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Integrative Morphological and Genetic Characterisation of the Fish Parasitic Copepod *Ergasilus mirabilis* Oldewage & van As, 1987: Insights into Host Specificity and Distribution in Southern Africa

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Abstract: Ergasilids are external parasites typically found on the gills and fins of their hosts. In Africa, 19 species of *Ergasilus* von Nordmann, 1832 are reported. Of those, *Ergasilus mirabilis* Oldewage & van As, 1987 is one of the least host-specific, with a wide distribution range in southern Africa. As with most species in the genus, genetic data are not available to support the morphological placement of this species within the genus. Specimens representing *E. mirabilis* were obtained from the gills of *Clarias gariepinus* (Burchell, 1822) collected from several localities in South Africa and Zambia. Fish were dissected and gills screened using standard techniques. Following a comprehensive morphological study using light and scanning electron microscopy, additional morphological characters are reported. Furthermore, novel data on partial 18S, 28S (rRNA), and COI (mtDNA) gene regions are presented. This is the first integrative study on the morphology of *E. mirabilis* with supporting genetic data, as well as new distribution records from the KuShokwe Pan in the Phongolo River floodplain and the Vaal River in South Africa, and from the Barotse floodplain in Zambezi River, Zambia. An updated overview is provided for the species of *Ergasilus* from Africa, including hosts, distribution, and genetic information.

Keywords: freshwater biodiversity; integrative taxonomy; parasitic copepod; sharptooth catfish; Vaal River; Zambezi River

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1. Introduction

Parasitic copepods within the family Ergasilidae (Cyclopoida: Copepoda) are globally distributed parasites that mainly infest bony freshwater fishes, with few species found in brackish and marine hosts [1]. They feed on the host's tissue and typically attach themselves to the gills, fins, and occasionally the urinary bladder of their hosts [2–8]. The attachment of ergasilids may result in the compression of gill tissue [9], host immune responses such as increased production of mucous and rodlet cells [10,11] and necrosis of the gill filament, ultimately making hosts susceptible to secondary infections (see [10,12] and the references therein). Due to their importance in biodiversity studies and the economic importance of some species (such as *Ergasilus sieboldi* von Nordmann, 1832 and *E. lizae* Krøyer, 1863) in the aquaculture and fisheries industry, there have been numerous publications focusing on the taxonomy, feeding, pathology, and lifecycle of ergasilids [13–23]. The general body morphology of a typical ergasilid, whether male or female, is cyclopiform with a swelling in the prosome somites of females, and members of the Ergasilidae are characterised by the loss of the maxillipeds in females [1].

Among the 30 accepted genera in the family Ergasilidae [24], *Dermoergasilus* Ho & Do, 1982 (brackish and marine); *Ergasilus* von Nordmann, 1832 (freshwater, brackish and marine); *Neoergasilus* Yin, 1956 (freshwater); and *Paraergasilus* Markewitsch, 1937 (freshwater) are known from Africa [2–4,6,25,26]. *Ergasilus* was the first genus to be described within the family based on specimens of *Ergasilus gibbus* von Nordmann, 1832 and *E. sieboldi*. Globally, there are 162 accepted *Ergasilus* species known from marine, brackish, and freshwater environments [27]. To date, 19 species have been described from Africa (Table 1).

Ergasilus represents the most speciose ergasilid genus in Africa [4,6] (see [28]). Of importance to southern Africa is the freshwater species, *E. mirabilis*, first recorded in 1987 [29], with the most recent report being by Douëllou and Erlwanger [30]. This species has been reported to parasitise a wide range of hosts (mostly clariids, mochokids, and mormyrids) (see Table 1). Among the clariids, *Clarias gariepinus* (Burchell, 1822) is one of several fish species in southern Africa that have been translocated beyond the natural geographic range (see [31]) and is a frequently reported host for *E. mirabilis* (see Table 1). Similar to other species of *Ergasilus*, this widely distributed copepod lacks genetic data. Globally, only 10% of species in this genus have genetic data available, and there are only eleven sequences available in GenBank from Africa (see Table 1).

The use of genetic information to complement the taxonomic placement (based on morphology) of an ergasilid species is limited in Africa. Therefore, almost four decades after its discovery, this study provides an extension of the distribution of *E. mirabilis* in southern Africa, using an integrative taxonomic approach (providing morphological notes supplemented with data for partial 18S, 28S (rRNA) and COI (mtDNA) gene regions). Furthermore, the present study provides up-to-date information on hosts, distribution, and molecular data available for all African *Ergasilus* species (Table 1).

Table 1. Updated information for all 19 African *Ergasilus* von Nordmann, 1832 species with information on host species, host families, distribution, and available GenBank data. Information from the present study is represented in bold. Abbreviations: TH—Type Host; TLOC—Type Locality.

Species	Hosts	Distribution	Host Families	Water Body	Genetic data	References
Ergasilus brevimanus (Sars, 1909) **Syn**: *Ergasiloides brevimanus* Sars 1909	**TH**: Unknown	**TLOC**: Mbete, south shore of Lake Tanganyika	-	Freshwater	-	Sars [32]
	-	Lake Malawi	-	Freshwater	-	Sars [32]
	-	Angola: Dilolo Lake	-	Freshwater	-	Marques [33]
Ergasilus caparti Míč, Řehulková & Seifertová, 2023	**TH**: *Neolamprologus brichardi* (Poll, 1974)	**TLOC**: Magara, Lake Tanganyika, Burundi	Cichlidae	Freshwater	-	Míč et al. [34]
	Eretmodus marksmithi Burgess, 2012; *Lamprologus callipterus* Boulenger, 1906; *Neolamprologus mondabu* (Boulenger, 1906); *Perissodus microlepis* Boulenger, 1898; *Spathodus erythrodon* Boulenger, 1900	Burundi: Mukuruka, Mvugo, Nyaruhongoka (Lake Tanganyika)	Cichlidae	Freshwater	OQ407469 (18S); OQ407474 (28S)	Míč et al. [34]
Ergasilus cunningtoni Capart, 1944	**TH**: *Campylomormyrus elephas* (Boulenger, 1898)	**TLOC**: Lake Tumba, Ubangi River, Democratic Republic of the Congo	Mormyridae	Freshwater	-	Capart [35]
	Cyphomyrus psittacus (Boulenger, 1897); *Distichodus atroventralis* Boulenger, 1898; *Marcusenius greshoffii* (Schilthuis, 1891); *M. moorii* (Günther, 1867); *Mormyrops nigricans* Boulenger, 1899; *Petrocephalus grandoculis* Boulenger, 1916; *Pollimyrus isidori* (Valenciennes, 1847); *Pterochromis congicus* (Boulenger, 1897), *Schilbe laticeps* (Boulenger, 1899); *S. tumbanus* (Pellegrin, 1926), *Synodontis nigriventris* David, 1936; *Tylochromis microdon* Regan, 1920	Democratic Republic of the Congo: Lake Tumba, Ubangi River, Ikela, Tshuapa River & Mokombe River	Cichlidae; Distichodontidae; Mochokidae; Mormyridae; Schilbeidae	Freshwater	-	Fryer [36,37]
	Brycinus leuciscus (Günther, 1867); *B. nurse* (Rüppell, 1832); *Distichodus rostratus* Günther, 1864; *Pellonula leonensis* Boulenger, 1916	Ghana: Lake Volta	Alestidae; Distichodontidae; Dorosomatidae	Freshwater	-	Paperna [38]
	Brycinus nurse (Rüppell, 1832); *Enteromius macrops* (Boulenger, 1911); *Hydrocynus vittatus* Castelnau, 1861; *Mormyrops anguilloides* (Linnaeus, 1758); *Mormyrus macrophthalmus* Günther, 1866; *Raiamas senegalensis* (Steindachner, 1870)	Nigeria: Galma River, Zaria	Alestidae; Cyprinidae; Mormyridae	Freshwater	-	Shotter [39]
	Chrysichthys auratus (Geoffroy Saint-Hilaire, 1809)	Nigeria: Tiga Lake, Kano	Claroteidae	Freshwater	-	Ndifon & Jimeta [40]

Table 1. *Cont.*

Species	Hosts	Distribution	Host Families	Water Body	Genetic data	References
Ergasilus egyptiacus Abdel-Hady, Bayoumy & Osman, 2008	**TH**: *Coptodon zillii* (Gervais, 1848)	**TLOC**: Lake Temsah	Cichlidae	Freshwater	-	Abdel-Hady et al. [41]
Ergasilus flaccidus Fryer, 1965	**TH**: *Oreochromis tanganicae* (Günther, 1894)	**TLOC**: Lake Tanganyika	Cichlidae	Freshwater	-	Fryer [42]
Ergasilus ilani Oldewage & Van As, 1988	**TH**: *Mugil cephalus* Linnaeus, 1758	**TLOC**: Mgobezeleni Estuary, Sodwana Bay, South Africa	Mugilidae	Brackish; Freshwater	-	Oldewage & van As [3]
	M. cephalus Linnaeus, 1758	South Africa: Kowie River Estuary, Eastern Cape	Mugilidae	Brackish; Freshwater	-	Oldewage & van As [4]
	Chelon richardsonii (Smith, 1846)	South Africa: Berg River and Verlorevlei River, Western Cape	Mugilidae	Freshwater	-	Oldewage & van As [4]
Ergasilus inflatipes Cressey in Cressey & Collette, 1970	**TH**: *Strongylura senegalensis* (Valenciennes, 1864)	**TLOC**: Volta River, Ghana	Belonidae	Freshwater	-	Cressey & Collette [43]
	S. senegalensis (Valenciennes, 1864)	Ivory Coast: Ébrié Lagoon	Belonidae	Brackish; Marine	-	Cressey & Collette [43]
Ergasilus kandti van Douwe, 1912	**TH**: Unknown	**TLOC**: Lake Albert		Freshwater	-	van Douwe [44]
	Pseudosimochromis curvifrons (Poll, 1942)	Lake Tanganyika	Cichlidae	Freshwater	-	Capart [35]
	Lates niloticus (Linnaeus, 1758)	Mali: Niger River	Latidae		-	Capart [45]
	Pterochromis congicus (Boulenger, 1897)	Democratic Republic of the Congo: Lake Tumba, Ubangi River	Cichlidae	Freshwater	-	Fryer [36]
	Lamprologus lemairii Boulenger, 1899; *Lates niloticus* (Linnaeus, 1758); *Limnotilapia dardennii* (Boulenger, 1899); *Oreochromis tanganicae* (Günther, 1894); *Plecodus paradoxus* Boulenger, 1898	Lake Albert & Lake Tanganyika	Cichlidae; Latidae	Freshwater	-	Fryer [42]
	Tylochromis bangwelensis Regan, 1920; *T. mylodon* Regan, 1920;	Democratic Republic of the Congo: Lake Mweru and Luapula River	Cichlidae	Freshwater	-	Fryer [37]
	T. polylepis (Boulenger, 1900)	Tanzania: Malagarasi Delta	Cichlidae	Freshwater	-	Fryer [37]
	Citharinus citharus (Geoffroy St. Hilaire, 1809); *Lates niloticus* (Linnaeus, 1758); *Synodontis membranaceus* (Geoffroy Saint-Hilaire, 1809); *Schilbe intermedius* Rüppell, 1832	Ghana: Lake Volta	Citharinidae; Mochokidae; Latidae; Schilbeidae	Freshwater	-	Paperna [38]
	Bagrus bajad (Forsskål, 1775); *Lates niloticus* (Linnaeus, 1758)	Lake Albert	Bagridae	Freshwater	-	Thurston [46]
	L. niloticus (Linnaeus, 1758)	Egypt: Lake Nasser	Latidae	Freshwater	-	Hamouda et al. [47]

Table 1. *Cont.*

Species	Hosts	Distribution	Host Families	Water Body	Genetic data	References
Ergasilus lamellifer Fryer, 1961	**TH**: Various *Haplochromis* species	**TLOC**: Lake Victoria and the Victoria Nile	Cichlidae	Freshwater	-	Fryer [48]
	Parailia pellucida (Boulenger, 1901)	Ghana: Lake Volta	Schilbeidae	Freshwater	-	Paperna [38]
	Astatoreochromis alluaudi Pellegrin, 1904; *Haplochromis bicolor* Boulenger, 1906; *H. degeni* (Boulenger, 1906); *H. guiarti* (Pellegrin, 1904); *H. longirostris* (Hilgendorf, 1888); *H. nuchisquamulatus* (Hilgendorf, 1888); *H. obesus* (Boulenger, 1906); *H. obliquidens* (Hilgendorf, 1888); *H. retrodens* (Hilgendorf, 1888)	Lake Victoria and the Victoria Nile	Cichlidae	Freshwater	-	Thurston [46]
	Haplochromis spp.; *Haplochromis heusinkveldi* Witte & Witte-Maas, 1987; *H. hiatus* Hoogerhoud & Witte, 1981; *H. iris* Hoogerhoud & Witte, 1981; *H. macrognathus* Regan, 1922; *H. ptistes* Greenwood & Barel, 1978; *H. pyrrhocephalus* Witte & Witte-Maas, 1987; *H. teegelaari* Greenwood & Barel, 1978	Lake Victoria	Cichlidae	Freshwater	-	Witte & van Oijen [49]
	H. nyererei Witte-Maas & Witte, 1985	Tanzania: Makobe Island in the western Speke Gulf, Lake Victoria	Cichlidae	Freshwater	-	Maan et al. [50]
	H. nyererei Witte-Maas & Witte, 1985; *H. pundamilia* (Seehausen & Bouton, 1998)	Tanzania: Makobe Island, south-eastern Lake Victoria	Cichlidae	Freshwater	-	Maan et al. [51]
	Haplochromis chilotes (Boulenger, 1911); *Haplochromis mbipi* (Lippitsch & Bouton, 1998); *Haplochromis nyererei* Witte-Maas & Witte, 1985; *Haplochromis omnicaeruleus* (Seehausen & Bouton, 1998); *Haplochromis pundamilia* (Seehausen & Bouton, 1998); *Haplochromis rufocaudalis* (Seehausen & Bouton, 1998); *Haplochromis sauvagei* (Pfeffer, 1896); *Neochromis* sp.; *Pundamilia* sp.	Tanzania: Lake Victoria	Cichlidae	Freshwater	-	Karvonen et al. [52]; Gobbin et al. [53]
	Clarias gariepinus (Burchell, 1822); *Haplochromis* spp.; *Oreochromis esculentus* (Graham, 1928); *Protopterus aethiopicus* Heckel, 1851	Kenya: Lake Kanyaboli	Cichlidae; Clariidae; Protopteridae	Freshwater	-	Mwamburi et al. [54]
	Oreochromis niloticus (Linnaeus, 1758)	Kenya: Lake Victoria	Cichlidae	Freshwater	-	Mwainge et al. [55]; Outa et al. [56]

Table 1. *Cont.*

Species	Hosts	Distribution	Host Families	Water Body	Genetic data	References
Ergasilus latus Fryer, 1960	**TH**: *Oreochromis niloticus* (Linnaeus, 1758); *Sarotherodon galilaeus* (Linnaeus, 1758)	**TLOC**: Lake Turkana, Kenya	Cichlidae	Freshwater	-	Fryer [57]
	S. nigripinnis (Guichenot, 1861); *Pelmatolapia cabrae* (Boulenger, 1899)	Kitona, Moanda, and Bulambemba, near the Congo River mouth; Nile River	Cichlidae	Brackish; Freshwater	-	Fryer [37,58]
	Coptodon guineensis (Günther, 1862); *C. zillii* (Gervais, 1848); *Oreochromis niloticus* (Linnaeus, 1758); *Sarotherodon melanotheron* Rüppell, 1852	Ghana: Volta Basin and Peshi Lagoon	Cichlidae	Brackish; Freshwater	-	Paperna [38]
	Auchenoglanis occidentalis (Valenciennes, 1840); *Coptodon zillii* (Gervais, 1848); *Oreochromis niloticus* (Linnaeus, 1758); *Sarotherodon galilaeus* (Linnaeus, 1758); *Schilbe mystus* (Linnaeus, 1758)	Nigeria: Galma River	Claroteidae; Cichlidae; Schilbeidae	Freshwater	-	Shotter [39]
	Chrysichthys nigrodigitatus (Lacepède, 1803)	Nigeria: Cross River estuary	Claroteidae	Brackish	-	Obiekezie et al. [59]
	Mugil cephalus Linnaeus, 1758; *Neochelon falcipinnis* (Valenciennes, 1836)	Republic of Benin: Ganvie, Djdje and Zogbo, Lake Nokoue Lagoon	Mugilidae	Brackish	-	Aladetohun et al. [60]
	M. cephalus Linnaeus, 1758; *N. falcipinnis* (Valenciennes, 1836)	Nigeria: Makoko, Mcquin, and University of Lagos lagoon	Mugilidae	Brackish	-	Aladetohun et al. [61]
	Sarotherodon melanotheron Rüppell, 1852	Ghana: Oyibi, Fosu, Apabaka, Kpeshie, Sakumo, and Keta Lagoons	Cichlidae	Brackish	-	Rokicki et al. [62]
	Lates niloticus (Linnaeus, 1758)	Egypt: Lake Nasser	Latidae	Freshwater	-	Hamouda et al. [47]
	Sarotherodon melanotheron Rüppell, 1852	Côte d'Ivoire: Ebrie Lagoon	Cichlidae	Brackish	-	Adou et al. [63]
Ergasilus lizae Krøyer, 1863 **Syn**: *Ergasilus nanus* Beneden, 1870	**TH**: *Mugil liza* Valenciennes, 1836	**TLOC**: New Orleans, USA	Mugilidae	Marine	-	Krøyer [64]
	Alosa fallax (Lacepéde, 1803); *Barbus barbus* (Linnaeus, 1758); *Chelon ramada* (Risso, 1827); *C. saliens* (Risso, 1810); *Mugil cephalus* Linnaeus, 1758; *Solea solea* (Linnaeus, 1758)	Tunisia: Gulf of Gabès & Lake Ichkeul	Alosidae; Cyprinidae; Mugilidae; Soleidae	Brackish; Marine	-	Raïbaut et al. [65]
	M. cephalus Linnaeus, 1758	Algeria: Gulf of Annaba, East coast	Mugilidae	Marine	-	Boualleg et al. [66]
	M. cephalus Linnaeus, 1758; *Neochelon falcipinnis* (Valenciennes, 1836)	Republic of Benin: Ganvie, Djdje and Zogbo, Lake Nokoue Lagoon	Mugilidae	Brackish	-	Aladetohun et al. [60]
	Mugil cephalus Linnaeus, 1758; *Neochelon falcipinnis* (Valenciennes, 1836)	Nigeria: Makoko, Mcquin, and University of Lagos lagoon	Mugilidae	Brackish	-	Aladetohun et al. [61]

Table 1. *Cont.*

Species	Hosts	Distribution	Host Families	Water Body	Genetic data	References
Ergasilus lizae Krøyer, 1863 **Syn**: *Ergasilus nanus* Beneden, 1870	*Synodontis schall* (Bloch & Schneider, 1801)	Nigeria: Nsidung beach, Cross River Estuary	Mochokidae	Brackish	-	Eyo & Effanga [67]
	Clarias gariepinus (Burchell, 1822)	Nigeria: Lake Gerio, Yola, Adamawa	Clariidae	Freshwater	-	Amos et al. [68]
	Coptodon zillii (Gervais, 1848)	Egypt: Lake Maruit	Cichlidae	Freshwater	-	Mitwally et al. [69]
Ergasilus macrodactylus (Sars, 1909) **Syn**: *Ergasiloides macrodactylus* Sars, 1909	**TH**: Unknown	**TLOC**: Sumbu, south-western shore of Lake Tanganyika		Freshwater	-	Sars [32]
	Brycinus imberi (Peters, 1852); *Haplochromis* spp.; *Lethrinops* spp.; *Tilapia* spp.	Lake Malawi	Alestidae; Cichlidae	Freshwater	-	Fryer [70]
	Eretmodus marksmithi Burgess, 2012; *Gnathochromis permaxillaris* (David, 1936); *Lamprologus callipterus* Boulenger, 1906; *Perissodus microlepis* Boulenger, 1898; *Tanganicodus irsacae* Poll, 1950	Burundi: Magara, Mvugo, Nyaruhongoka (Lake Tanganyika)	Cichlidae	Freshwater	OQ407465 (18S) OQ407470 (28S)	Míč et al. [34]
Ergasilus megacheir (Sars, 1909) **Syn**: *Ergasiloides megacheir* Sars, 1909	**TH**: Unknown	**TLOC**: Sumbu, south-western shore of Lake Tanganyika	-	Freshwater	-	Sars [32]
	Pseudosimochromis curvifrons (Poll, 1942)	Lake Tanganyika	Cichlidae	Freshwater	-	Capart [35]
	Pterochromis congicus (Boulenger, 1877)	Democratic Republic of the Congo: Lake Tumba	Cichlidae	Freshwater	-	Fryer [36]
	Bathybates fasciatus Boulenger, 1901; *Bathybates minor* Boulenger, 1906; *Cyphotilapia frontosa* (Boulenger, 1906); *Haplotaxodon microlepis* Boulenger, 1906; *Limnotilapia dardennii* (Boulenger, 1899); *Plecodus paradoxus* Boulenger 1898; *Synodontis granulosus* Boulenger, 1900; *S. multipunctatus* Boulenger, 1898	Lake Tanganyika	Cichlidae; Mochokidae	Freshwater	-	Fryer [42]
	Shuja horei (Günther, 1894); *Simochromis diagramma* (Günther, 1894)	Burundi: Magara, Nyaruhongoka (Lake Tanganyika)	Cichlidae	Freshwater	OQ407466 (18S) OQ407471 (28S)	Míč et al. [34]

Table 1. *Cont.*

Species	Hosts	Distribution	Host Families	Water Body	Genetic data	References
Ergasilus mirabilis Oldewage & van As, 1987	**TH**: *Synodontis leopardinus* Pellegrin, 1914	**TLOC**: Phongolo flood plains on the Makatini Flats, South Africa	Mochokidae	Freshwater	-	Oldewage & Van As [29]
	Brycinus imberi (Peters, 1852); *Clarias gariepinus* (Burchell, 1822); *C. ngamensis* Castelnau, 1861; *Enteromius afrohamiltoni* (Crass, 1960); *Glossogobius giuris* (Hamilton, 1822); *Hydrocynus vittatus* Castelnau, 1861; *Labeo rosae* Steindachner, 1894; *Schilbe intermedius* Rüppell, 1832; *Synodontis zambezensis* Peters, 1852	South Africa: Limpopo River & Phongolo River System	Alestidae; Clariidae; Cyprinidae; Gobiidae; Schilbeidae	Freshwater	-	Oldewage & Van As [4]
	Clarias gariepinus (Burchell, 1822); *C. ngamensis* Castelnau, 1861; *Hemichromis elongatus* (Guichenot, 1861); *Hepsetus odoe* (Bloch, 1794); *Marcusenius macrolepidotus* (Peters, 1852); *Schilbe intermedius* Rüppell, 1832; *S. mystus* (Linnaeus, 1758); *Synodontis leopardinus* Pellegrin, 1914; *S. macrostigma* Boulenger, 1911; *S. nigromaculatus* Boulenger, 1905	Namibia: Zambezi River, Caprivi	Cichlidae; Clariidae; Hepsetidae; Mochokidae; Mormyridae; Schilbeidae	Freshwater	-	Oldewage & Van As [4]
	Synodontis zambezensis Peters, 1852	Mozambique: Lake Malawi	Mochokidae	Freshwater	-	Oldewage & Van As [4]
	Cyphomyrus discorhynchus (Peters, 1852)	Zimbabwe: Lake Kariba	Mormyridae	Freshwater	-	Oldewage & Van As [4]
	Clarias gariepinus (Burchell, 1822); *Marcusenius macrolepidotus* (Peters, 1852); *Petrocephalus catostoma* (Günther, 1866); *Synodontis nigromaculatus* Boulenger, 1905	Namibia: Kwando River, Caprivi	Clariidae; Mochokidae; Mormyridae	Freshwater	-	Avenant-Oldewage & Oldewage [5]
	Cyphomyrus discorhynchus (Peters, 1852)	Zimbabwe: Lake Kariba	Mormyridae	Freshwater	-	Douëllou & Erlwanger [30]
	Clarias gariepinus **(Burchell, 1822)**	**South Africa: Kushokwe Pan**	**Clariidae**	**Freshwater**	**-**	**Present study**
	C. gariepinus **(Burchell, 1822)**	**South Africa: Vaal River**	**Clariidae**	**Freshwater**	**OR449753 (18S); OR449755 (28S); OR448769 (COI)**	**Present study**
	C. gariepinus **(Burchell, 1822)**	**Zambia: Zambezi River**	**Clariidae**	**Freshwater**	**OR449754 (18S); OR449756 (28S); OR448770 (COI)**	**Present study**
Ergasilus nodosus Wilson, 1924	**TH**: *Bagrus bajad* (Forsskål, 1775)	**TLOC**: White Nile, Omdurman, Sudan	Bagridae	Freshwater	-	Wilson [71]
	Bagrus sp.	Ghana: Sielo Tuni Stream	Bagridae	Freshwater	-	Fryer [36]

Table 1. *Cont.*

Species	Hosts	Distribution	Host Families	Water Body	Genetic data	References
Ergasilus parasarsi Míč, Řehulková & Seifertová, 2023	**TH**: *Simochromis diagramma* (Günther, 1894)	**TLOC**: Magara, Lake Tanganyika, Burundi	Cichlidae	Freshwater	-	Míč et al. [34]
	Eretmodus marksmithi Burgess, 2012; *Gnathochromis permaxillaris* (David, 1936); *Lamprologus callipterus* Boulenger, 1906; *Ophthalmotilapia nasuta* (Poll & Matthes, 1962); *Perissodus microlepis* Boulenger, 1898; *Tanganicodus irsacae* Poll, 1950	Burundi: Mukuruka, Nyaruhongoka (Lake Tanganyika)	Cichlidae	Freshwater	OQ407467 (18S) OQ407473 (28S)	Míč et al. [34]
Ergasilus parvus Míč, Řehulková & Seifertová, 2023	**TH**: *Spathodus erythrodon* Boulenger, 1900	**TLOC**: Magara, Lake Tanganyika, Burundi	Cichlidae	Freshwater	-	Míč et al. [34]
	Bathybates ferox Boulenger, 1898; *Eretmodus marksmithi* Burgess, 2012; *Lamprologus callipterus Boulenger, 1906; Neolamprologus brichardi* (Poll, 1974); *Neolamprologus mondabu* (Boulenger, 1906)	Burundi: Bujumbura fish market, Nyaruhongoka (Lake Tanganyika)	Cichlidae	Freshwater	OQ407468 (18S) OQ407472 (28S)	Míč et al. [34]
Ergasilus sarsi Capart, 1944	**TH**: *Tylochromis mylodon* Regan, 1920	**TLOC**: Katanga, Democratic Republic of the Congo	Cichlidae	Freshwater	-	Capart [35]
	Clarias ngamensis Castelnau, 1861; *Marcusenius macrolepidotus* (Peters, 1852); *Synodontis nigromaculatus* Boulenger, 1905	Lake Bangwelu	Clariidae; Mochokidae; Mormyridae	Freshwater	-	Fryer [72]
	Thoracochromis moeruensis (Boulenger, 1899); *Tylochromis bangwelensis* Regan, 1920; *T. mylodon* Regan, 1920	Democratic Republic of the Congo: Lake Mweru and Luapula River	Cichlidae	Freshwater	-	Fryer [37]
	Clarias gariepinus (Burchell, 1822)	Ghana: Mawli River	Clariidae	Freshwater	-	Paperna [38]
	Clarias anguillaris (Linnaeus, 1758); *Heterobranchus bidorsalis* Geoffroy Saint-Hilaire, 1809	Nigeria: River Galma, small lakes around Zaria	Clariidae	Freshwater	-	Shotter [39]
	Clarias gariepinus (Burchell, 1822)	Nigeria: Bagauda fish farm, Kano	Clariidae	Freshwater	-	Bichi & Yelwa [73]
	Lamprichthys tanganicanus (Boulenger, 1898)	Democratic Republic Congo: Lake Tanganyika	Procatopodidae	Freshwater	-	Kilian & Avenant-Oldewage [12]
	Oreochromis niloticus (Linnaeus, 1758)	Egypt: Mariotteya Stream	Cichlidae	Freshwater	-	Mahmoud et al. [74]
	O. niloticus (Linnaeus, 1758)	Egypt: River Nile Branch (Bahr Nashart), Drainage canal (Damroo Drainage canal), and Fish farm	Cichlidae	Freshwater	-	El-Seify et al. [75]

Table 1. *Cont.*

Species	Hosts	Distribution	Host Families	Water Body	Genetic data	References
Ergasilus sieboldi von Nordmann, 1832 **Syn**: *Ergasilus baicalensis* Messjatzeff, 1928 **Syn**: *Ergasilus depressus* Sars, 1863 **Syn**: *Ergasilus esocis* Sumpf, 1871 **Syn**: *Ergasilus hoferi* Borodin, 1915 **Syn**: *Ergasilus surbecki* Baumann, 1913 **Syn**: *Ergasilus trisetaceus* von Nordmann, 1832	**TH**: pike, bream, and carp	**TLOC**: Europe	Cyprinidae; Percidae	Marine	-	von Nordmann [76]
	-	Angola: Dilolo Lake	-	Freshwater	-	Marques [34]
	Cyprinus carpio (Linnaeus, 1758)	Algeria: Foum El Khanga reservoir, Souk Ahras	Cyprinidae	Freshwater	-	Boucenna et al. [77]
	Luciobarbus callensis (Valenciennes, 1842)	Algeria: Beni-Haroun Dam, Mila city	Cyprinidae	Freshwater	-	Boucenna et al. [78]
	Bagrus bajad (Fabricius, 1775)	Egypt: Lake Nasser	Bagridae	Freshwater	-	Hamouda [79]
	Sparus aurata Linnaeus, 1758	Egypt: Semi-intensive marine fish farms	Sparidae	Marine	OM812074 (28S)	Abdel-Radi et al. [80]
	Carassius carassius (Linnaeus, 1758)	Algeria: Beni-Haroun Dam, Mila city	Cyprinidae	Freshwater	-	Berrouk et al. [25,81]

2. Materials and Methods

2.1. Sampling

As part of a larger parasitology project, a total of 157 *Clarias gariepinus* specimens were caught between 2018 and 2020 from ten localities in southern Africa (Figure 1), using various sampling methods: rod and reel, baited longlines, gill nets, and fyke nets (see [82]). This study received the necessary ethical clearance (Ethics No. NWU-00159-18-A5) and permits: Ezemvelo KZN Wildlife (KwaZulu-Natal, permit Nos. OP 1075/2017, OP 1582/2018); Department of Rural, Environmental and Agricultural Development (North West, permit no. HO 20/02/18-057 NW); the Department of Economic, Small Business Development, Tourism and Environmental Affairs (DESTEA, Free State, permit no. JM 4066/2018); the Department of Economic Development, Environmental Affairs and Tourism (Eastern Cape, permit no. CRO 20/18CR, CRO 22/18CR) and CapeNature (Western Cape, permit no. CN44-31-6790); and permission for joint research in the Upper Zambezi Basin, Zambia. Host nomenclature is from FishBase [83].

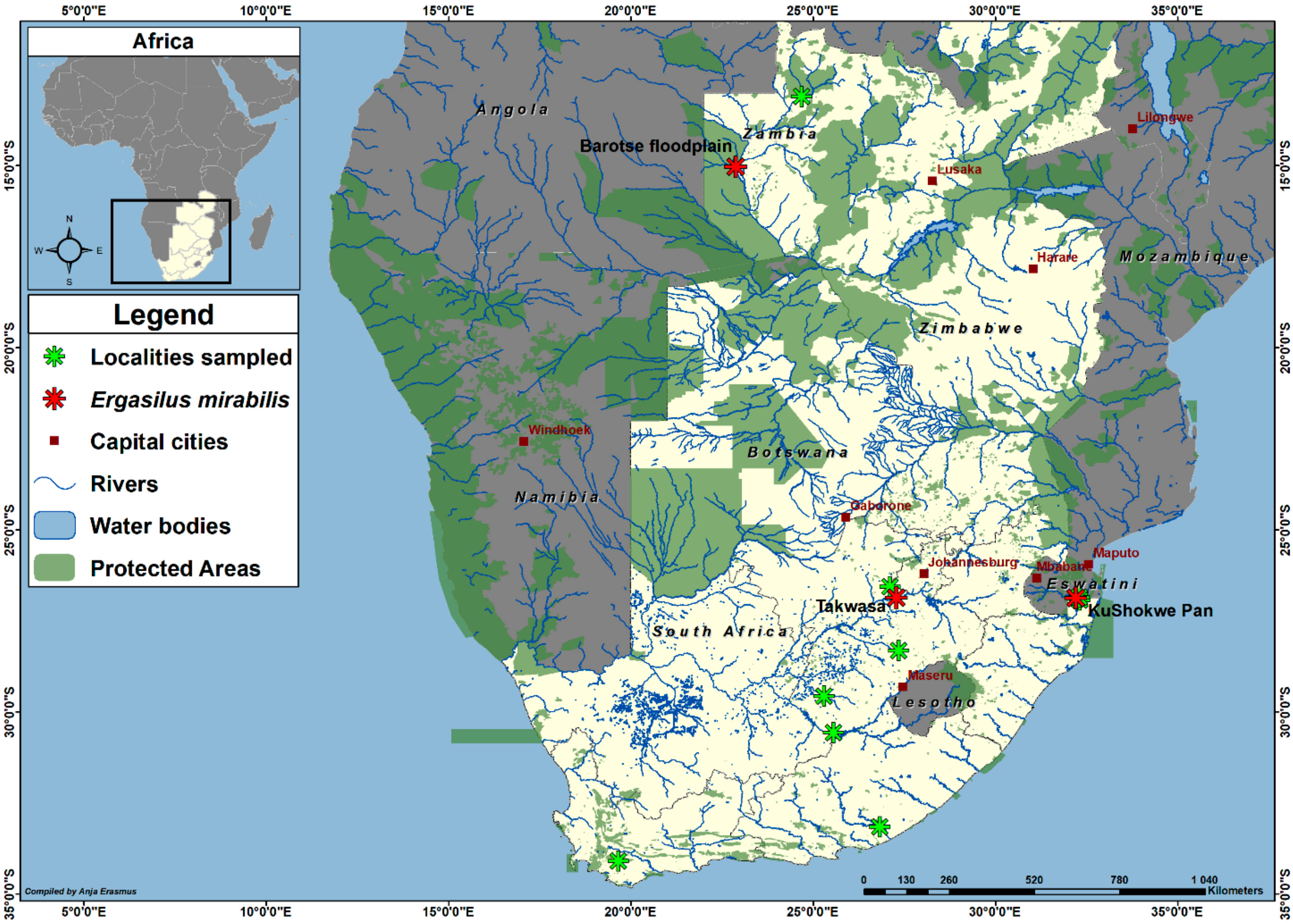

Figure 1. Map of all sampling localities from this study, with star icons in red representing sites where adult female ergasilids were collected.

2.2. Morphological Analysis

Fish gills were removed and screened for parasites with the aid of a Zeiss Stemi 305 compact stereomicroscope (Zeiss, Oberkochen, Germany), and collected copepods were preserved in 70% ethanol for further analysis. Photomicrographs were taken with a ZEISS Axiocam ERc 55 camera attached to the Zeiss Stemi 508 stereomicroscope. Measurement was given in millimetres and expressed as mean ± standard deviation (with range in parentheses). The total lengths of specimens were measured from the anterior margin of the cephalosome to the posterior margin of the caudal rami.

Selected specimens were cleared in lactic acid, stained with lignin pink, and dissected. Specimens were temporarily mounted with glycerine and studied using a Nikon Eclipse *Ni* microscope (Nikon Instruments, Tokyo, Japan), further applying the z-dimensional stacking function for differential interference contrast micrographs of different taxonomic structures. Drawings of specimens and dissected appendages were made with the aid of a drawing tube. Terminologies for the description of body somites and cephalic appendages in this manuscript follow Boxshall [20].

For scanning electron microscopy (SEM), 13 adult females were studied. Specimens were dehydrated through a graded ethanol series, followed by a series of graded Hexamethyldisilazane, and allowed to dry. Specimens were mounted on aluminium stubs using carbon tape, gold palladium, and observed using a JEOL Winsem JSM IT 200. Photomicrographs of selected features were taken at 5Kva.

2.3. Infestation Rates

Infestation levels were expressed as prevalence (P), mean abundance (MA), and mean intensity (MI), following definitions from Bush et al. [84]; calculations for each are provided in parentheses.

2.4. Molecular Analysis

Genomic DNA extraction was performed using non-ovigerous females from the Zambezi River and egg strings from the Vaal River. The extraction followed the protocol of the Macherey-Nagel NucleoSpin® Tissue extraction kit (GmbH & Co. KG, Sandton, South Africa), with a pre-lysis period of 3–4 h. For partial gene amplification, three gene regions were targeted: two ribosomal RNA gene regions (18S and 28S) and one mitochondrial DNA gene region (cytochrome *c* oxidase I or COI). Polymerase Chain Reactions (PCR) for 18S and 28S utilised primers (18SF, 18SR; and 28SF, 28SR) prepared by Song et al. [85]. COI reactions used the universal mitochondrial primers LCO1490, HCO2198 [86] (see Table 2). Amplification reactions for each gene region were carried out in 25 µL volumes using: 12.5 µL of DreamTaq PCR Master Mix (2X) (ThermoFischer Scientific, Waltham, MA, USA), 1.25 µL of 10 µM of each primer, 3 µL of DNA product and 7 µL of double distilled water. Thermocycling conditions followed Song et al. [85] for the 18S and 28S rRNA gene regions and Hayes et al. [87] for the COI gene regions. Positive PCR products were verified by 1% agarose gel electrophoresis and sent to the commercial sequencing company Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa) for purification and sequencing in both directions.

Table 2. List of primers used for DNA amplification of *Ergasilus mirabilis* Oldewage & van As, 1987 with sequences and references, used in the amplification of partial 18S, 28S, and COI genes in this study.

Gene Regions	Primers	Sequences	Sources
18S	18SF	5′-AAG GTG TGM CCT ATC AAC T-3′	Song et al. [85]
	18SR	5′-TTA CTT CCT CTA AAC GCT C-3′	
28S	28SF	5′-ACA ACT GTG ATG CCC TTA G-3′	
	28SR	5′-TGG TCC GTG TTT CAA GAC G-3′	
COI	LCO1490	5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′	Folmer et al. [86]
	HCO2198	5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′	

Using Geneious Prime v. 2022.2.2 (Biomatters, Auckland, New Zealand), newly generated forward and reverse sequences were assembled, aligned, edited, and trimmed. Using the nucleotide Basic Local Alignment Search Tool (BLAST) *Lernaea cyprinacea* Linnaeus, 1758 (Lernaeidae Cobbold, 1879) was used as the outgroup for all three gene regions (Table 3).

Due to the limited number of COI sequences available, unpublished sequences of *Ergasilus* species that occur in Africa and were available in the Barcode of Life Database (BOLD) were also included in the COI alignment (see Table 3).

Following the default parameters implemented by MAFFT v7.490 [88,89], the alignments for novel sequences were generated and trimmed. Genetic divergences among aligned specimens were calculated in Geneious Prime v. 2022.2.2 and expressed as percentage similarities and differences in the number of bases. An estimation of the best nucleotide substitution model for each dataset was determined using the Akaike Information Criterion (AIC) implemented in the jModelTest 2.1.4 [90,91]. The suggested model for all datasets (18S, 28S, COI) was the general time-reversible model incorporating invariant sites and gamma-distributed among site rate variations (GTR+I+G). For phylogenetic analyses, Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were run using this suggested model of nucleotide evolution. Bayesian Inference (BI) analyses were executed on the computational resource CIPRES Science Gateway v 3.3 [92] adapting MrBayes v. 3.2.7a. set parameters [93], running two independent Markov Chain Monte Carlo (MCMC) runs of four chains for 10 million generations and sampling tree topologies every 1000 generations. Burn-in parameters were set to the first 25,000 generations. Maximum Likelihood analyses were run using PhyML v. 3.0 [94], on the ATGC bioinformatics platform with estimated model parameters and bootstrap values of 1000 repetitions. Nodal support for ML analyses was estimated at 100 bootstrap repetitions. Phylogenetic trees for BI and ML outputs were visualised in FigTree v 1.4.4 software [95].

Table 3. List of GenBank and Barcode of Life Database (BOLD) Ergasilidae sequences included in the phylogenetic analyses. The taxa in bold fonts are sequences generated from the present study, all other sequences are GenBank and BOLD sequences. *Lernaea cyprinacea* Linneaus, 1758 (in grey shade) was used as the outgroup.

Taxon	Host	Locality	GenBank Accession Numbers			Reference
			18S	28S	COI	
Acusicola margulisae	*Amphilophus citrinellus*, *Parachromis managuensis*, *Oreochromis* sp., *Poecilia exicana*	Nicaragua	MN852694	MN852851	MN854870	Santacruz et al. [96]
Ergasilus anchoratus	*Pseudobagrus fulvidraco*	China	DQ107564	DQ107528	-	Song et al. [85]
Ergasilus briani	*Misgurnus anguillicaudatus*	China	DQ107572	DQ107532	-	Song et al. [85]
Ergasilus caparti	*Neolamprologus brichardi*	Burundi	OQ407469	OQ407474	-	Míč et al. [34]
Ergasilus hypomesi	*Acanthogobius hasta*	China	DQ107573	DQ107539	-	Song et al. [85]
* *Ergasius lizae*	*Fundulus diaphanus*	Canada	-	-	ECTCR024-14	BOLD [97]
Ergasilus macrodactylus	*Gnathochromis permaxillaris*	Burundi	OQ407465	OQ407470	-	Míč et al. [34]
Ergasilus megacheir	*Simochromis diagramma*	Burundi	OQ407466	OQ407471	-	Míč et al. [34]
Ergasilus mirabilis	***Clarias gariepinus***	**Vaal River, South Africa**	**OR449753**	**OR449755**	**OR448769**	**Present study**
Ergasilus mirabilis	***Clarias gariepinus***	**Zambezi River, Zambia**	**OR449754**	**OR449756**	**OR448770**	**Present study**
Ergasilus parasarsi	*Simochromis diagramma*	Burundi	OQ407467	OQ407473	-	Míč et al. [34]
Ergasilus parvus	*Spathodus erythrodon*	Burundi	OQ407468	OQ407472	-	Míč et al. [34]
** *Ergasilus parasiluri*	*Tachysurus fulvidraco*	China	DQ107567	DQ107536	-	Song et al. [85]
Ergasilus peregrinus	*Siniperca chuatsi*	China	DQ107577	DQ107531	-	Song et al. [85]
Ergasilus scalaris	*Tachysurus dumerili*	China	DQ107565	DQ107538	-	Song et al. [85]
Ergasilus sieboldi	*Perca fluviatilis*	Czech Republic	MW810238	MW810242	-	Kvach et al. [98]
Ergasilus sieboldi	*Sparus aurata*	Egypt	-	OM812074	-	Abdel-Radi et al. [80]
Ergasilus sp.	Free-living	South Korea	-	-	KR049035	Baek et al. [99]
Ergasilus sp.	*Mugil liza*	Argentina	-	-	KU557411	Castro-Romero et al. [100]
Ergasilus tumidus	*Acanthorhodeus taenianalis*	China	DQ107569	DQ107535	-	Song et al. [85]
Ergasilus wilsoni	Free-living	South Korea	-	-	KR049036	Baek et al. [99]
Ergasilus yaluzangbus	*Gymnocypris stewartii*	China	DQ107578	DQ107540	-	Song et al. [85]
*** *Ergasilus yandemontei*	*Odontesthes hatcheri*	Argentina	MT969345	-	-	Waicheim et al. [23]
Neoergasilus japonicus	*Lepomis gibbosus*	Czech Republic	MH167969	MH167967	-	Ondračková et al. [101]
Neoergasilus japonicus	*Lepomis gibbosus*	Czech Republic	MH167970	MH167968	-	Ondračková et al. [101]
Neoergasilus japonicus	*Lepomis gibbosus*	Czech Republic	MW810236	MW810240	-	Kvach et al. [98]

Table 3. *Cont.*

Taxon	Host	Locality	GenBank Accession Numbers			Reference
			18S	28S	COI	
Neoergasilus japonicus	*Lepomis gibbosus, Scardinius erythrophthalmus*	Czech Republic	MW810237	MW810241	-	Kvach et al. [98]
Neoergasilus japonicus	Collected by plankton net	USA	-	-	MZ964935	Vasquez et al. [102]
Neoergasilus japonicus	Free-living	South Korea	-	-	KR049037	Baek et al. [99]
Paraergasilus brevidigitus	*Cyprinus carpio*	China	DQ107576	DQ107530	-	Song et al. [85]
Paraergasilus longidigitus	*Abramis brama, Perca fluviatilis, Scardinius erythrophthalmu*	Czech Republic	MW810239	MW810243	-	Kvach et al. [98]
Paraergasilus medius	*Ctenopharyngodon idellus*	China	DQ107574	DQ107529	-	Song et al. [85]
Sinergasilus major	*Ctenopharyngodon idella*	China	DQ107560	DQ107524	-	Song et al. [85]
Sinergasilus major	*Silurus glanis*	Hungary	MZ047814	MZ047815	-	Dos Santos et al. [103]
Sinergasilus polycolpus	*Hypophthalmichthys molitrix*	China	DQ107563	DQ107525	-	Song et al. [85]
Sinergasilus polycolpus	*Hypophthalmichthys molitrix*	China	-	-	KR263117	Feng et al. [104]
Sinergasilus undulatus	*Cyprinus carpio*	China	DQ107561	DQ107526	-	Song et al. [85]
Sinergasilus undulatus	*Cyprinus carpio*	China	-	-	MW080644	Hua et al. [105]
Lernaea cyprinacea	*Carassius auratus, Cyprinus carpio, Chanodichthys ilishaeformis*	China	MH982195	MH982204	MH982220	Hua et al. [106]

* Taxon from the Barcode of Life Database (BOLD); ** *Ergasilus parasiluri* (published on GenBank as its synonym *Pseudergasilus parasiluri*); *** *Ergasilus yandemontei* (Published on GenBank as *Ergasilus* sp.).

3. Results

3.1. Taxonomy

Order Cyclopoida Burmeister, 1834
Family Ergasilidae Burmeister, 1835
Genus *Ergasilus* von Nordmann, 1832
Type species: *Ergasilus gibbus* von Nordmann, 1832 and *Ergasilus sieboldi* von Nordmann, 1832.

Generic remarks.

Individuals from the genus *Ergasilus* are characterised by an elongate cyclopoid body form. Antennules are usually six-segmented and ornamented with setae, although a few species have five-segmented antennules, i.e., *E. flaccidus*, *E. ilani*, *E. inflatipes*, *E. nodosus* from Africa; *E. pitalicus* Thatcher, 1984 from Brazil; and *E. wilsoni* Markewitsch, 1933 from the Black Sea. The antennae of *Ergasilus* species are typically devoid of any cuticular covering and its terminal segment is sclerotised, with a single point. The fourth swimming legs usually have only two-segmented exopodites.

In addition to the characteristics listed above, individuals of the genus *Ergasilus* are further differentiated by several characteristics from the four other African genera. Individuals from the genus *Dermoergasilus* have a characteristic cuticular membrane covering the antennae, which is absent in species of *Ergasilus.* Species of the genus *Neoergasilus* are characterised by short and strongly curved antennae, as opposed to the long slender antennae found in most species of *Ergasilus.* Furthermore, the first legs of individuals of *Neoergasilus* have a triangular protrusion at the posterior margin of the basiopodite (in between the exopod and the endopod), and the second segment of the exopod is characterised by a spatulate spine, extending parallel to the length of the third exopod segment. These features of leg 1 are absent in individuals from the genus *Ergasilus.* Lastly, species of *Ergasilus* are characterised by a single claw, compared to *Paraergasilus*, which has three prongs for its terminal antennal segment.

***Ergasilus mirabilis* Oldewage & Van As, 1987**
Figures 2–6

Type host: Synodontis zambezensis Peters, 1851 (incorrectly identified as *Synodontis leopardinus* Pellegrin, 1914).

Other hosts: Brycinus imberi (Peters, 1852); *Clarias gariepinus* (Burchell, 1822); *Clarias ngamensis* Castelnau, 1861; *Cyphomyrus discorhynchus* (Peters, 1852); *Enteromius afrohamiltoni* (Crass, 1960); *Glossogobius giuris* (Hamilton, 1822); *Hemichromis elongatus* (Guichenot, 1861); *Hepsetus cuvieri* (Castelnau, 1861); *Hydrocynus vittatus* Castelnau, 1861; *Labeo rosae* Steindachner, 1894; *Marcusenius macrolepidotus* (Peters, 1852); *Petrocephalus catostoma* (Günther, 1866); *Schilbe intermedius* Rüppell, 1832; *Schilbe mystus* (Linnaeus, 1758); *Synodontis macrostigma* Boulenger, 1911; *Synodontis nigromaculatus* Boulenger, 1905.

Type locality: Phongolo River, northern Natal, South Africa.

Other localities: Mozambique—Lake Malawi; South Africa—**Kushokwe Pan (present study)**, Limpopo River; **Vaal River (present study)**; Namibia—the Zambezi region (previously known as Caprivi strip): Chobe River, Kwando River, Lake Liambezi, Lake Lisikili, Zambezi River; Zambia—**Barotse floodplain (present study)**; Zimbabwe—Lake Kariba [3–5].

Material examined.

A total of 184 ergasilids (151 adult females and 33 copepodites/males) were collected. Only adult females were examined: 13 were used for SEM; nine for dissection; eight adult females and five egg strings were used for DNA extraction; 10 were deposited

in the parasitological collections of the National Museum, Bloemfontein, South Africa (NMB: P-969); the remaining specimens are in the possession of the Water Research Group, North-West University, Potchefstroom, South Africa.
Zambia: One hundred and sixty-four copepods (164; 146 females, 25 examined) were collected from the Barotse floodplain, Zambezi River, Western Province, Zambia (15°12′01.59″ S 22°58′09.27″ E), from four *C. gariepinus*, col. 2019 M. Truter.
South Africa: Seventeen copepods (17; three females, three examined) were collected from the Vaal River (Takwasa Youth Camp), Venterskroon, North West Province, South Africa (26°52′02.7″ S 27°17′36.0″ E) from nine *C. gariepinus*, col. 2019 M. Truter. Another three copepods (two females, two examined) copepods were collected from the KuShokwe Pan, Phongolo floodplain in the Ndumo Game Reserve, KwaZulu-Natal Province, South Africa (26°52′19.5″ S 32°12′53.1″ E) from three *C. gariepinus*, col. 2018 M. Truter.

Representative DNA sequences. GenBank accession numbers and numbers of bases (bp) for Vaal River and Barotse floodplain, Zambezi River specimens are given as follows: (18S)—1367 & 1373 bp long sequences of two specimens, OR449753–OR449754; (28S)—668 & 694 bp long sequences of two specimens, OR449755–OR449756; (COI)—692 & 693 bp long sequences of two specimens, OR448769–OR448770.

Infestation rates. From all the localities sampled, *E. mirabilis* was only collected from three sites and the infestation rates (of copepodites and adults) are given as follows:
South Africa: Kushokwe Pan—prevalence 20% (3/15), mean intensity 1 (3/3), mean abundance 0.2 (3/15); Vaal River—prevalence 50% (9/18), mean intensity 1.8 (17/9), mean abundance 0.9 (17/18).
Zambia: Barotse floodplain—prevalence 23.5% (4/17), mean intensity 41 (164/4), mean abundance 9.6 (164/17).

Description of adult female (Figures 2–6).
Measurements (n = 20) are given as total length (anterior margin of prosome to posterior margin of caudal rami, excluding caudal rami setae) 1.35 ± 0.14 (1.05–1.58) mm, cephalosome length 0.51 ± 0.07 (0.36–0.63) mm, cephalosome width 0.42 ± 0.04 (0.34–0.50) mm.

Body cyclopiform (Figures 2a, 4a, and 5a). Prosome comprising cephalosome, thorax with four pedigerous somites; urosome comprising reduced fifth pedigerous somite, non-pedigerous genital double-somite, three free abdominal somites, and caudal rami. Cephalosome (Figures 2a and 4a,b) quadrangular in shape, almost as broad as long. Dorsolateral depression between cephalosome and first thoracic segment present; first thoracic segment and cephalosome not fused. Ornamentation present on dorsal side of cephalosome (Figures 2a and 4b), comprises an inverted T-structure situated post-medially, between two oval sculptures situated anteriorly and posteriorly on cephalosome; paired eyespots and depression of antennae attachment visible above anterior oval ornamentation; paired sensory pores and papillae observed between inverted T and posterior oval sculpture with numerous sensory papillae and pores scattered over the dorsal surface of cephalosome. Thorax five-segmented (Figures 2a and 4a,c). Segments one to four wider than long and progressively smaller, fifth segment reduced. Paired sensory papillae observed mid-dorsally on segments two to four (Figure 4c,d), 2–4 sensory papillae on dorsolateral margins of segments two to four (Figure 4c,e). Genital double-somite (Figure 3a) 1.50 times as wide as long, five times as long as first abdominal somite, bearing a pair of multiseriate egg sacs dorsally (Figures 2a, 4a, and 5a). Two robust spines situated dorsolaterally, close to egg sac attachment pore (Figure 6d). Abdomen (see Figure 3a) three-segmented, first abdominal somite widest, second somite shortest, and third somite incised dorsoventrally forming attachment for caudal rami. All abdominal somites with a posterior row of ventral spinules. Caudal rami elongated, approximately twice as long as wide with four setae: one long median seta with an array of spines (Figure 6e,f); a single shorter dorsolateral seta, 0.2 times as long as median seta; and two even shorter ventrolateral setae, 0.1 times as long as the median seta. Two sensory pores, and spinules on the posterior-ventral margins on each ramus.

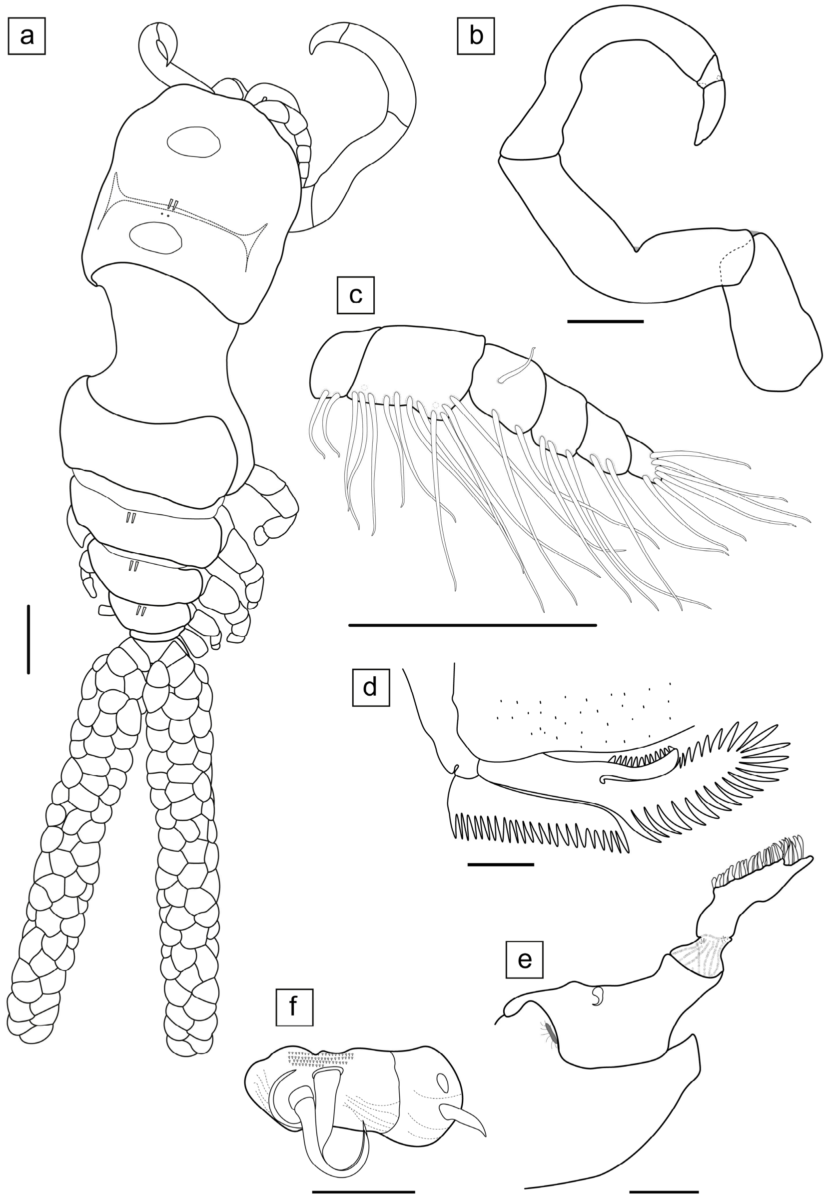

Figure 2. Illustrations of adult female *Ergasilus mirabilis* Oldewage & van As, 1987: (**a**) full image, dorsal view; (**b**) antenna; (**c**) antennule; (**d**) mandible; (**e**) maxilla; (**f**) maxillule. Scale bars: (**a**–**c**) 100 µm; (**d**–**f**) 10 µm.

Antennule (Figures 2c and 6a) six-segmented, armed with long and short setae, bearing a ring of spines on the dorsal surface of the first antennular segment (Figure 6b). Sensory pores at the proximal and distal dorsolateral margin of the second antennular segment, setal formula from proximal to distal segments given as 2–11–3–3–2–6. Antenna (Figure 2b) four-segmented, slender, smooth, and unarmed; second segment the longest; third segment sickle-shaped; fourth segment greatly reduced; terminal claw curved and sharply pointed.

Mouth tube positioned ventrally on cephalosome with row of spines on lateral side (Figure 5b); labrum with studs towards posterior margin (Figure 5c). Mandible (Figures 2d and 5e) comprises two stout segments with three blades; endopod splits into a shorter anteriorly toothed blade and a longer medial blade ornamented with teeth along anterior and posterior margins; distal blade (exopod) ornamented with teeth on posterior margin. Maxillule (Figures 2f and 5d) ornamented with spines on dorsal surface, reduced to two-segmented lobe with two simple setae on distal margin of exopod and single simple seta on distal margin of endopod. Maxilla (Figures 2e and 5c) three-segmented with terminal process of numerous teeth on convex margin of distal segment, single seta on medial segment, proximal segment ornamented with large maxillary pore.

Legs 1–4 (Figures 3b–d and 4f) with similar basic morphology as in other species of *Ergasilus*. Setae for legs 1–4 plumose except basiopodites ornamented with short simple setae (Figure 4f); legs 2 and 3 with similar armature formulae. Spinules present on lateral margins of exo- and endopodites of legs 1–4. Armature of legs 1–4 given in Table 4. Leg 5 with four setae; one short seta at base of segment, three terminal setae of unequal length on free segment, median seta longest (Figures 3e and 6c).

Table 4. Spine-setae formula on swimming legs of *Ergasilus mirabilis* Oldewage & van As, 1987. Number of spines in Roman numerals, number of setae in Arabic numerals.

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	I-0	I-0; I-1; II-5	0-1; 0-1; II-4
Leg 2	0-0	I-0	I-0; 0-1; 0-6	0-1; 0-2; I-4
Leg 3	0-0	I-0	I-0; 0-1; 0-6	0-1; 0-2; I-4
Leg 4	0-0	I-0	I-0; 0-5	0-1; 0-2; I-3

Male: Not described.

Variability.

Compared to the original description by Oldewage and van As [29], specimens from this study showed some variability in the number of antennular setation, mandible dentation, spines on the mouth tube and maxillules, as well as the number of spines and setae on legs 1–5, with the addition of two spines on the genital double somite (see Remarks for details).

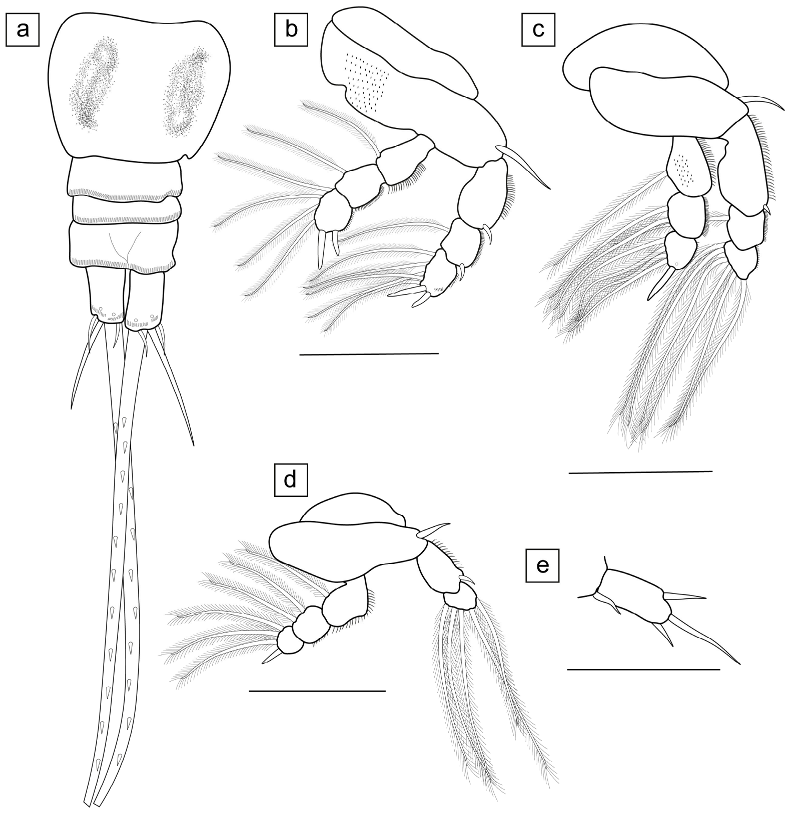

Figure 3. Illustrations of adult female Ergasilus mirabilis Oldewage & van As, 1987: (**a**) genital double somite, three abdominal somites, and caudal rami with setae; (**b**) leg 1; (**c**) leg 2; (**d**) leg 4; (**e**) leg 5. Scale bars: (**a**–**e**) 100 μm.

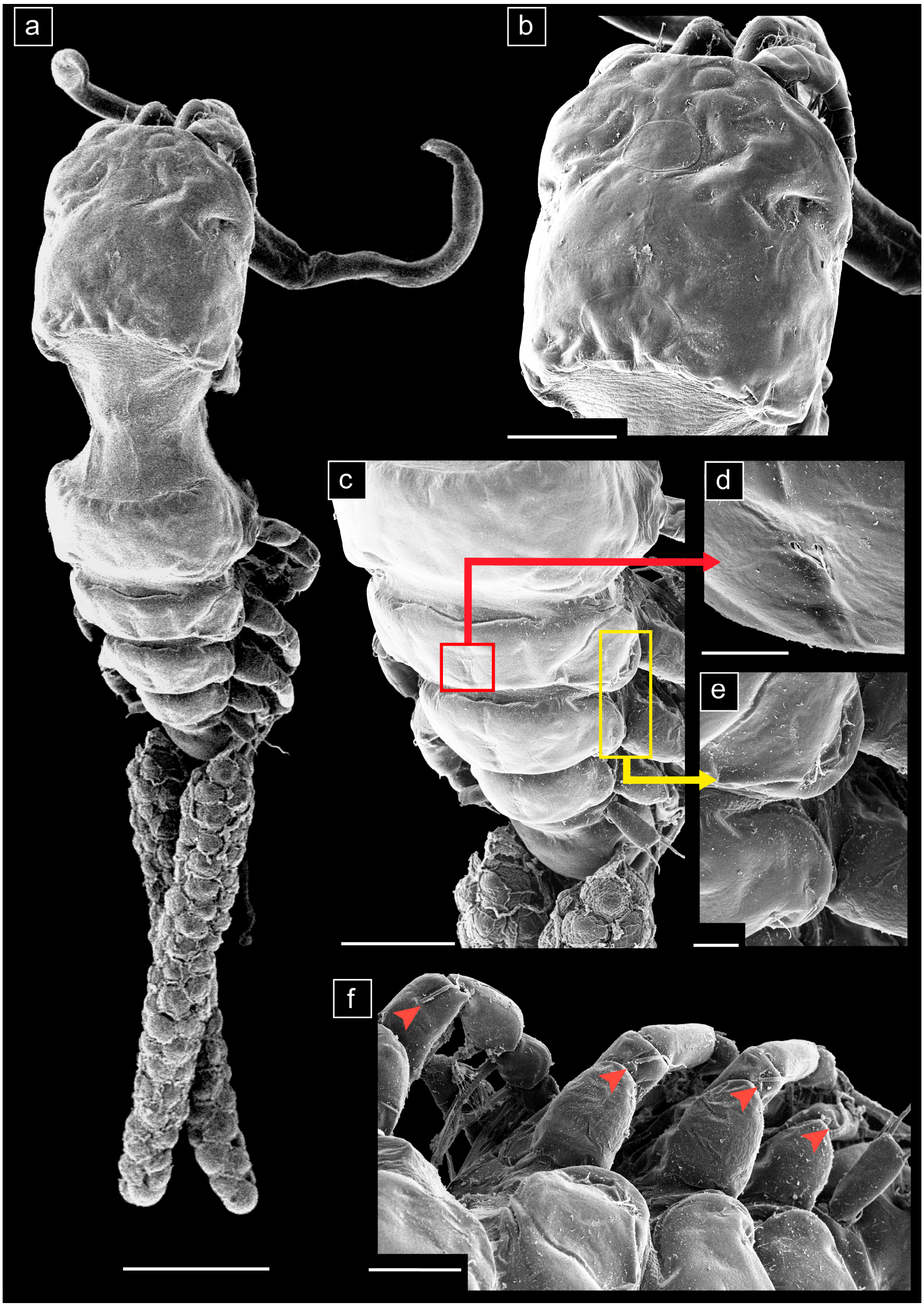

Figure 4. Scanning electron microscope photomicrographs of adult female Ergasilus mirabilis Oldewage & van As, 1987 showing features from the dorsal view: (**a**) habitus; (**b**) cephalosome showing ornamentation, sensory pores, and sensory papillae; (**c**) thoracic segments highlighting paired mid-dorsal sensory papillae on segments 2–4 (red square) and dorsolateral sensory papillae (yellow square); (**d**) zoomed in paired mid-dorsal sensory papillae; (**e**) zoomed in dorsolateral sensory papillae; (**f**) simple setae (red arrowheads) on basiopodite of legs 1–4. Scale bars: (**a**–**c**,**f**) 100 µm; (**d**–**e**) 50 µm.

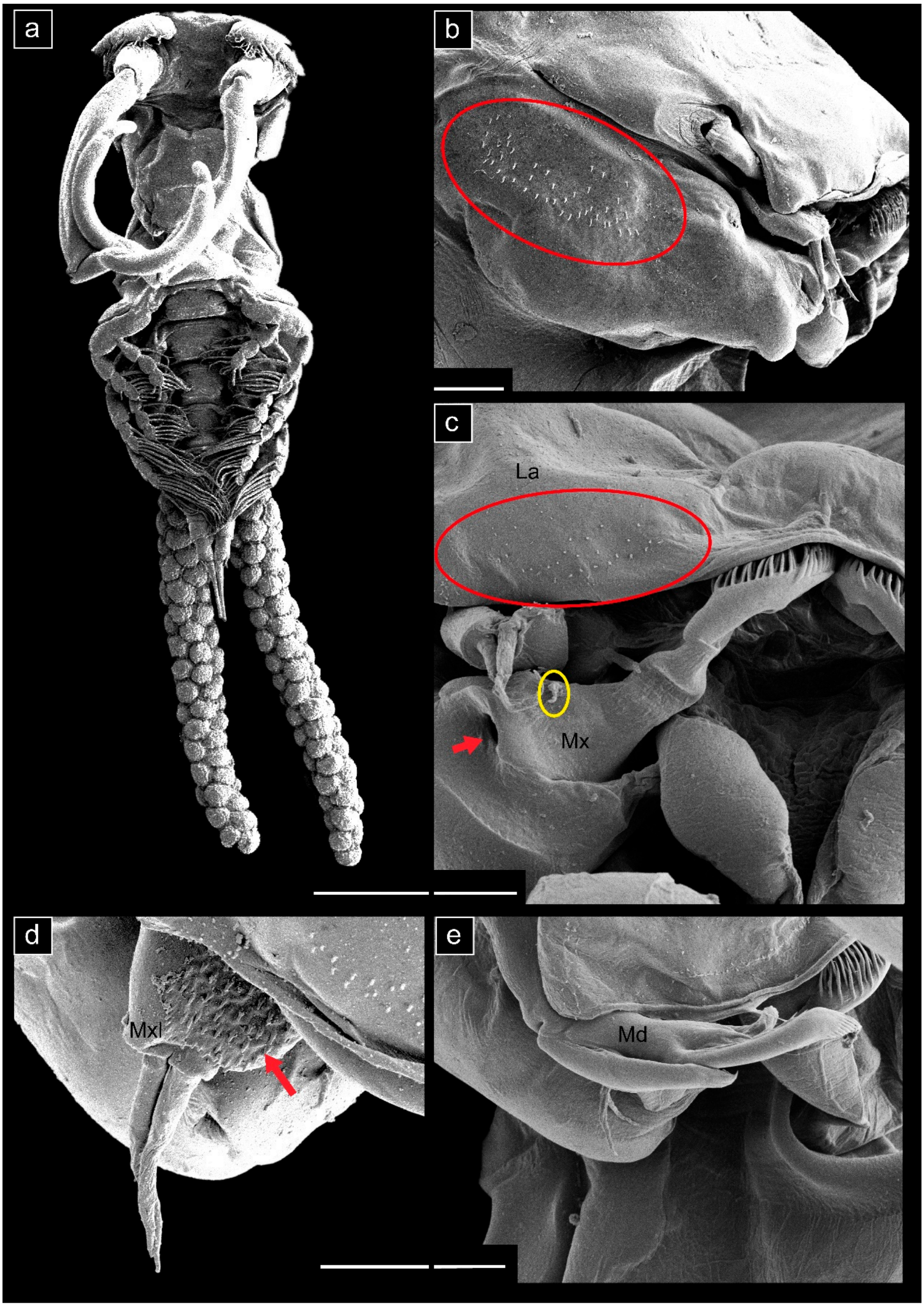

Figure 5. Scanning electron microscope photomicrographs of the full ventral image (**a**) and mouth parts (**b**–**e**) of Ergasilus mirabilis Oldewage & van As, 1987: (**a**) Full ventral image; (**b**) Mouth tube with lateral spines, red circle; (**c**) Studded labrum (red circle), maxilla with maxillary pore (red arrow) and single maxillary seta (yellow circle); (**d**) maxillule with rows of spines (red arrow); (**e**) mandible. Scale bars: (**a**) 200 µm; (**b**) 20 µm; (**c**–**e**) 10 µm. Abbreviations: La—labrum; Md—mandible; Mx—maxilla; Mxl—maxillule.

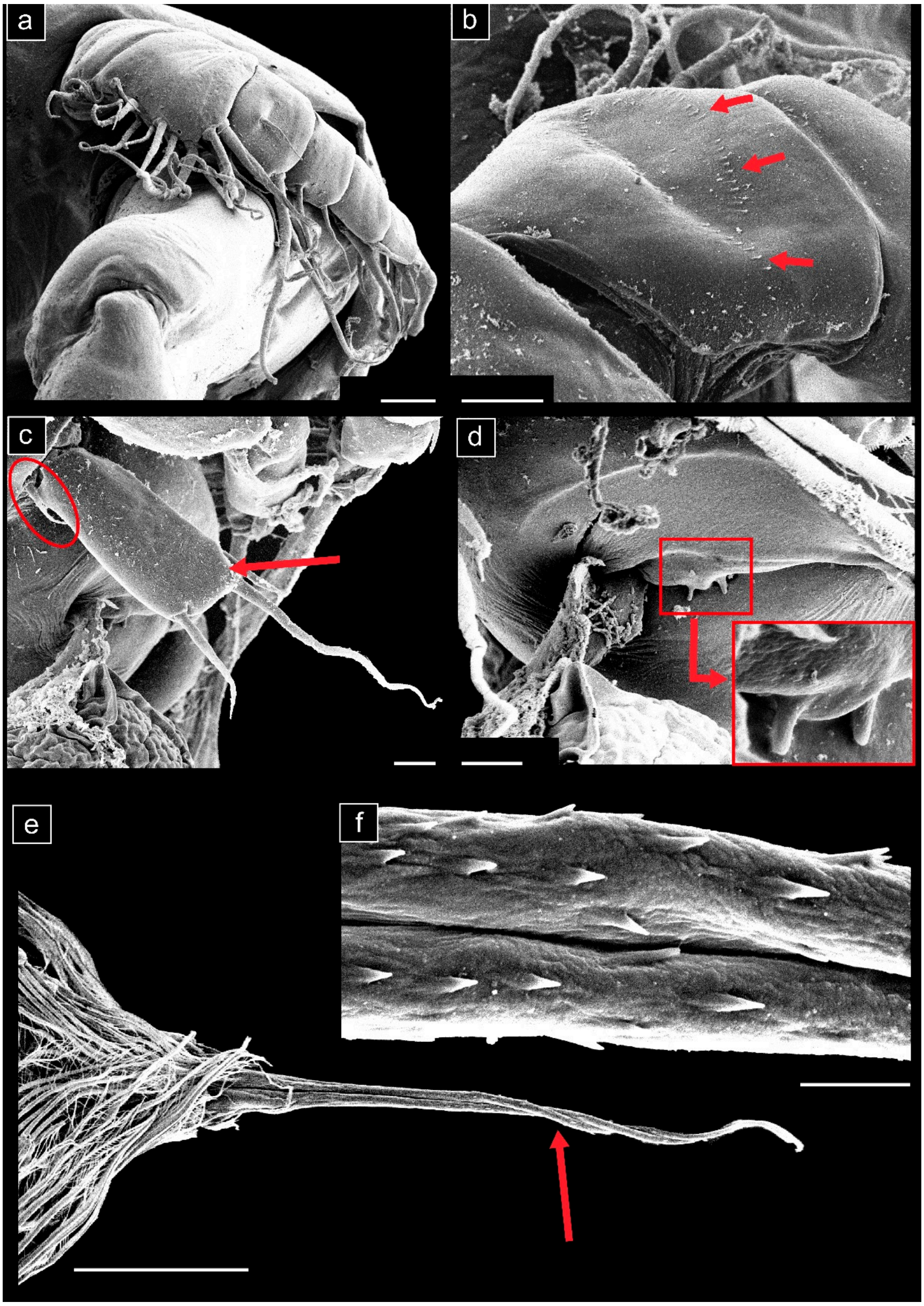

Figure 6. Scanning electron microscope photomicrographs of Ergasilus mirabilis Oldewage & van As, 1987: (**a**) antennule; (**b**) first antennular segment with ring of spines (red arrows); (**c**) leg 5 (red arrow) with basal seta (red circle); (**d**) Two robust spines situated dorsolaterally on genital double somite (inset showing a magnified image of the robust spines); (**e**) Elongated median setae (red arrow) of caudal rami; (**f**) Enlargement of median setae with array of spines. Scale bars: (**a**) 20 µm; (**b**–**d**) 10 µm; (**e**) 100 µm; (**f**) 5 µm.

Remarks.

The specimens from the present study were identified as *Ergasilus* mirabilis based on a combination of specific morphological characteristics. Representative specimens from

South Africa (Kushokwe Pan in the Phongolo floodplain, and the Vaal River) and Zambia (Barotse floodplain, Zambezi River) were morphologically similar when comparing data from SEM and light microscopy. Specimens were characterised by a quadrangular-shaped cephalosome with two oval structures, positioned anteriorly and posteriorly, respectively, to an inverted T-structure; paired sensory papillae on the cephalosome; as well as the six-segmented antennules armed with setae, four-segmented smooth antennae, and paired sensory papillae observed dorsomedially on the thoracic somites 2–4.

On the cephalosome, numerous sensory pores and papillae were observed on specimens from this study (Figure 4b). Oldewage and van As [29] reported a total of 19 setae on the antennular segments; the current study found 27 setae, as well as additional ornamentation. Denticulation at all margins of the medial blade of the mandible, as noted by Oldewage and van As [29] was not observed in the specimens from the current study (Figure 2d). Furthermore, several rows of spines on the lateral and dorsal axis of the mouth tube and maxillules, respectively, were observed in the current study. The genital double-somite in the present study was separated from the thoracic segments, following nomenclature by Boxshall [20], therefore five thoracic segments (Figures 2a and 4c) were reported, differing from the six segments observed by Oldewage and van As [29]. Furthermore, two robust spines, were observed on the genital double somite, located close to the egg string attachment pore in the newly studied material (Figure 6d). When comparing leg armature, the basiopodite of legs 1–4 possessed a single simple seta each (Figure 4f), which was not mentioned in the original description. The third exopodite of leg 1 had two spines and five plumose setae (Figure 3b); compared to six plumose setae and no spines reported by Oldewage and van As [29]. Legs 2 and 3 of the newly examined material also had similar spine-setae formulae, which was not the case with *E. mirabilis* from the original description. Additionally, leg 4 had one, two, and three setae on the first, second, and third endopodal segments, respectively, with a spine on the third endopoal segment (Figure 2d). No setae were observed on the first and second endopodites, and six setae without spines were reported on the third endopodite of leg 4 by Oldewage and van As [29]. The original description only noted two setae for leg 5, while four setae (Figures 3e and 6c) were observed from the present study.

Compared to all other species from Africa, *E. mirabilis* is most similar to *E. cunningtoni* (see [35] for *E. cunningtoni* description). The cephalosome of *E. cunningtoni* is shorter than the sum of its thoracic segments and has cephalothoracic ornamentation similar to that of *E. mirabilis*. However, and in accordance with the original description of *E. mirabilis*, the species described in this study also differs from *E. cunningtoni* in having a more quadrangular cephalosome than the triangular shape seen with *E. cunningtoni*. The digitiform process observed on the antennae of *E. cunningtoni* is absent in the species described in this study. Additionally, the second proximal segment of the antennae of *E. cunningtoni* has a definite notch that is absent in *E. mirabilis*.

Regarding the clariid host, *E. sarsi* is the only African species that has been reported from *C. gariepinus* apart from *E. mirabilis*. The smooth antennae and ornamentation on the cephalosome are similar to *E. mirabilis*; however, the triangular-shaped cephalosome and possession of only two abdominal segments differentiate it from *E. mirabilis* (see [35] for *E. sarsi* description).

3.2. Molecular Analysis

A total of six sequences were generated from this study, two each for partial 18S, 28S, and COI gene regions, with representatives from the Vaal and Zambezi rivers, respectively. Tree topologies for the ML and BI analyses for all gene regions were congruent. Strong bootstrap and posterior probability support values were obtained along branch nodes for the 18S and 28S analyses (Figures 7 and 8), while posterior probability support values for the ML analyses of the COI gene region were low (Figure 9).

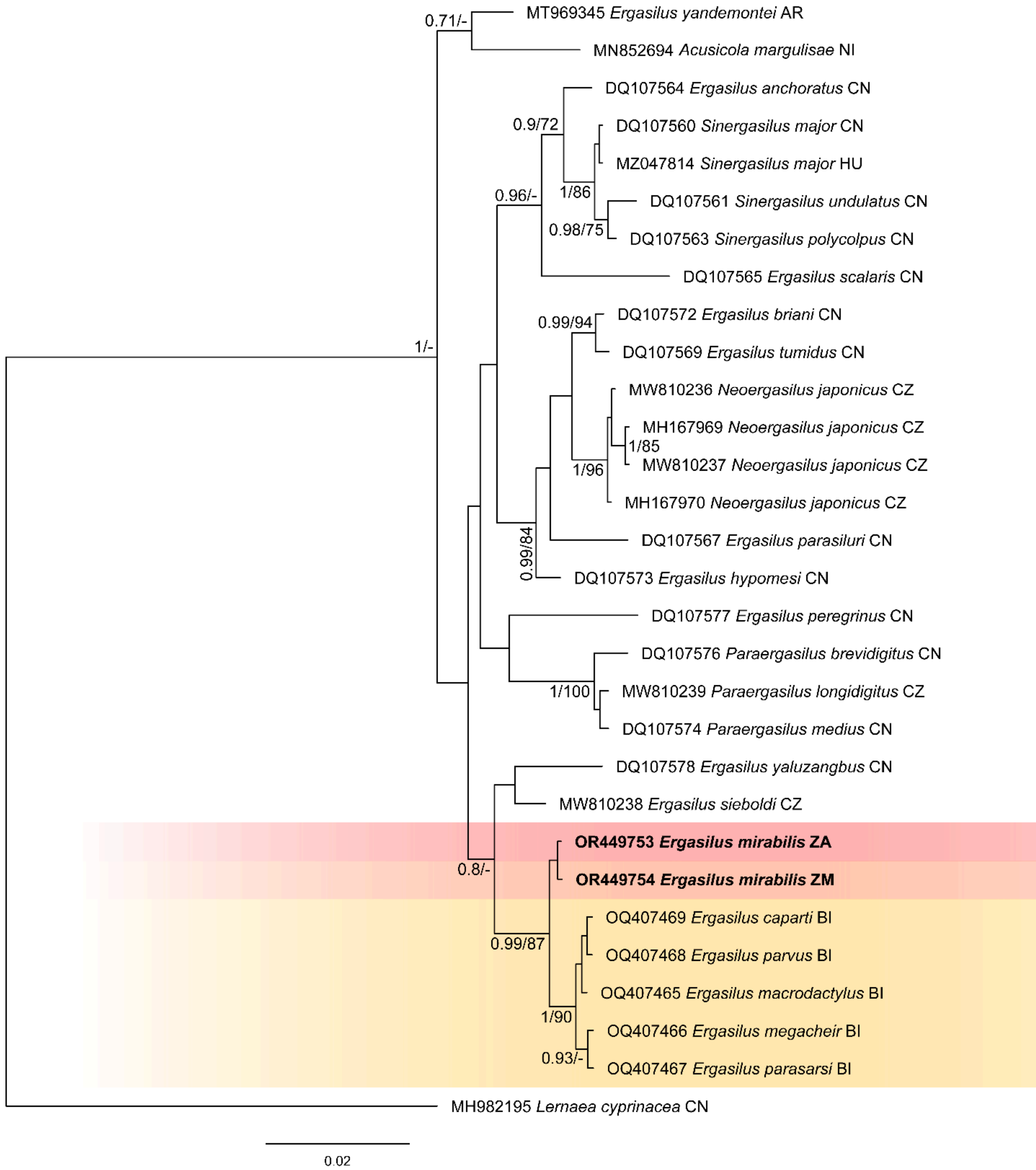

Figure 7. Phylogenetic tree of Ergasilidae copepods based on partial 18S rRNA gene alignments. Newly generated sequences for *Ergasilus mirabilis* Oldewage & van As, 1987 are provided in bold. Sub-Saharan species are presented in graded shades. Nodal support presented above or below branches for Bayesian Inference (>0.7) and Maximum Likelihood (>70%) analyses (BI/ML). *Lernaea cyprinacea* Linnaeus, 1758 was used as the outgroup. Abbreviations: AR—Argentina, BI—Burundi, CN—China, CZ—Czech Republic, HU—Hungary, NI—Nicaragua, ZA—South Africa (Vaal River), ZM—Zambia (Zambezi River).

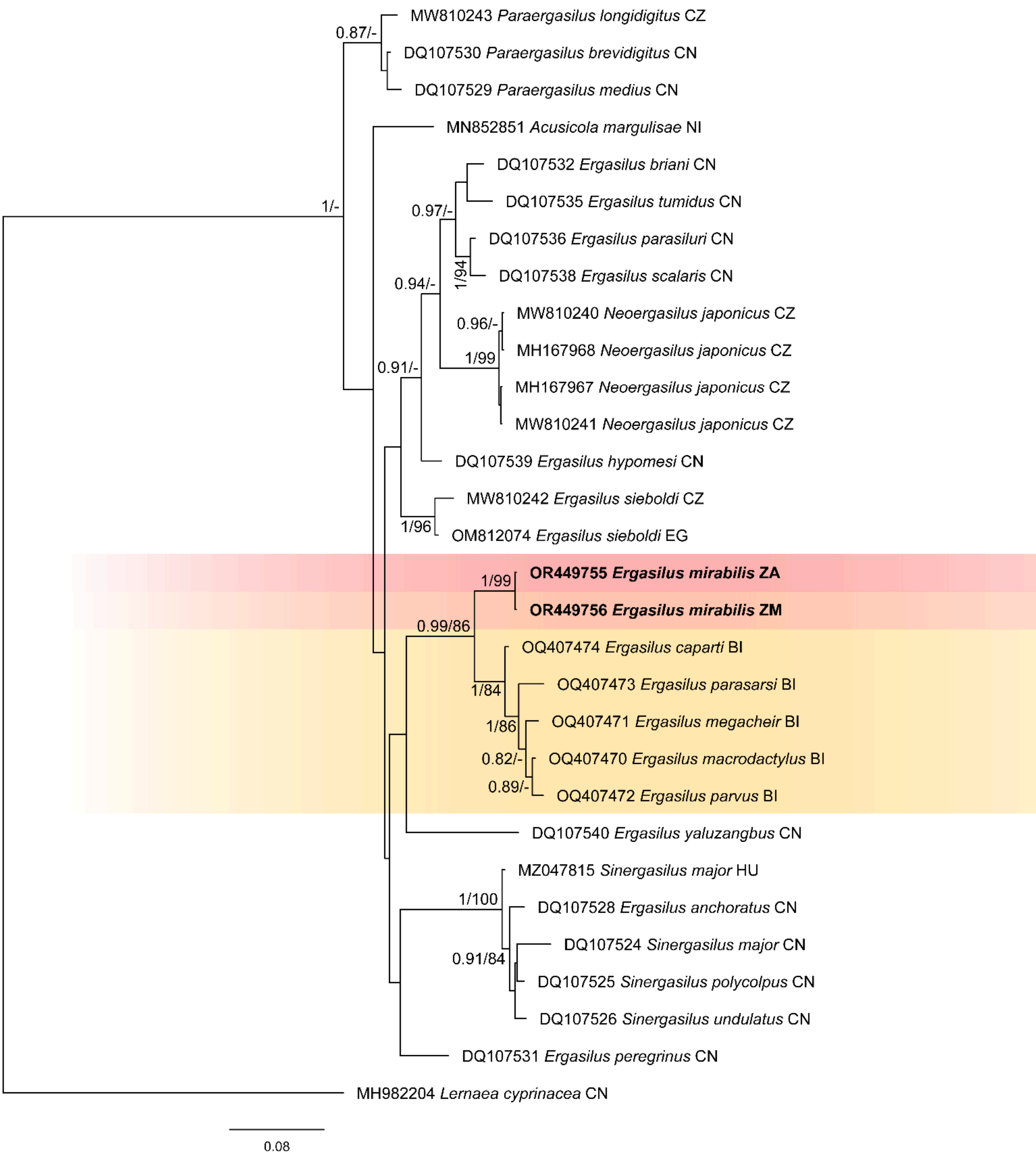

Figure 8. Phylogenetic tree of Ergasilidae copepods based on partial 28S rRNA gene alignments. Newly generated sequences for *Ergasilus mirabilis* Oldewage & van As, 1987 are provided in bold. Sub-Saharan species are presented in graded shades. Nodal support presented above or below branches for Bayesian Inference (>0.7) and Maximum Likelihood (>70%) analyses (BI/ML). *Lernaea cyprinacea* Linnaeus, 1758 was used as the outgroup. Abbreviations: BI—Burundi, CN—China, CZ—Czech Republic, EG—Egypt, HU—Hungary, NI—Nicaragua, ZA—South Africa (Vaal River), ZM—Zambia (Zambezi River).

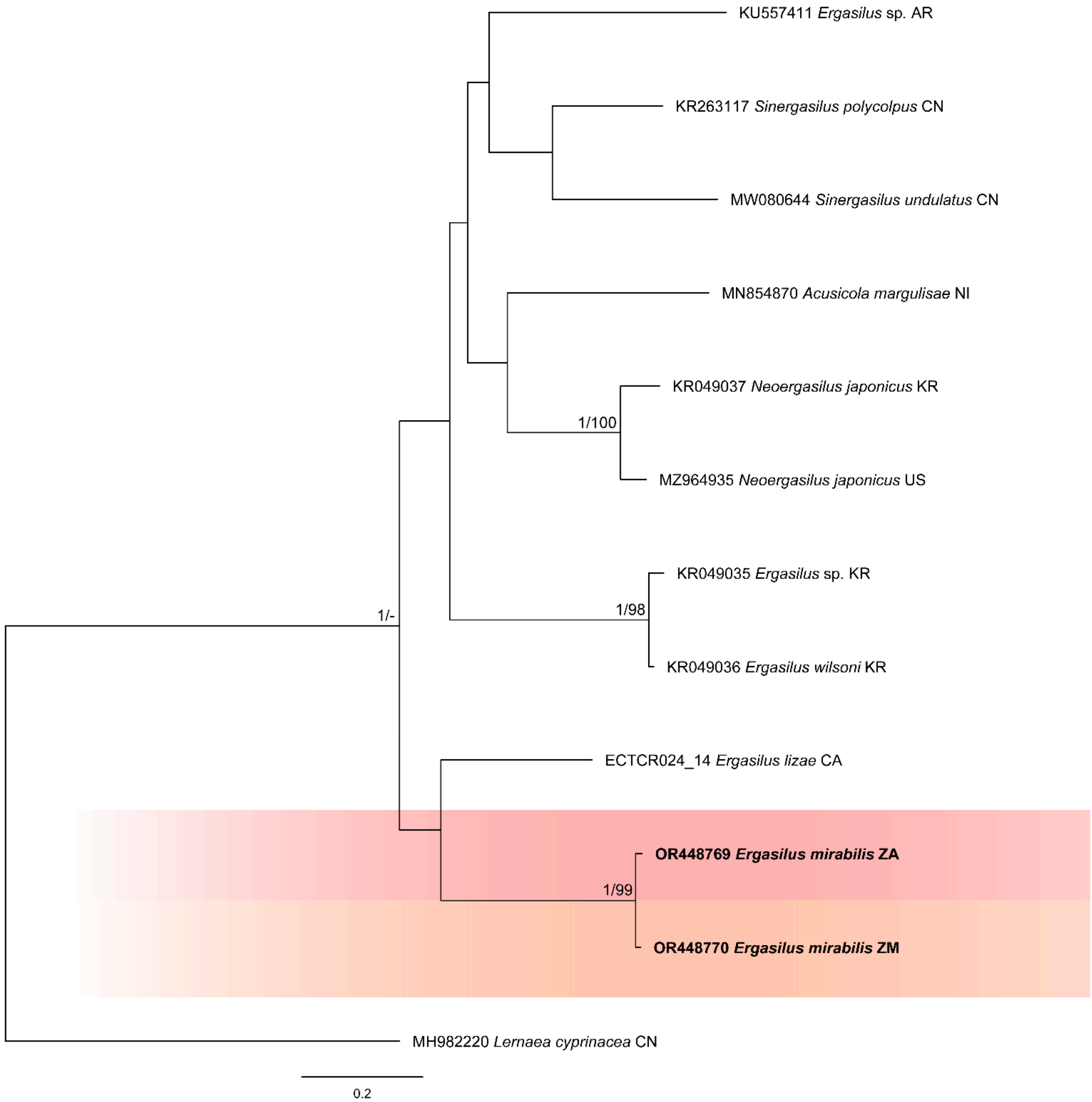

Figure 9. Phylogenetic tree of Ergasilidae copepods based on partial COI mtDNA gene alignments. Newly generated sequences for *Ergasilus mirabilis* Oldewage & van As, 1987 are provided in bold. Sub-Saharan species are presented in graded shades. Nodal support presented above or below branches for Bayesian Inference (>0.7) and Maximum Likelihood (>70%) analyses (BI/ML). *Lernaea cyprinacea* Linnaeus, 1758 was used as the outgroup. Abbreviations: AR—Argentina, CA—Canada, CN—China, KR—South Korea, NI—Nicaragua, US—United States of America, ZA—South Africa (Vaal River), ZM—Zambia (Zambezi River).

For the 18S phylogenetic analyses, alignments of GenBank and novel sequences resulted in a final alignment of 1398 bases. Newly generated partial 18S sequences from the Vaal River (South Africa) and Barotse floodplain (Zambia) specimens were 100% identical and most similar to the African sequences of ergasilids from Lake Tanganyika, with percentage similarity ranging from 99.60 to 99.70% (3–4 bp difference) (see

Supplementary Table S1). The *E. mirabilis* sequences from the present study clustered as a sister clade to the *Ergasilus* sequences from Lake Tanganyika (Burundi): *E. caparti*, *E. macrodactylus*, *E. megacheir*, *E. parasarsi*, and *E. parvus* (Figure 7), further confirming the placement of *E. mirabilis* in the genus *Ergasilus*, and as a member of the African clade, although a different species.

The final alignment implemented for the partial 28S gene region resulted in a length of 752 bases. Similar to the 18S gene region, the 28S sequences from the Vaal River and Barotse floodplain (Zambezi River) specimens were 100% identical, and most similar to the ergasilid sequences from Lake Tanganyika with a percentage similarity range of 93.11–95.10% (32–45 bp difference) (see Supplementary Table S2). All newly generated sequences clustered as a sister clade with Lake Tanganyika sequences, but separate from the *E. sieboldi* sequence from Egypt, which claded with the other available *E. sieboldi* sequence from the Czech Republic (Figure 8). As with the 18S tree, the phylogenetic relationship confirms the identity of the newly generated sequences as a different species from its congeners, and further highlights the evolutionary relationship with the sub-Saharan species (from Lake Tanganyika).

With the COI analyses, a total of 12 sequences were aligned with an invertebrate mitochondrial translation for the COI gene region, resulting in an alignment length of 692 bases. The sequences used included selected GenBank sequences and one BOLD sequence (*E. lizae*, an ergasilid also found in Africa) submitted from Canada. Newly generated partial COI sequences showed a 98.55% similarity (10 bp) to each other. From the translations, the codons having these 10 nucleotide differences all translated to the same amino acids (silent mutations) (see Supplementary Table S3). The newly generated sequences differed by more than 100 bases from all other COI Ergasilidae sequences in the alignment (see Supplementary Table S4). Some of these nucleotide differences were silent mutations and others were missense mutations. Novel sequences of *E. mirabilis* clustered in a clade with *E. lizae* (Figure 9).

4. Discussion

4.1. Morphology and Phylogenetics

In the present study, very little variation in the morphological characteristics was observed between specimens from the Vaal River, Kushokwe Pan, and the Barotse floodplain, and all specimens were morphologically identified as *E. mirabilis*. Subtle variations were observed when comparing these specimens with the original description of *E. mirabilis*. These differences may be attributed to slight mutation over time and across regions; subspecies variation [107]; and observational errors [108], as seen with other ergasilid genera. Minor variations within a species of *Ergasilus* can be expected, with some setation in smaller species or older descriptions being unreliable [20]. Boxshall [20] highlighted these inconsistencies when comparing the setation on the swimming legs in original descriptions of *E. xenomelanirisi* Carvalho, 1955 and *E. jiangxiensis* Liu, 1998 with the pattern observed in other species of Ergasilidae. The author further explained that details such as antennular setation may differ from older descriptions because setae could have broken off or been overlooked, and the aesthetasc setae are difficult to observe. Furthermore, the presence or absence of sensory papillae and pores may be overlooked when confirming the identity of a species.

The phylogenetic analyses of the present study corroborate the morphological identity of this species as belonging to the family Ergasilidae. The separate clades formed by newly generated sequences for all datasets (18S, 28S, and COI partial gene regions) further confirm its identity as an *Ergasilus* species different from its congeners used in the alignments. As previously reported, less divergence was recorded for the ribosomal genes than for the faster evolving mitochondrial DNA gene region, COI (see [109]). With the ribosomal phylogenetic analyses, the Tanganyikan (Burundi) sequences were the closest evolutionarily to specimens from this study, forming a sub-Saharan evolutionary clade. With the COI phylogenetic tree, newly generated sequences formed a sister clade with *E. lizae*, a brackish water parasite of mullet that has a global distribution, including Africa. So, even though the *E. lizae* sequence used in this study was from Canada rather than Africa, it is noteworthy that the newly generated *E. mirabilis* sequences showed the closest evolutionary relationship to *E. lizae*. The present study suggests a possible evolutionary relationship between species ancestry and geographical distribution, but with the limited amount of genetic data available this concept cannot be further explored. Additionally, the specimens from the Vaal and Zambezi rivers, which are two completely different river systems in southern Africa, were molecularly similar (100% identical for ribosomal genes). It can therefore be said that the molecular analysis from this study supports the distribution reports and affirms the status of *E. mirabilis* as a pan-southern African species.

From this study, the evolutionary positions of certain genera in Ergasilidae are consistent with Song et al. [85]: monophyly for both *Sinergasilus* Yin, 1949 and *Paraergasilus*, and polyphyly for *Ergasilus*. However, more genetic and morphological studies are needed for species belonging to the genus *Ergasilus*, and ultimately the family Ergasilidae, to enable a more robust analysis of genera within the family.

4.2. Host Preference and Distribution Range

Ergasilus mirabilis was originally described from the leopard squeaker *Synodontis leopardinus* (Mochokidae) in the Phongolo River, South Africa [29]. However, the distribution of *S. leopardinus* appears to be restricted to the Kunene, Okavango, and other rivers in the Upper Zambezi system [110], while the only known species of Synodontis in the Phongolo River system is the plain squeaker *Synodontis zambezensis* (see [111,112]). A year after its description in 1987, *E. mirabilis* was reported on 16 fish species across various regions in southern Africa, including *S. leopardinus* from the Phongolo and Zambezi River systems by the same authors [4] (see Table 1). According to FishBase [110] and Skelton [111], *S. leopardinus* is not present in the Phongolo River system, and this species has not been reported in this system other than the record of it as host of *E. mirabilis* by Oldewage and Van As [4,29]. Therefore, the record of *S. leopardinus* as the type host of *E. mirabilis* from the Phonoglo River was most probably a misidentification of *S. zambezensis* (known from the system) and therefore the type host of *E. mirabilis* may, in fact, be *S. zambezensis* and not *S. leopardinus*.

A total of 16 fish species belonging to nine families are reported as hosts for *E. mirabilis*, with distributions across major rivers and tributaries in southern Africa (see Table 1). Currently, most of the *E. mirabilis* records in southern Africa are associated with three fish families: Clariidae, Mochokidae, and Mormyridae. *Clarias gariepinus* (Clariidae) is the most widely distributed fishes in southern Africa [111] and is consequently one of the most reported host species for *E. mirabilis* (see Table 1). From the data presented in Table 1 for *E. mirabilis*, the presence of the parasite appears to align with the natural southern distribution limit of *C. gariepinus* (the Vaal River) and northward into the upper Zambezi River system.

Therefore, the present study confirms *C. gariepinus* as a host for *E. mirabilis* and supports the distribution record from the Zambezi River system with the Barotse floodplain as a new site from the upper Zambezi system, and adds the Kushokwe Pan as a new site in the Phongolo system. Additionally, this study provides the first record of this ergasilid species in the Vaal River in South Africa.

Generally, *E. mirabilis* is capable of parasitising various fish host species across multiple functional feeding groups, including bottom feeders, pelagic species, predators, and scavengers, due to its specialised hook morphology, ensuring firm attachment to the hosts' gill filaments [4,9]. Host preference in species of *Ergasilus* could be multifactorial and may not depend solely on the availability of host species in a river system (see [5]). Future studies on this copepod are required to understand the mechanism of host selection by *E. mirabilis*, influenced by factors such as host availability, seasonality, and environmental conditions [5,60,113].

4.3. Infestation Intensities and Parasitisation

The attachment and feeding activities of ergasilids can affect host tissue, interfere with respiration, cause irritation, and make fish susceptible to secondary infections [2,11,12,114]. In the current study, the highest infestation prevalence was recorded from the Vaal River in South Africa (50%), which is less than the 81% infestation prevalence (an average of six parasites per host) reported by Avenant-Oldewage and Oldewage [5], from the Kwando River system in Namibia [5]. The highest mean intensity (41) from the present study was recorded from the Barotse floodplain, Zambia, with up to 146 adult females collected from a single *C. gariepinus* host. Although prevalence from this study appears to be lower than what was reported in previous studies, the infestation of 146 parasite individuals is the highest infestation report for *E. mirabilis* parasitisation on a single host, to date. Other reports include an infestation of approximately seven parasites per host [9]; and a total of 106 individuals of *E. mirabilis* reported from a single Zambesi parrotfish, *Cyphomyrus discorhynchus* (Peters, 1852) (syn. *Hippopotamyrus discorhynchus* (Peters, 1852) by Douëllou and Erlwanger [30] in Lake Kariba, Zimbabwe.

Records of heavy parasitisation by other *Ergasilus* species have also been noted. Paperna and Zwerner [115,116], for instance, reported infestations of up to 2757 *E. labracis* Krøyer, 1863 individuals on a single striped bass host, *Morone saxatilis* (Walbaum, 1792), as well as, several developmental stages of *E. labracis* on *M. saxatilis* with an overall prevalence of 90%, respectively. Furthermore, severe parasitisation by *E. sieboldi*, which is currently a challenge in aquaculture, was reported to have led to mortality in a cultured sea bream population in Egypt (see [80]).

Although higher levels of infestation have been reported for other *Ergasilus* species compared to *E. mirabilis*, future studies are recommended to investigate the potential for high infestation by *E. mirabilis* in capture environments, since all currently available records of parasitisation by *E. mirabilis* are from natural or wild caught populations (see [8,80,115,116]).

5. Conclusions

With a combination of morphological and molecular techniques, the identity of the species from this study is confirmed as *Ergasilus mirabilis*. The present study verifies *C. gariepinus* as a host for *E. mirabilis* and provides an overall summary of the knowledge available for the 19 species of *Ergasilus* in Africa. Novel data are provided on the distribution of *E. mirabilis* in southern Africa, and a geographic range expansion is reported from the Vaal River, from which it was previously thought to be absent (see [4]). An additional locality record is reported for *E. mirabilis* from KuShokwe Pan in the Phongolo floodplain, and from the Barotse floodplain in the upper Zambezi River system. Phylogenetic analyses of all datasets showed that the newly generated sequences belonged to the Ergasilidae, but clustered separately in clades with sequences of other *Ergasilus* species. An evolutionary relationship between species ancestry and parasite distribution is suggested with *Ergasilus* species, as seen with the sub-Saharan species, but more genetic data are needed to further understand this relationship. This study serves as the first integrative study of *E. mirabilis*, using morphological and molecular techniques, with partial 18S, 28S, and COI gene regions; moreover, adding six new sequences for an African ergasilid to the very limited genetic data available for the Ergasilidae. These novel sequences are the first available sequences for *E. mirabilis*, and the first sequences of species of *Ergasilus* from southern Africa.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15090965/s1, Table S1: Genetic divergences among aligned 18S rRNA sequences expressed as percentage identities (below diagonal) and differences in the number of nucleotides (above diagonal). Represented as GenBank/Sequence ID, Taxon and Country. Sequences from the present study in bold and grey shade. *Lernaea cyprinacea* (MH982195) was used as the outgroup. Abbreviations: AR—Argentina, BI—Burundi; CN—China, CZ—Czech Republic, HU—Hungary, KR—South Korea, NI—Nicaragua, ZA—South Africa (Vaal River), ZM—Zambia (Zambezi River); Table S2: Genetic divergences among aligned 28S rRNA sequences expressed as percentage identities (below diagonal) and differences in the number of nucleotides (above diagonal).

Represented as GenBank/Sequence ID, Taxon and Country. Sequences from the present study in bold and grey shade. *Lernaea cyprinacea* (MH982204) was used as the outgroup. Abbreviations: BI—Burundi; CN—China, CZ—Czech Republic, EG—Egypt, HU—Hungary, NI—Nicaragua, ZA—South Africa (Vaal River), ZM—Zambia (Zambezi River); Table S3: Sites of amino acid variation in the alignment of partial COI *Ergasilus mirabilis* Oldewage & van As, 1987 sequences from the Vaal River (VR), South Africa and the Zambezi River (ZR), Zambia from this study, using invertebrate mitochondrion translation and stating what amino acids the codons translate; Table S4: Genetic divergences among aligned COI mtDNA sequences expressed as percentage identities (below diagonal) and differences in the number of nucleotides (above diagonal). Represented as GenBank/BOLD/Sequence ID, Taxon and Country. Sequences from the present study in bold and grey shade. *Lernaea cyprinacea* (MH982220) was used as the outgroup. Abbreviations: AR—Argentina, CA—Canada, CN—China, KR—South Korea, NI—Nicaragua, US—United States of America, ZA—South Africa (Vaal River), ZM—Zambia (Zambezi River).

Author Contributions: Conceptualization, P.P.F., N.J.S., L.L.V.A. and K.A.H.; methodology, P.P.F., N.J.S., L.L.V.A. and K.A.H.; software, P.P.F.; validation, P.P.F. and K.A.H.; formal analysis, P.P.F.; investigation, P.P.F., N.J.S. and K.A.H.; resources, P.P.F., N.J.S., L.L.V.A., M.T. and K.A.H.; data curation, P.P.F.; writing—original draft preparation, P.P.F.; writing—review and editing, P.P.F., N.J.S., L.L.V.A., M.T. and K.A.H.; visualization, P.P.F., N.J.S., L.L.V.A. and K.A.H.; supervision, N.J.S., L.L.V.A. and K.A.H.; project administration, P.P.F., N.J.S., L.L.V.A. and K.A.H.; funding acquisition, N.J.S., L.L.V.A. and K.A.H.; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study received necessary ethical clearance from The AnimCare Animal Research Ethics Committee of The North-West University (Ethics No. NWU-00159-18-A5).

Data Availability Statement: All sequences generated from this study have been submitted in the GenBank database under the following Accession numbers OR449753–OR449756 (for 18S and 28S), and OR448769–OR448770 (for COI). Adult female copepods from this study have been deposited in the collections of the National Museum, Bloemfontein, South Africa (NMB: P-969).

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