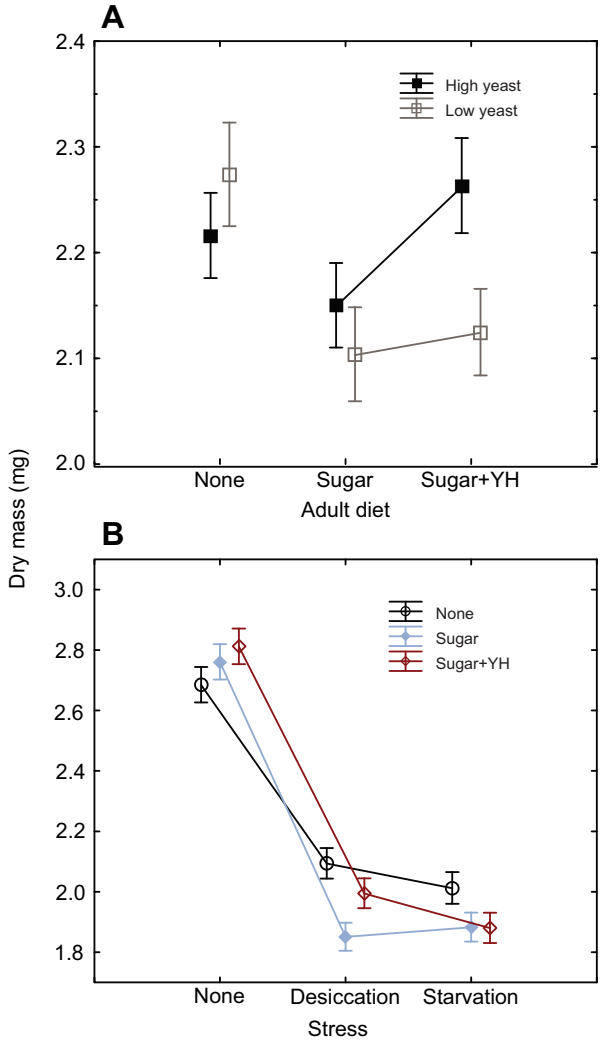

Fig. 4. Effect of adult diet or stress on dry mass of adult *C. cosyra*. (A) Dry mass of flies from larvae fed a high yeast or low yeast diet, which were then fed different adult diets. (B) Dry mass of flies fed different adult diets and then exposed to either starvation or desiccation stress assays. Adult diets as described in Fig. 1. Flies receiving no stress represent the condition of flies before being desiccated or starved to death. Mean dry mass is predicted from a generalised linear model with Gaussian distribution and log link, and initial wet body mass at its mean (8.56 mg). For each combination of larval diet, adult diet, sex and stress, n=15. Error bars indicate ±1 s.e.

than those that had been desiccated (parameter estimate: female=0.092±0.006, male=0.082±0.007) or starved to death (parameter estimate: female=0.104±0.006, male=0.096±0.007).

Water-soluble carbohydrate content of *C. cosyra* was not significantly affected by larval diet (GLZ: Wald χ^2=0.32, d.f.=1, P=0.573), or adult diet nested within larval diet (GLZ: Wald χ^2=6.64, d.f.=4, P=0.156). Within adult diet treatment, stress had a significant effect on carbohydrate content per unit dry mass (GLZ: Wald χ^2=176.27, d.f.=6, P<0.001). Initial carbohydrate content of newly emerged, unfed adult *C. cosyra* did not differ from carbohydrate content after desiccation or starvation to death (Fig. 5A). In contrast, initial carbohydrate content of flies fed sugar or sugar+YH for 10 days after emergence was approximately double the levels remaining after desiccation and starvation. Regardless of the adult diet, there was no difference in the carbohydrate remaining per unit dry mass after desiccation or starvation. The effect of sex nested within stress had no effect on water-soluble carbohydrate content (GLZ: Wald χ^2=3.93, d.f.=3, P=0.269).

Total glycogen content of *C. cosyra* per unit dry mass was not significantly affected by larval diet (GLZ: Wald χ^2=0.01, d.f.=1, P=0.926) or adult diet nested within larval diet (GLZ: Wald χ^2=8.10, d.f.=4, P=0.088). Total glycogen content was significantly affected by stress nested within adult diet (GLZ: Wald χ^2=36.37, d.f.=5, P<0.001). Newly emerged, unfed *C. cosyra* had very low glycogen content, even before being desiccated or starved to death (Fig. 5B). No glycogen was detected in newly emerged flies that were starved to death. A similar result was found for those desiccated to death, with the exception of a single individual (total glycogen: 1.58 µg; 0.85 µg mg^{-1} dry mass). Unstressed flies fed sugar for 10 days after emergence had a glycogen content not significantly different from that of flies starved to death, but

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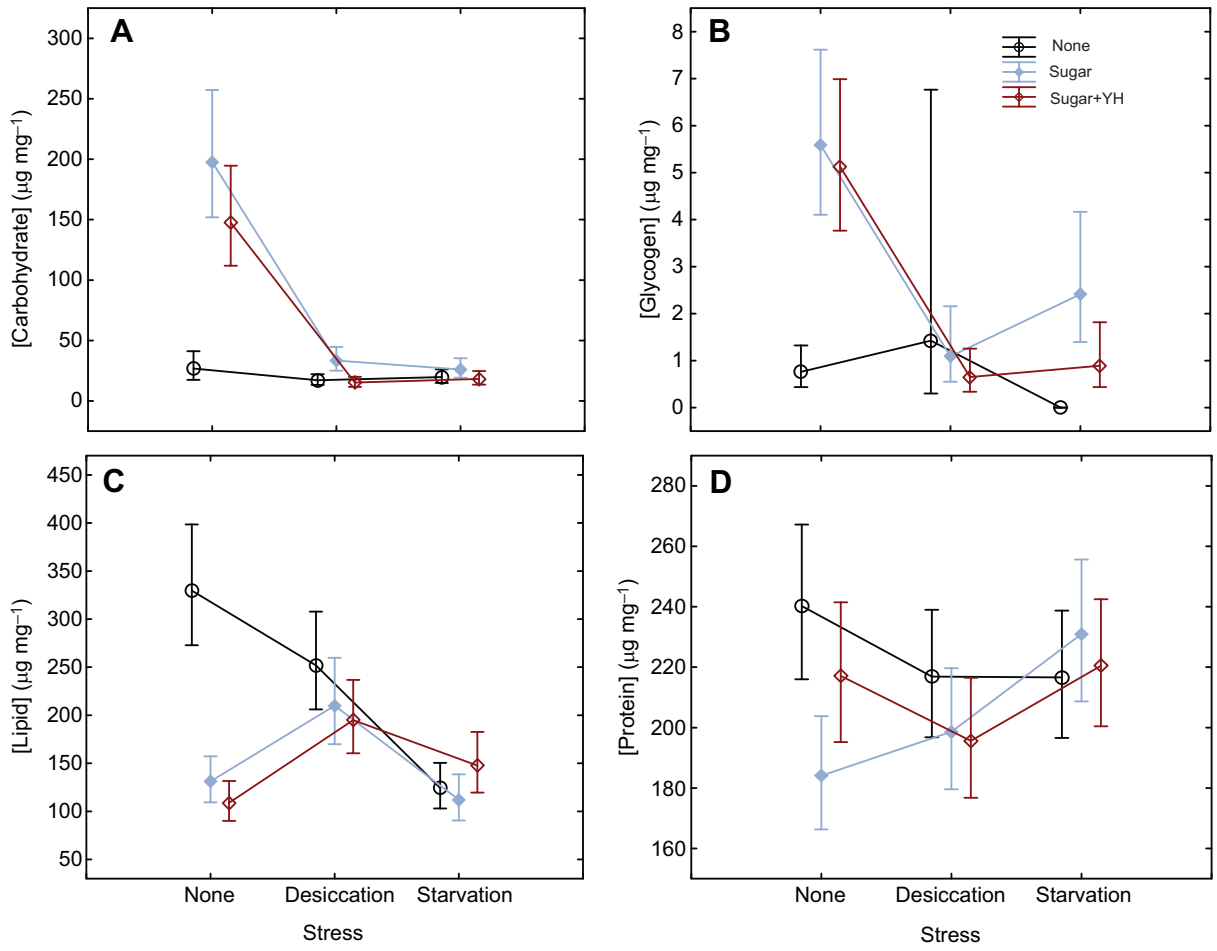

Fig. 5. Effect of adult diet and stress on carbohydrate, glycogen content, lipid and protein content of adult *C. cosyra*. Concentration of each water-soluble carbohydrate (A), total glycogen (B), total lipid (C) and soluble protein (D) is expressed per unit dry mass. Adult diets as described in Fig. 1. Flies receiving no stress represent the condition of flies before being desiccated or starved to death. For each combination of larval diet, adult diet, and stress, n=6–18. Means are predicted from generalized linear models with gamma distribution and log link function. Error bars indicate ±1 s.e.

glycogen content of desiccated flies fed the same adult diet were significantly lower than those that were starved. When fed sugar+YH for 10 days after adult emergence, unstressed *C. cosyra* had significantly higher initial glycogen content than those that were desiccated or starved to death. Sex nested within stress significantly affected glycogen content (GLZ: Wald χ^2=9.41, d.f.=3, P=0.024). Females had significantly higher glycogen content than males after desiccation (female=2.14±0.38 µg mg^{-1}, male=0.46±0.41 µg mg^{-1}), but there was no difference in glycogen content between the sexes before stress (female=2.47±0.15 µg mg^{-1}, male=3.16±0.17 µg mg^{-1}) or after being starved to death (female=0.90±0.37 µg mg^{-1}, male=1.26±0.37 µg mg^{-1}).

There was no significant effect of larval diet (GLZ: Wald χ^2=0.28, d.f.=1, P=0.597), or adult diet nested within larval diet (GLZ: Wald χ^2=36.37, d.f.=4, P=0.383) on total lipid content of *C. cosyra*. However, the effect of stress within adult diet did significantly affect total lipid content per unit dry mass (GLZ: Wald χ^2=61.809, d.f.=6, P<0.001). Among newly emerged, unfed *C. cosyra*, initial lipid content and lipid content of flies that had been desiccated to death was significantly higher than in flies starved to death (Fig. 5C). Among flies fed an adult diet of sugar only for 10 days after emergence, lipid content of those desiccated to death was significantly higher than lipid content in unstressed flies or those starved to death. The apparent elevation of total lipid content following the desiccation assay was due to lower depletion of lipids relative to total dry mass loss in these flies (Fig. 4B) in comparison with starved flies. When fed an adult diet of sugar+YH, initial lipid content was significantly higher than in flies that had been desiccated or starved to death. Total lipid content was not significantly affected by sex nested within stress (GLZ: Wald χ^2=1.01, d.f.=3, P=0.799).

Soluble protein content of adult *C. cosyra* was significantly affected by larval diet (GLZ: Wald χ^2=4.689, d.f.=1, P=0.030), with protein content per unit dry mass in adults reared on a low yeast larval diet being lower (204.77±0.02 µg mg^{-1}) than when they were reared on a high yeast larval diet (221.03±0.02 µg mg^{-1}). The effect of adult diet nested within larval diet did not have a significant effect on soluble protein content (GLZ: Wald χ^2=2.01, d.f.=4, P=0.734). Stress nested within adult diet had a significant effect on soluble protein content (GLZ: Wald χ^2=15.30, d.f.=6, P=0.018). Among newly emerged *C. cosyra* that were not fed as adults, there was no difference in protein content before or after desiccation or starvation to death (Fig. 5D). Similarly, stress treatment did not affect protein content among flies fed for 10 days on sugar+YH after emergence. Among adults fed only sugar for 10 days, initial protein content per unit dry mass was significantly lower than in flies starved to death, with desiccated flies intermediate between those treatments. Sex nested within stress had a significant effect on soluble protein

content (GLZ: Wald χ^2=20.47, d.f.=3, P<0.001). Among unstressed flies, there was no difference in protein content per unit dry mass of females (206.75±0.04 µg mg^{-1}) and males (218.52±0.04 µg mg^{-1}). However, after desiccation, female protein content per unit dry mass (187.07±0.04 µg mg^{-1}) was less than in males (221.37±0.04 µg mg^{-1}), and the same pattern between the sexes was found after starvation (female: 201.84±0.04 µg mg^{-1}; male: 245.51±0.04 µg mg^{-1}).

DISCUSSION

There was no evidence for compensation by the adult diet for poor stress resistance resulting from the larval diet. Regardless of adult diet over a period of 10 days following emergence, *C. cosyra* was at greater risk of desiccation and starvation than newly emerged adults. Other studies on dietary compensation across arthropod life stages have focused on its effect on fitness traits such as body size, fecundity, egg size and longevity (Aluja et al., 2008, 2009; Bauerfeind and Fischer, 2009; Kleinteich et al., 2015; Michaud and Jyoti, 2007). With the exception of the bridge spider (*Larinioides sclopetarius*) (Kleinteich et al., 2015), juvenile deprivation of food or feeding on a less nutritious diet led to reduced juvenile survival, slower development and lower adult body mass. This was also noted in our study among *C. cosyra* fed on the low yeast larval diet. In the case of the butterfly *B. anynana*, subsequent adult feeding on nutritious diets led to improved female fitness (Bauerfeind and Fischer, 2009; Geister et al., 2008), whereas the improved number of egg clutches and fecundity of the lady beetle *Coleomegilla maculate*, depended on a switch between the source of protein consumed between the larval and adult life stage (Michaud and Jyoti, 2007). Male *A. ludens* fed a high quality adult diet mated sooner, secured more copulations, had shorter copulations and inhibited female re-mating for longer than males receiving a poor adult diet, regardless of reduced size resulting from a poor larval diet (Aluja et al., 2009).

While effects on fitness traits have been examined, there have been no explicit tests for the potential compensatory effects of adult diet on insect environmental tolerance traits. When insects have been reared on a constant larval diet but experienced differences in their adult diet, the effect on environmental tolerance traits is equivocal. Following a short starvation treatment as adults, *D. melanogaster* were more desiccation and starvation resistant (Bubliy et al., 2012). However, starvation-acclimated *D. melanogaster* also suffered reduced survival. In *C. capitata*, a starvation pre-treatment had no significant effect on chill-coma recovery time or heat knockdown time (Mitchell et al., 2017). In contrast, in the same species and in the Natal fly (*Ceratitis rosa*), a short starvation treatment significantly improved the critical thermal maximum and minimum (Nyamukondiwa and Terblanche, 2009).

Development on a low yeast larval diet rather than a high yeast larval diet led to improved desiccation and starvation resistance in newly emerged adult *C. cosyra*. This was despite development on a high yeast larval diet leading to higher body water content on adult emergence. Similar results have been found in *D. melanogaster*, where adults reared from a ‘field diet’ of decomposing apples rather than a standard diet used in the laboratory were more resistant to starvation, while also being smaller and leaner (Kristensen et al., 2016). In addition, rearing on a standard laboratory diet led to higher adult tolerance of high temperatures in comparison with *D. melanogaster* reared on the field diet (Kristensen et al., 2016). When reared on a protein-enriched larval diet, adult *D. melanogaster* exhibit higher heat and desiccation tolerance, but impaired recovery from chill-coma, in comparison with those fed a carbohydrate-enriched diet (Andersen et al., 2010). The same patterns were found in *Drosophila ananassae* (Sisodia and Singh, 2012). These results are surprising because initial body mass is positively associated with water content and survival of water stress in tephritid flies in this and other studies (Tejeda et al., 2014; Weldon et al., 2016, 2013). A higher pool of standing water should provide a protection from dehydration. However, in addition to differences in desiccation resistance in *C. cosyra*, water content at death was very similar in flies reared on high and low yeast larval diets. This suggests that water loss rates were higher in flies reared on the high yeast diet. These results point to the possibility of a stressful larval environment leading to correlated improvement in adult performance, at least in the short term. This ‘acclimation’ or ‘hardening’ could be associated with increased lipid reserves (discussed in more detail below) or reduced metabolic rate (Bubliy et al., 2012). Investigation of the mechanisms leading to this cross-life stage hardening or acclimation in stress tolerance will be a fascinating area for future research.

Decreased desiccation resistance in older *C. cosyra* corresponded to a decrease in dehydration tolerance at an age of 10 days, as indicated by higher water content at death following both desiccation and starvation resistance assays. This result aligns with previous studies showing that desiccation resistance declines with age in another tephritid, the Queensland fruit fly (*Bactrocera tryoni*) (Weldon and Taylor, 2010). The causes underlying this observation still require further study but several plausible explanations can be posited. It may be that older tephritids exhibit lower desiccation resistance due to higher rates of water loss as a result of elevated metabolic rate to sustain foraging, sexual development, mating behaviour and reproduction. It has been reported in *C. capitata* that transcription of genes associated with growth, development and energy metabolism is elevated in young females and males (Gomulski et al., 2012). Similarly, in *B. dorsalis*, starch, sucrose and galactose metabolism pathways, and D-glucose levels (obtained by conversion from more complex carbohydrates) were associated with improved male competitive abilities during mating (Cheng et al., 2014). Reduced desiccation resistance in older tephritids may be caused by changes in the water-proofing properties of the cuticle (Gibbs, 1998) during the adult life stage. This could occur due to abrasion and damage to the cuticle as flies age (Johnson, 2000; Johnson and Gibbs, 2004). Alternatively, the composition of cuticular lipids may change as flies mature. Flexibility in the composition of cuticular hydrocarbons within the adult life stage has been found in *D. melanogaster*, where rapid desiccation hardening led to a reduced proportion of unsaturated and methylated hydrocarbons relative to controls (Stinziano et al., 2015). In blood-feeding insects, blood feeding leads to plasticisation of the cuticle through the breakdown of weak intermolecular bonds between proteins, and cuticular lipids increase in abundance in preparation for dry off-host conditions (Benoit and Denlinger, 2010).

There was little evidence for a role of metabolic water from lipid or glycogen stores in extending desiccation resistance of *C. cosyra*. This result is similar to that reported for *C. cosyra* by Weldon et al. (2016). However, lipid reserves do appear to play a role in starvation resistance, with high levels of lipids in newly emerged *C. cosyra* being associated with high starvation resistance early in life. Lipids are the main store of energy in insects, with lipids being converted to carbohydrates, particularly trehalose in the hemolymph and glycogen in the cells, to support energetic requirements during periods of low food intake (Arrese and Soulages, 2010). Body lipid reserves were also positively correlated with starvation resistance in four distinct populations of *Drosophila simulans* (Ballard et al., 2008). In *D. melanogaster* selected for starvation resistance, high

levels of starvation resistance relative to those from unselected lines were evident immediately after adult emergence, which suggested that metabolites permitting this phenotype were obtained during the larval stage (Chippindale et al., 1996). Subsequently, it has been shown that the contents of fat cells acquired during larval feeding permit survival after eclosion and before feeding in adult *D. melanogaster* (Aguila et al., 2013, 2007). Protein and carbohydrate content had no noticeable association with desiccation or starvation resistance. Inclusion of yeast in the larval diet and hydrolysed yeast in the adult diet of tephritids is usually linked with their need for protein (or more specifically, amino acids) during larval (Kaspi et al., 2002; Nestel et al., 2016), pupal (Nestel et al., 2004) and adult reproductive development (Taylor et al., 2013; Yuval et al., 2007). This was supported in *C. cosyra* with higher soluble protein content resulting from both the high yeast larval diet and inclusion of hydrolysed yeast in the adult diet. Development of *C. cosyra* was slower in the low yeast larval diet, so the limiting effect of fewer amino acids may have been overcome to some extent by compensatory feeding to acquire sufficient resources for successful pupal development, as in *C. capitata* (Nestel et al., 2016).

The findings of this study indicate that caution must be taken when extrapolating results from laboratory-reared insects to the field. In our study, the low yeast larval diet had a protein content similar to a preferred host fruit for *C. cosyra*, but the high yeast larval diet is the standard diet used to rear *C. cosyra* and other tephritid species in the laboratory. However, the standard diet is too rich and results in adults with lower stress resistance. Unfed flies lived longer when fed on a protein-restricted diet as larvae. Similarly, adults of a neriid fly, *Telostylinus angusticollis*, had shorter lifespans when fed protein-restricted diets as larvae (Runagall-McNaull et al., 2015). As noted earlier, similar results have also been found in *D. melanogaster* (Kristensen et al., 2016). Consequently, it is important to consider the influence of the larval diet when measuring stress resistance traits of newly emerged adults. Our results may also have implications for other species reared on high protein larval diets for sterile insect technique (SIT) programmes. The success of SIT programmes to achieve population suppression or extirpation relies on high survival rates of released insects in the potentially stressful field environment (Lance and McInnis, 2005), so the benefits of a larval diet that trades high rates of larval development for lower stress resistance need to be quantified. This will also affect the cost of fly production because yeast is the most expensive tephritid larval diet component (Nestel and Nemny-Lavy, 2008).

Conclusions

Larval diet has a short-term effect on desiccation and starvation resistance of *C. cosyra*. Adult diet does not compensate for deficiencies in desiccation or starvation resistance resulting from a larval diet suboptimal for these tolerance traits. On the contrary, over the period of time that *C. cosyra* were fed adult diets, their desiccation and starvation resistance declined irrespective of the diet offered to them. Water content plays a role in the ability of *C. cosyra* to withstand water loss, but this may be moderated by water loss rates and there is a need to investigate the pathways for water loss in this and other tephritid species. This study provided no evidence for metabolic water from stored nutrients extending survival under water stress, but showed that elevated lipid reserves likely contribute to starvation resistance.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.W.W., S.M., S.W.N.; Methodology: C.W.W., S.M., F.D., E.E.d.R., A.M., S.W.N.; Validation: C.W.W., E.E.d.R.; Formal analysis: C.W.W., F.D.; Investigation: S.M., F.D., E.E.d.R., K.M.; Resources: C.W.W., A.M., S.W.N.; Data curation: C.W.W.; Writing - original draft: C.W.W., E.E.d.R.; Writing - review & editing: C.W.W., S.M., K.M., A.M., S.W.N.; Visualization: C.W.W.; Supervision: C.W.W., S.W.N.; Project administration: C.W.W.; Funding acquisition: C.W.W., A.M., S.W.N.

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Data availability

Data are deposited and publicly available on UPSpace, the digital repository of the University of Pretoria: http://hdl.handle.net/2263/66649.

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.192534.supplemental

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