A key for the identification of African piscine parasitic Ergasilidae (Copepoda: Poecilostomatoida)

W.H. Oldewage* and J.G. van As

Research Unit for Fish Biology, Rand Afrikaans University, P.O. Box 524, Johannesburg, 2000 Republic of South Africa

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A synopsis of the major morphological features of representatives of the family Ergasilidae Thorell, 1859 (Copepoda: Poecilostomatoida), parasitic on freshwater and estuarine fish of the African continent, is presented. The three genera representing this family; *Ergasilus* von Nordmann, *Paraergasilus*, Markewitsch and *Dermoergasilus* Ho et Do, are clearly distinguishable from their original descriptions. A guide for the preparation of specimens for light microscopical examination and a key for identification of the species are given.

'n Samevatting van die belangrikste morfologiese kenmerke van Afrika-verteenwoordigers van die familie Ergasilidae Thorell, 1859 (Copepoda: Poecilostomatoida), parasities op varswater- en estuarine vis, word gegee. Die drie genera binne die familie, nl. *Ergasilus* von Nordmann, *Paraergasilus* Markewitsch en *Dermoergasilus* Ho et Do, is duidelik onderskeibaar aan die hand van die oorspronklike beskrywings. Metodes vir die voorbereiding van monsters vir ligmikroskopiese ondersoek en 'n sleutel vir die identifikasie van die spesies word verskaf.

*To whom correspondence should be addressed

Introduction

The first summary of the occurrence of parasitic Crustacea of African freshwater fishes was that of Cunnington (1920), who listed 17 species from the great lakes of central Africa. An updated review was subsequently given by Fryer (1968), listing a total of 81 species and sub-species of the families Lernaeidae, Arguillidae, Cymothoidae, Caligidae, and introduced Lernaeopididae and Ergasilidae. Fryer (1968) lists 10 species of Ergasilidae. A marine species, Ergasilus monodi Brian, 1927 has also been recorded from estuarine environments on the African continent (Brian 1927). Although Sars (1909) recorded Ergasilus brevimanus (Sars 1909), this appears to have been a misidentification, judging from the morphology thereof, as well as the fact that it was sampled as a free-living individual in a plankton sample. In this regard it should be noted that the original drawings are included in the illustrations accompanying this paper in order to facilitate comparison with other species should this be necessary. Drawings of Ergasilus sieboldi von Nordmann, 1932, the type species of the genus, are also included as a reference.

The first African Ergasilidae were described by Sars (1909), who found three species of *Ergasiloides* (Sars) in Lake Tanganyika, which has since been synonimized with *Ergasilus* von Nordmann (Fryer 1968). Work subsequent to Fryer (1968) has further enlarged the African repertoire and there are currently 16 species of *Ergasilus*, two species of *Paraergasilus* Markewitsch and one species of *Dermoergasilus* Ho et Do parasitic on African fish, as listed below.

Systematic list of African piscine parasitic Ergasilidae

Class: Crustacea Sub-class: Copepoda

Order:	Cyclopodea
Suborder:	Poecilostomatoida
Family:	Ergasilidae Thorell, 1859
2	Dermoergasilus Ho et Do, 1982
·	D. mugilis Oldewage & van As. 1987a
	Paraergasilus Markewitsch 1937
	P minutus (Erver 1056)(syn: Trigasilus
	minutus (Tryct, 1950)(syn. Trigasnus
	B laccoparia Banarma 1060
	P. lagoonaris Paperna, 1969
	Ergasilus von Nordmann, 1932
	E. nanus van Beneden, 1870
	E. macrodactylus (Sars, 1909)
	E. megacheir (Sars, 1909)
	E. kandti van Douwe, 1912
	E. monodi Brian, 1927
	E. nodosus Wilson, 1928
	E. cunningtoni Capart, 1944
	E. sarsi Capart, 1944
	E. latus Fryer, 1960
	E. lamellifer Fryer, 1961
	E. inflatipes Cressev, 1971
	E. mirabilis Oldewage & van As 1987
	E ilani Oldewage & van As 1087a
	D. nam Oldewage & Vall As, 170/a

Sampling and preparation of specimens for identification

Ergasilids occur mainly on the gill filaments of their hosts, but have also been recorded from the skin (Rogers & Hawke 1978) and nasal fossae (Yamashita 1980). The parasites are generally less than 1 mm long and can only be found using a dissection microscope at a low magnification of approximately $20 \times .$

For sampling ergasilids, the fish is killed and the gills removed. Individual arches are separated, kept wet in water from which the fish was sampled and examined microscopically. Parasites are recognized by their shape, movement or proliferation of gill tissue around the site of attachment and removed by hooking a bent, sharpened tungsten needle under the second antennae and pulling away from the gill filament.

To facilitate later identification, it is preferable to clean specimens at this stage, by either brushing gently with a fine (000), soft brush or allowing it to swim freely in filtered water for 30-60 min. Specimens are fixed in 5% buffered neutral formalin. Although alcohol fixation has been suggested (Singhal, Jeet & Davies 1984; Thatcher & Boeger 1983; Cloutman & Becker 1977), this method has proved to be unsuitable for later histological and scanning electron microscopical work. In order to negate the long-term effects of formalin preservation it is, however, recommended that specimens be preserved in either 70% ethanol or 50% propanol after 24-h formalin fixation.

In some cases the cleaning methods mentioned above are not satisfactory. In such cases the method suggested by Johnson (1969) may be employed, using sodium hypochlorite and KOH. We found, however, that some care is necessary when using the suggested concentration of 5,25% sodium hypochlorite, as fine setae are frequently softened and break as a result of handling. For cleaning we suggest that fully hydrated specimens be placed consecutively in 2% sodium hypochlorite and 2% KOH for 1–2 min each, with intermediate and final washes in water.

Although African ergasilids are generally easily distinguished on gross morphological features, dissection is sometimes necessary for positive identification. Two methods may be employed to prepare a specimen for examination. Using the first method, dehydrated, cleaned specimens are transferred to a clearing agent, such as phenol (Thatcher & Boeger 1983), methyl salicylate (Thatcher 1984), or lactophenol (Rogers 1969). The specimens are placed in a drop of clearing agent on a microscope slide and a coverslip gently placed over it. A piece of suitable absorbent tissue is placed against the edge of the coverslip and the specimen 'squashed' (Thatcher 1984) as a result of the withdrawal of fluid. As the preparation cannot be dissembled, a semi-permanent preparation can be made by sealing the



Figure 1 Dorsal views of the cephalothorax and true thoracic segments of African *Ergasilus* (redrawn from original descriptions): (a) *E. brevimanus*, (b) *E. mirabilis*, (c) *E. monodi*, (d) *E. ilani*, (e) *E. sars*, (f) *E. nodosus*, (g) *E. nanus*, (h) *E. inflatipes*, (i) *E. megacheir*, (j) *E. cunningtoni*, (k) *E. kandti*, (l) *E. macrodactylus*, (m) *E. sieboldi*.

Figure 2 Ventral views of the abdomen of African Ergasilus (redrawn from original descriptions): (a) E. lamellifer, (b) E. latus, (c) E. inflatipes, (d) E. cunningtoni, (e) E. macrodactylus, (f) E. ilani, (g) E. sieboldi, (h) E. sarsi, (i) E. inflatipes, (j) E. mirabilis, (k) E. kandti, (l) E. monodi, (m) E. nodosus, (n) E. megacheir, (o) E. brevimanus.

edge of the coverslip with a suitable commercial sealant. This method should yield clear, flat specimens, which can be studied at higher magnification under a light microscope.

In cases where improper fixation or mechanical damage makes the above-mentioned technique unsatisfactory, a dissection of the specimen may be performed. Dehydrated specimens are placed in a 25% glycerine solution in 70% ethanol and set aside for approximately 1 h to allow gradual evaporation of the ethanol (Pennak 1963), as the increased viscosity of the medium facilitates the dissection. Specimens are dissected under a dissection microscope using sharpened tungsten needles. A needle is inserted into the centre of the cephalothorax and abdomen and appendages (swimming legs, first antennae and second antennae) removed. The preparation may be permanently mounted under a coverslip and sealed with a suitable commercial sealant.

Staining has been suggested prior to identification



Figure 3 Lateral views of the second antenna of African *Ergasilus:* (redrawn from original descriptions): (a) *E. mirabilis,* (b) *E. macrodactylus,* (c) *E. sarsi,* (d) *E. kandti,* (e) *E. cunningtoni,* (f) *E. nodosus,* (g) *E. flaccidus,* (h) *E. sieboldi,* (i) *E. nanus,* (j) *E. monodi,* (k) *E. inflatipes,* (l) *E. brevimanus,* (m) *E. ilani,* (n) *E. lamellifer, (o) E. latus,* (p) *E. megacheir.*

(Singhal *et al.* 1984; Johnson 1969: Fernando & Hanek 1971), but has been found to be time consuming and ineffective. Examination of unstained specimens at magnifications of $20-400 \times$ under a phase-contrast microscope yields excellent results and fine detail is clearly visible.

Identification may be done using the following key and confirmed from the accompanying illustrations



Figure 4 The rudimentary fifth leg of African Ergasilus (redrawn from original descriptions): (a) E. kandti, (b) E. ilani, (c) E. inflatipes, (d) E. latus, (e) E. sieboldi, (f) E. cunningtoni, (g) E. megacheir, (h) E. mirabilis, (i) E. flaccidus, (j) E. monodi, (k) E. sarsi (l) E. lamellifer, (m) E. nanus.



Figure 5 Egg-sacs of African Ergasilus (redrawn from original descriptions): (a) E. ilani, (b) E. cunningtoni, (c) E. inflatipes, (d) E. monodi, (e) E. nodosus, (f) E. nanus, (g) E. latus, (h) E. sarsi, (i) E. mirabilis.

(Figures 1–7), which have been arranged to facilitate the comparison of one morphological feature for all species where such detail is available.

Generic diagnosis

The three ergasilid genera occurring in Africa are readily distinguishable by the morphology of their second antennae. In *Ergasilus*, the second antennae are smooth and the terminal tip singular, as in *E. mirabilis* (Oldewage & van As 1987), although tiny spines may be present in some species. *Dermoergasilus mugilis*, the only species of this genus on the continent, has a thick, cuticular membrane covering the second antennae up to the exposed, terminal tip (Oldewage & van As 1987). In *Paraergasilus*, the sharp, sclerotized terminal segment of the second antenna is sub-divided into three prongs.

Key to the piscine parasitic Ergasilidae of Africa

- (a) Dermoergasilus
 - 1 (a) Second antennae with cuticular covering......D. mugilis.
 (b) Second antennae without cuticular
 - covering......(b).
- (b) Paraergasilus
 - 1 (a) Terminal segment of second antenna pointed and sclerotized.....(c)
 - (b) Terminal segment of second antenna consisting of three pointed processes......2
 - 2 (a) Three pointed processes longer than second proximal segment *P. minutus.*
 - (b) Three pointed processes shorter than second proximal segment *P. lagoonaris.*

(c) Ergasilus

- 1 (a) Cephalothorax longer than sum of (b) Cephalothorax shorter than sum of 3 (a) Two denticular processes on second proximal segment of second antenna.....E. nanus. (b) Second antenna smooth E. ilani. 4 (a) Denticular processes on the third or second proximal segments of the second antenna5 (b) Second antennae smoothE. monodi. 5 (a) Dorsal ornamentation on cephalothorax (b) Dorsal surface of cephalothorax smooth E. nodosus. 6 (a) Cephalothorax quadrangular in shape7 (b) Cephalothorax triangular or rounded in 7 (a) Furcal rami with two setae per ramusE. macrodactylus. 8 (a) Three abdominal segments E. megacheir.
 - (b) Four abdominal segments E. mirabilis.

- - (b) Three bristles on second proximal segment of second antennaE. inflatipes.
 - (c) Single process on second segment of second antenna..... E. kandti.
- 11 (a) Abdomen three segmented......E. sarsi.
 - (b) Abdomen four segmented E. latus.



Figure 6 Gross morphological features of the two species of *Paraergasilus* known from Africa (redrawn from original descriptions): (a)-(d) *Paraergasilus minutus* (a) Dorsal view of cephalothorax and true thoracic segments, (b) abdomen, dorsal, (c) second antenna, (d) rudimentary fifth leg; (e)-(h) *Paraergasilus logoonaris* (e) Dorsal view of cephalothorax and true thoracic segments, (f) second antenna, (g) rudimentary fifth leg, (h) egg-sacs.

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Figure 7 Morphology of the only African species of *Dermoergasilus, D. mugilis* (redrawn from original description): (a) dorsal view of cephalothorax and true thoracic segments, as well as the first and second antennae, (b) abdomen, ventral, (c) egg-sac, (d) rudimentary fifth leg.

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