REVIEW OF SOUTH AFRICAN GENERA OF THE FAMILY HEXABOTHRIIDAE PRICE, 1942, PARASITES OF CHONDRICHTHYAN FISHES

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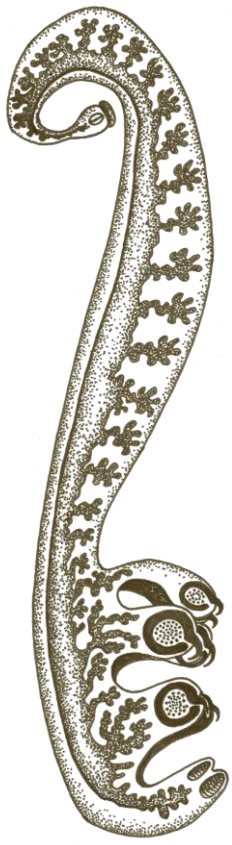

For my wife Melany and daughter Amy-Grace who kept me on my toes, my inspiration for completing this dissertation.

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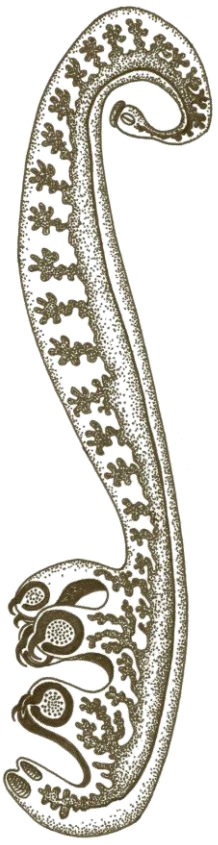

KEYWORDS

1. Hexabothriidae

2. *Callorhynchocotyle, Branchotenthes*

3. Taxonomic revision

4. Novel morphometrics

5. Chondrichthyes

6. Public aquaria

ABSTRACT

Review of South African genera of the family Hexabothriidae Price, 1942, parasites of chondrichthyan fishes

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The oligonchoinean monogenean family Hexabothriidae Price, 1942 currently consists of approximately 60 valid species, representing 15 genera. Hexabothriids are gill parasites of chondrichthyan fishes (sharks, rays and chimaeras). Some hexabothriid species have been reported as problematic in public aquaria, directly responsible for host pathology and subsequent host mortalities. However, without information on specific hexabothriid species and their host associations, accurate captive management of hexabothriids in public aquaria is hindered. Hexabothriid taxonomy is in a state of confusion. The historic taxonomic restoration of the priority of *Hexabothrium* sees the beginning of the taxonomic uncertainty of the hexabothriids, and is continued into the present literature particularly among lower-level taxa in Hexabothriidae. In addition, there is currently no consensus for a single accepted morphometric protocol for the discrimination of hexabothriid taxa, which leads to unnecessary ambiguity of character variable nomenclature, measurement and interpretation. A call for stability in the nomenclature and morphometric discrimination of species is therefore proposed. A novel morphometric protocol is tested for the sclerotised haptoral armature, supported by the proteolytic digestion of structures for optimal representation. Character variables, subjected to univariate and multivariate analyses were systematically accepted or rejected based on their potential to discriminating species of *Callorhynchocotyle* Suriano and Incorvaia, 1986. The hexabothriid genera *Callorhynchocotyle* and *Branchotenthes*, represented by South African taxa, are reviewed, using these variables. Four *Callorhynchocotyle* species and 2 *Branchotenthes* species are redescribed with the inclusion of some new voucher specimens.

DECLARATION

I declare that the dissertation, ***Review of South African genera of the family Hexabothriidae Price, 1942, parasites of chondrichthyan fishes,*** is my own work. It has not been submitted for any degree or examination in any other university, and all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name..... Date.....

Signed.....

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CONTENTS

CHAPTER 1
Introduction

1.1 Monogenea: Problematic parasites of fishes in captivity

The Class Monogenea consists of representatives in both the sub-classes Oligonchoinea (Bychowsky, 1937) and Polyonchoinea (Bychowsky, 1937) that cause problems to the health of fishes both in captivity and the wild. These problematic monogenean taxa have an ability to influence the economics of both inland and offshore aquaculture industries worldwide through both host-mortalities and the costs involved in their control and management. The most notorious of these is undoubtedly *Gyrodactylus salaris* Malmberg, 1957 which has been well documented as one of the most important pathogens of Atlantic salmon (*Salmo salar*) in Europe and parts of Russia. According to Bakke *et al*. (2007), *G. salaris* still poses one of the most significant threats to the continued existence of wild Atlantic salmon stocks in the Eastern Atlantic. Similarly, yellowtail kingfish (*Seriola* spp.) cultures in Japan, Australia, New Zealand and The Mediterranean have suffered economic losses from *Benedenia seriolae* (Yamaguti, 1934) and *Zeuxapta seriolae* (Meserve, 1938) which are monogeneans parasitising skin and gills respectively (Ernst *et al*. 2005; Mooney *et al*. 2006).

Hayward *et al*. (2007) identified 3 parasites including 2 monogenean species as emergent on the gills of Dusky kob (*Argyrosomus japonicus* Temminck and Schlegel, 1843) from Australia. The significance of the reports of these problematic parasites of *Seriola* spp. and *A. japonicus* is reflected in the current status of marine finfish culture in South Africa. Although *Seriola* spp., and *A. japonicus* are native to South African waters and are currently being investigated as prime culture species, no formal records of their respective monogenean parasites nor indications of potential economic losses have to date been reported. However, these parasites are present on these species, and they have caused isolated mortalities in South African public aquaria (Vaughan *et al*. 2008a).

Epidemiologically, public aquarium exhibit systems and finfish culture facilities are very similar. Factors influencing the successful transfer, reproduction and re-infection of monogeneans can be exacerbated in captivity. Reduced spatial arrangements between

susceptible hosts improves the invasion-success of vulnerable re-infective monogenean larvae, which in turn can increase in population size and mature, and from which exhibit-confined and cultured fishes have limited or no escape. The dynamic equilibrium between host and parasite could bias in favour of the parasite and disease outbreaks can occur (Reno 1998).

Although anecdotal information regarding problematic monogeneans in public aquaria is commonplace, peer-reviewed reports are few (Bullard *et al.* 2001, Chisholm and Whittington 2004, Chisholm *et al*. 2004, Jahn and Kuhn 1932, Janse and Borgsteede 2003, MacCallum 1927, Poynton *et al*. 1997, Thoney and Hargis 1991, Vaughan *et al*. 2008b, Vaughan and Chisholm 2009, Williams *et al*. 2008). One of the most notorious monogeneans affecting fishes in public aquaria is *Neobenedenia melleni* (MacCallum, 1927), which was originally reported from the New York aquarium by MacCallum (1927). Its notoriety originates from its apparent lack of host-specificity, a trait uncommon in most monogeneans. Recently however, Whittington *et al.* (2004) and Whittington (2004) provided molecular evidence to show that the likelihood was high that *N. melleni* was in fact a species complex made up of species that resemble each other morphologically.

Few accounts exist for problematic monogeneans of chondrichthyan fishes in public aquaria. Reports on the monocotylid genus *Dendromonocotyle* Hargis, 1955 affecting public aquarium-held stingrays are provided by Chisholm and Whittington (2004), Chisholm *et al.* (2004), Vaughan *et al.* (2008b) and Vaughan and Chisholm (2009). In addition, Poynton *et al.* (1997) and Bullard *et al.* (2001) discuss problematic monogeneans affecting captive Lemon sharks (*Negaprion brevirostris* Poey, 1868) and Bonnethead sharks (*Sphyrna tiburo* Linnaeus, 1758) respectively. The latter study deals with the hexabothriid monogenean *Erpocotyle tiburonis*[1] (Brooks, 1934), which parasitises the gill tissue of its host causing severe pathology of the secondary gill lamellae.

All hexabothriids are exclusive gill parasites of chondrichthyan fishes. Hexabothriidae Price, 1942 consists of approximately 60 species. Hexabothriids belong to the sub-class Polyonchoinea and it is believed that they feed on blood derived from the gill lamellae (Bullard *et al.* 2001). Hexabothriids are generally host-specific, which

[1]This species is currently unconfirmed as a member of *Erpocotyle* van Beneden and Hesse, 1863 (Boeger *et al*. 1989).

makes improving our knowledge of their host associations valuable for public aquaria housing many different chondrichthyan fishes.

Although Bullard *et al.* (2001) identified the most recent hexabothriid of public aquarium importance, Wiskin (1970) reported the first hexabothriid infection from the public aquarium at the Marine Biological Association Laboratory in Plymouth from which she obtained material to work on the ontogeny of *Rajonchocotyle emarginata* Olsson, 1876. Upon post-mortem investigation of a single host *Raja clavata* Linnaeus, 1758, more than 300 individual *R. emarginata* were discovered in the host gill mucous. Another report from Europe, that of Janse and Borgsteede (2003) mentioned problems experienced with a mixed monogenean infestation of captive *Aetobatus narinari* Euphrasen, 1790, including "a species of the Hexabothriinae" which was treated using the anthelmintic praziquantel. However, the hexabothriid was never identified. This relative paucity of reports on problematic hexabothriids affecting public aquarium-held chondrichthyan fishes is not a reflection of their low pathogenicity. On the contrary, the only conclusive reports on problematic hexabothriids in public aquaria, those of Bullard *et al.* (2001) and Wiskin (1970), identified hexabothriids as responsible for host mortalities. The reason that little current information on hexabothriids in public aquaria exists is that chondrichthyan fishes are all high-profile exhibit animals and constitute elaborate and dramatic collections that are usually hard to come by and expensive to replace. Routine examinations of these fishes is restricted by their size and temperament, but also because hexabothriids live exclusively within the folds of the gill lamellae and usually cannot be accessed or even monitored ante-mortem.

Hexabothriids are of particular importance to public aquaria because of their co-evolutionary association with chondrichthyan fishes (Boeger and Kritsky 1989) of which elasmobranchs make up the majority. Recent surveys of chondrichthyan by-catch along the West and South coasts of South Africa conducted aboard the research vessel *Africana* from 2006–2008 (as part of the present study), revealed the presence of 21 hexabothriid taxa from as many different hosts.

A better understanding of basic but also specific health requirements of chondrichthyans in public aquaria would provide information for their captive conservation. Recently, the publication of the Elasmobranch Husbandry Manual by Smith *et al*. (2004) highlighted the importance of improving the levels of health care and

husbandry to members of the Chondrichthyes, but emphasised a lack of generally available information. As part of this we need to explore the natural relationships between chondrichthyan hosts and parasites and apply this information to better improve their captive management.

The present study was conducted on the only 2 hexabothriid genera currently reported from South Africa. *Callorhynchocotyle* Suriano and Incorvaia, 1982, and *Branchotenthes* Bullard and Dippenaar, 2003, are currently only represented by a single South African species each. Both host species *Callorhinchus capensis* (Duméril, 1865) and *Rhina ancylostoma* Bloch and Schneider, 1801, are of particular importance to public aquaria and the information presented in this study could provide the foundation necessary to support future quarantine regimes based on host-parasite profiling. In addition, it is hoped the results of this dissertation will pave the way for the progression of hexabothriid information for other chondrichthyan hosts earmarked as future aquarium exhibit target species in South Africa.

1.2 Hexabothriidae Price, 1942: Taxonomic history and problems

Price (1942) proposed the name Hexabothriidae for the previously assigned family Onchocotylidae Stiles and Hassall, 1908 after the discovery that the assigned type genus *Onchocotyle* Diesing, 1850 was the antedated synonym of *Hexabothrium* Nordmann, 1840. Price's (1942) proposal for Hexabothriidae was based on the taxonomic priority of the type species *Hexabothrium appendiculatum* (Kuhn, 1829) although he indicated that the result of such an amendment would be considerable confusion in the literature. The taxonomic restoration of the priority of *Hexabothrium* sees the beginning of the taxonomic uncertainty of the hexabothriids, and is continued into the present literature particularly among higher-level taxa in Hexabothriidae.

Coupland (1962) raised issues of confusion over *Rajonchocotyle emarginata* (Olsson, 1876), a hexabothriid from the gills of *Raja clavata* Linnaeus, 1758. The original description of this parasite, *Onchocotyle emarginata* Olsson, 1876 distinguished it from 3 other monogenean species from 3 different elasmobranch hosts (Coupland 1962). However, a subsequently described species of hexabothriid from the gills of the same host species by Baylis and Jones (1933) was assigned the name *Onchocotyle appendiculata* Baylis and Jones, 1933. This specimen was later confirmed by Price

(1940) as *O. emarginata*, which he subsequently re-described as *Rajonchocotyloides emarginata* (Olsson, 1876).

Rajonchocotyloides Price, 1940 was differentiated from *Rajonchocotyle* Cerfontaine, 1899 on the basis that the former possesses vitellaria that extend into the haptoral appendix. Although Sproston (1946) collected the same species from *R. clavata* and identified it as *O. emarginata*, she did not agree with the allocation of the species to *Rajonchocotyloides* of Price (1940) and assigned her specimens to *Rajonchocotyle* of Cerfontaine (1899). Dawes (1947) collected the same species from the same host after Sproston (1946), yet included the species under Price's (1940) genus *Rajonchocotyloides.* Finally, Coupland (1962) concluded that the correct taxonomic designation of the hexabothriid from *R. clavata* was indeed *Rajonchocotyle emarginata* (Olsson, 1876), because the (diagnostic character) presence or absence of vitellaria within the haptoral appendix in this species was dependant upon the age and maturity of the individual specimens (Sproston 1946). Boeger and Kritsky (1989) subsequently considered *Rajonchocotyloides* a synonym of *Rajonchocotyle* based on the lack of additional differential characters. Similarly, *Neoerpocotyle* Price, 1942 (then considered a synonym of *Squalonchocotyle* Cerfontaine, 1899) was considered by Boeger and Kritsky (1989) to be a synonym of *Erpocotyle* van Beneden and Hesse, 1863 for the same reason.

Apart from initial confusion with the above taxa, Boeger and Kritsky (1989) in their revision of the Hexabothriidae, considered 8 hexabothriid taxa to be *incertae sedis*[2] because they could not be recognised as members of any known genera according to their revised diagnoses at that time.

Fifteen hexabothriid genera are considered valid. These include *Erpocotyle; Rajonchocotyle; Squalonchocotyle; Heteronchocotyle* Brooks, 1934; *Hexabothrium; Pseudohexabothrium* Brinkmann, 1952; *Rhinobatonchocotyle* Doran, 1953; *Dasyonchocotyle* Hargis, 1955; *Neonchocotyle* Ktari & Maillard, 1972; *Protocotyle* Euzet & Maillard, 1974; *Epicotyle* Euzet & Maillard, 1974; *Pristonchocotyle* Watson and Thorson, 1976, *Paraheteronchocotyle* Mayes, Brooks & Thorson, 1981;

[2]*incertae sedis* = unconfirmed species, although valid, excluded as members of currently accepted genera of Boeger and Kritsky (1989).

Callorhynchocotyle, and *Branchotenthes*. According to Boeger and Kritsky (1989), the genera *Hexabothrium*, *Erpocotyle*, and *Heteronchocotyle* are parasites of carcharinoid sharks, while *Dasyonchocotyle*, *Paraheteronchocotyle*, and *Neonchocotyle* are parasites of myliobatoid rays (Table 1.1). The genera *Squalonchocotyle*, *Protocotyle*, *Callorhynchocotyle*, *Rhinobatonchocotyle* and *Epicotyle* are parasites of squaloid, hexanthoid, holocephalan, rhinobatoid and torpedinoid hosts respectively, and the genera *Rajonchocotyle*, and *Pseudohexabothrium* are from rajoid hosts (Table 1.1). *Branchotenthes* parasitises rhinobatoid hosts (Bullard and Dippenaar, 2003; Glennon *et al.* 2005) (Table 1.1). *Pristonchocotyle* was not included in the revision of the family of Boeger and Kritsky (1989) although it is still a valid hexabothriid taxon. This genus, of which there are currently 2 valid species, parasitises the gills of *Pristis* spp.

Agrawal *et al.* (1996) described the second species of *Pseudohexabothrium*, *P. taeniurae* Agrawal, Chisholm and Whittington, 1996, from the gills of its host *Taeniura lymma* Forsskål, 1775. This addition sees *Pseudohexabothrium* as parasitic of both myliobatoid as well as rajoid hosts (Table 1.1). Similarly, Neifar *et al*. (2001) described *Heteronchocotyle gymnurae* Neifar, Euzet and Hassine, 2001 from *Gymnura altavela* Linnaeus, 1758, a myliobatoid ray, not a carcharinoid (Table 1.1). The appearance of this species on a marine myliobatoid ray host, not a carcharhinoid, may have evolutionary significance when compared to its sister genus (*sensu* Boeger and Kritsky 1989) *Paraheteronchocotyle* which parasitises freshwater rays in the Amazon River system.

In the morphological phylogeny analysis of Boeger and Kritsky (1989), there are 3 clades that appear to be cohesive, providing justification of sub-family taxa within the Hexabothriidae. That said, the authors concluded that the proposal of subfamilial taxa within the Hexabothriidae at that time was probably premature based on the lack of information on specific and generic hexabothriid diversity. The clades represented by Boeger and Kritsky (1989) included: Clade 1. The genera: *Epicotyle* - *Neonchocotyle* - *Callorhynchocotyle*, Clade 2. The genera: *Heteronchocotyle* - *Paraheteronchocotyle* - *Rhinobatonchocotyle*, and Clade 3. The genera: *Rajonchocotyle* - *Protocotyle* - *Squalonchocotyle*.

Presently there is no phylogenetic resolution for the relationship between the genera *Rhinobatonchocotyle* and the recently discovered genus *Branchotenthes*. However, *B. octohamatus* Glennon *et al*., 2005 remains currently unique among the

hexabothriids in that its larva possess only 8 marginal hooklets whereas all other described hexabothriid larvae possess 10 (Glennon *et al.* 2005). The number of marginal hooklets present in representatives of the Class Monogenea is considered an important character in their classification and systematics (Glennon *et al.* 2005).

Table 1.1 Current distributions of valid hexabothriid genera to chondrichthyan fishes host groups

Host group	Valid hexabothriid genera
Hexanthoid	*Protocotyle* [a]
Squaloid	*Squalonchocotyle* [a]
Carcharhinoid	*Hexabothrium* [a]
	Erpocotyle [a]
	Heteronchocotyle [a]
Pristoid	*Pristonchocotyle* [b]
Torpedinoid	*Epicotyle* [a]
Rajoid	*Rajonchocotyle* [a]
	Pseudohexabothrium [a]
Rhinobatoid	*Rhinobatonchocotyle* [a]
	Branchotenthes [c]
Myliobatoid	*Dasyonchocotyle* [a]
	Paraheteronchocotyle [a]
	Neonchocotyle [a]
	Pseudohexabothrium [d]
	Heteronchocotyle [e]
Holocephalan	*Callorhynchocotyle* [a]

[a]Boeger and Kritsky (1989)
[b]Watson and Thorson (1976)
[c]Bullard and Dippenaar (2003)
[d]Agrawal *et al*. (1996)
[e]Neifar *et al*. (2001)

Boeger and Kritsky (1989) indicated that the hexabothriid taxa *Squalonchocotyle squali* MacCallum, 1931, and *Erpocotyle tiburonis* Brooks, 1934 (from *Squalus acanthias* Linnaeus, 1758, and *Sphyrna tiburo* Linnaeus, 1758 respectively) were unconfirmed species and required further study of fresh material because it was likely they were members of other genera. The hexabothriid taxon *Squalonchocotyle impristi* Hargis, 1955 was considered *incertae sedis* by Boeger and Kritsky (1989). However, since they had not considered the hexabothriid taxon *Pristonchocotyle* in their placement of confirmed hexabothriid genera, it is possible that *S. impristi* belongs to this genus. The recent publications on *E. tiburonis* by Bullard *et al.* (2001) and *S. squali* by Martorelli *et al.* (2008) appear to ignore the unconfirmed status of these hexabothriid taxa (*sensu*

Boeger and Kritsky 1989) and should therefore not be seen as valid until the amendment of their respective generic diagnoses has been addressed. These species therefore remain *incertae sedis*. Additionally, the hexabothriid taxon *S. squali* has been described a fourth time by Martorelli *et al.* (2008), following its original description (MacCallum, 1931), a new combination description (Price 1942), and a further redescription by Dillon and Hargis (1968) from the additional host *Squalus lebruni* (Vaillant, 1888).

A single accepted standard for the discrimination of species in terms of exact measurements taken and nomenclature in Hexabothriidae, is lacking. Martorelli *et al.* (2008) indicated a difference between the relationships between the body length to haptoral appendix length and the body length to sucker complex sclerite length of their *S. squali* material and that of the redescription of Dillon and Hargis (1968). This highlights a potentially fundamental error in the interpretation of nomenclature and measurement protocols by different authors.

For example, Dillon and Hargis (1968) measured the sucker complex sclerites of *S. squali*, referring to: "the measurement of curved structures" taken "across the lines subtending the greatest arcs described by those structures," and, "the length of clamps is regarded as the greatest dimension of the sclerotized framework; the width is taken as the greatest dimension at right angles to the length."(Fig.1.1A). Although Dillon and Hargis (1968) described several monogenean species from 3 different families, of which some have haptoral clamps, Hexabothriidae has curved sucker complex sclerites. Martorelli *et al.* (2008) made no reference to how they measured the length or width of the haptoral sclerites of their *S. squali* material, though they did indicate that the generic classification structure of their redescription followed that of Boeger and Kritsky (1989). The latter authors defined the sucker complex sclerite length as the central circumferential distance between the "point tip" and base of the shaft (Fig. 1.1B). It is therefore likely that the discrepancies encountered by Martorelli *et al.* (2008) between their new *S. squali* material and that of the redescription of Dillon and Hargis (1968) are merely the result of ambiguity in measurement nomenclature since measurement protocols were not consistent.

The nomenclature used to indicate specific taxonomic structures in the hexabothriid literature can be somewhat confusing and ambiguous especially when making reference to the haptoral sucker complex sclerites. These seemingly small

discrepancies in the literature have the potential to create further confusion in descriptions using 2 or more different sets of measurements for the same structures in comparison.

Although Price (1942) used the terms "large crescentic hooks" and "large root and blade" without reference to either separate shaft or hook measurements for the haptoral sucker complex sclerites, Wiskin (1970) was the first to separate the haptoral sucker complex sclerites of hexabothriids into the hook and the shaft, (Fig. 1.1C) and the hamulus into hamulus hook and hamulus shaft (Fig. 1.2). Brinkmann (1971) subsequently gave reference to "hook-like sclerites with prong" to indicate the sucker complex sclerite hook but this is misleading since the word prong defines one of two or more projecting points of a forked structure not a singular curved point. It is the opinion of the author that all publications on hexabothriids subsequent to Wiskin (1970) should refer correctly to these structures in nomenclature and measurement protocols should reflect these specific structures. This is not the case however.

Boeger *et al.* (1989) in their revision of *Callorhynchocotyle,* confused the sucker complex sclerite hook with the true sucker complex sclerite shaft (Fig. 1.1D), subsequently adopted by Beverley-Burton and Chisholm (1990) for measurements of *C. hydrolagi*.

Inconsistencies in measurement protocols are evident in much of the literature. Boeger and Kritsky (1989) published the revision of Hexabothriidae wherein they defined the sucker complex sclerite length for determining robustness (the ratio of sucker complex sclerite width divided by length) as the centre circumferential distance from the hook tip to the shaft base (Fig. 1.1B). This is particularly confusing as the total sucker complex sclerite length was defined by Boeger *et al*. (1989) as the straight line distance between the furthermost points on the length of the sucker complex sclerite (Fig. 1.1D).

Neifar *et al.* (2001) indicated sucker complex sclerite total length as the outer circumferential measurement from hook tip to shaft tip and similarly uses the outer circumferential length of the hook which he named the claw, as the hook length (Fig. 1.1E).

The shaft length used by Glennon *et al*. (2005) was the same as that of Domingues *et al*. (2007) yet differs again from Boeger *et al.* (1989) (Fig. 1.1F). The width measurement given for the sucker complex sclerites in both Glennon *et al.* (2005)

and Domingues *et al.* (2007) differs slightly although determined similarly in both publications (Fig. 1.1F). The maximum sclerite width of Glennon *et al.* (2005) is determined roughly through the thickest part of the sucker complex sclerite shaft. The sucker complex sclerite width defined by Domingues *et al*. (2007) is measured across the middle of the shaft, seemingly without reference to any morphometrically measured origin and is clearly not an indication of maximum width, which should be measured closer to the hook in this species if following the example of Glennon *et al*. (2005).

Domingues *et al.* (2007) makes reference to the use of morphological characters of Boeger and Kritsky (1989) for his hexabothriid taxon, however it is clear that Boeger and Kritsky (1989) make reference only to sucker complex sclerite width in similarity which is used in ratio to sucker complex sclerite length to determine robustness (as noted above).

A confusion of terms in the nomenclature of soft structures is also evident. Glennon *et al.* (2005) proposed a change in terminology for *vasa efferentia* and vas deferens to sperm ducts and common sperm duct respectively in hexabothriids after the incorrect use of the former term in the generic diagnosis of *Branchotenthes* (Bullard and Dippenaar 2003). Glennon *et al*. (2005) emphasised that the term *vasa efferentia* is defined as many convoluted ducts and is not the character being described by Bullard and Dippenaar (2003). The term vas deferens was changed to common sperm duct in order to avoid confusing the term with that of *vasa efferentia*. However, the latter change is unnecessary after the initial change proposed to the former and as such remains unchanged for the dissertation.

Seemingly careless editorial errors in the current hexabothriid literature also add to taxonomic confusion. Wiskin (1970) refers to *Rajonhocotyle emarginata* not *Rajonchocotyle emarginata* in discussing the history of low success rates in hatching hexabothriid eggs *in vitro*. Similarly, the species redescription of Domingues *et al*. (2007) of the species they named *Paraheteronchocotyle amazonense* Mayes, Brooks and Thorson, 1981, is an erroneous misspelling of *P. amazonensis* Mayes, Brooks and Thorson, 1981. *Paraheteronchocotyle amazonense* is therefore an invalid pseudo-synonym. In discussing additional reports of monogeneans affecting lemon sharks (*Negaprion brevirostris* Poey, 1868), Poynton *et al*. (1997) mentions "*Heterocotyle hypoprioni* (Hexabothriidae)" though *Heterocotyle* Scott, 1904 is in fact a member of

Monocotylidae Taschenberg, 1879 and not of the Hexabothriidae and forms part of the sub-class Polyonchoinea, not the sub-class Oligonchoinea. Although similar phonetically the correct hexabothriid genus is *Heteronchocotyle*.

Suriano and Incorvaia (1982) refer to *Callorhynchocotyle marplatensis* Suriano and Incorvaia, 1982 as *C. callorhynchi* and *C. callorhynchy* in the original description. Additionally, editorial errors in Beverley-Burton and Chisholm (1990) (*pers. comm*. Leslie A. Chisholm) for the comparative measurements of the cirrus of representatives of *Callorhynchocotyle,* for *C. callorhynchi* (Manter, 1955) have the potential to cause comparative differences in subsequent reviews. These measurements for *C. callorhynchi* are roughly 10 times smaller than they should be and are discussed accordingly in the genus review in Chapter 4.

Dillon and Hargis (1968) noted that there was a significant difference in the sucker complex sclerite hook lengths between then *Erpocotyle callorhynchi* (Manter, 1955) (junior synonym of *C. callorhynchi*) from *Callorhinchus capensis* in South Africa and *C. milii* Bory de Saint-Vincent, 1823 from New Zealand but failed to question the possibility they were separate species. The population of *E. callorhynchi* from *C. milii* in New Zealand was subsequently re-described as *Callorhynchocotyle amatoi* Boeger, Kritsky and Pereira, 1989 based on the differences between the morphology of the sucker complex sclerites alone. This raises an interesting question regarding the minimum requirements for the discrimination of taxa in Hexabothriidae but could also indicate the strong possibility of using these characters in future for discrimination of other hexabothiid species and genera as long as consistent measurements can be obtained. It should however be noted that errors in all the hamulus measurements of the 3 *Callorhynchocotyle* species in Boeger *et al.* (1989) as identified by Beverley-Burton and Chisholm (1990) raises questions regarding the accuracy of current hexabothriid descriptions.

Most authors on hexabothriid taxa have noted the importance of the haptoral sclerites in discriminating between species albeit with a lack of consensus on how this should be done. But, Beverley-Burton and Chisholm (1990) have expressed concerns over the use of sclerite measurements from fixed haptors that are rarely completely flattened in museum-deposited reference material for species of *Callorhynchocotyle.* The traditional difficulty with accurate hexabothriid sucker complex sclerite measurements

may originate from the shape of these sclerite structures which are not flat but naturally curved in such a way that the hook and shaft base form a weak "S" curve as illustrated by Cerfontaine (1899) for *Rajonchocotyle alba* Cerfontaine, 1899. Depending on its orientation upon fixation this could influence measurement variability. However, the problem of obtaining consistent measurements from sclerites from alcohol-fixed material could be resolved if they could be removed from the haptor proper prior to final flat fixation in mounting medium.

Investigations of novel techniques to assist with the morphometric discrimination of other monogenean taxa using the sclerotised parts of the haptoral armature are well documented. Initial proteolytic digestion of the sclerotised hard parts from the surrounding haptoral tissue for morphometric discrimination of gyrodactylid monogeneans was performed by Harris *et al*. (1999) and Harris and Cable (2000). Subsequently, Shinn *et al.* (2001) discussed the use of proteolytic digestion of the sclerotised parts of the haptor from species of *Gyrodactylus* von Nordmann, 1832. This provides support for a higher level of accuracy for statistically supported morphometrics in assisting with species discriminations (Shinn *et al*. 2004). More recently, Vaughan *et al*. (2008b) used a modified proteolytic digestion technique of Harris *et al*. (1999) to assist with the correct orientation of sclerotised structures from the haptors of species of *Dendromonocotyle* as well as sclerotised parts of their reproductive structures.

Shinn *et al.* (1996) indicated that the shape of the marginal hooklet sickle of gyrodactylids made it a useful feature for discriminating species, but that different combinations of the hamulus shape and marginal hooklet shape gave better resolution describing species in *Gyrodactylus*.

Unlike representatives of *Gyrodactylus*, all hexabothriids possess 6 sucker complex sclerites (paired sclerites of the 3 sucker sclerite complexes) and a

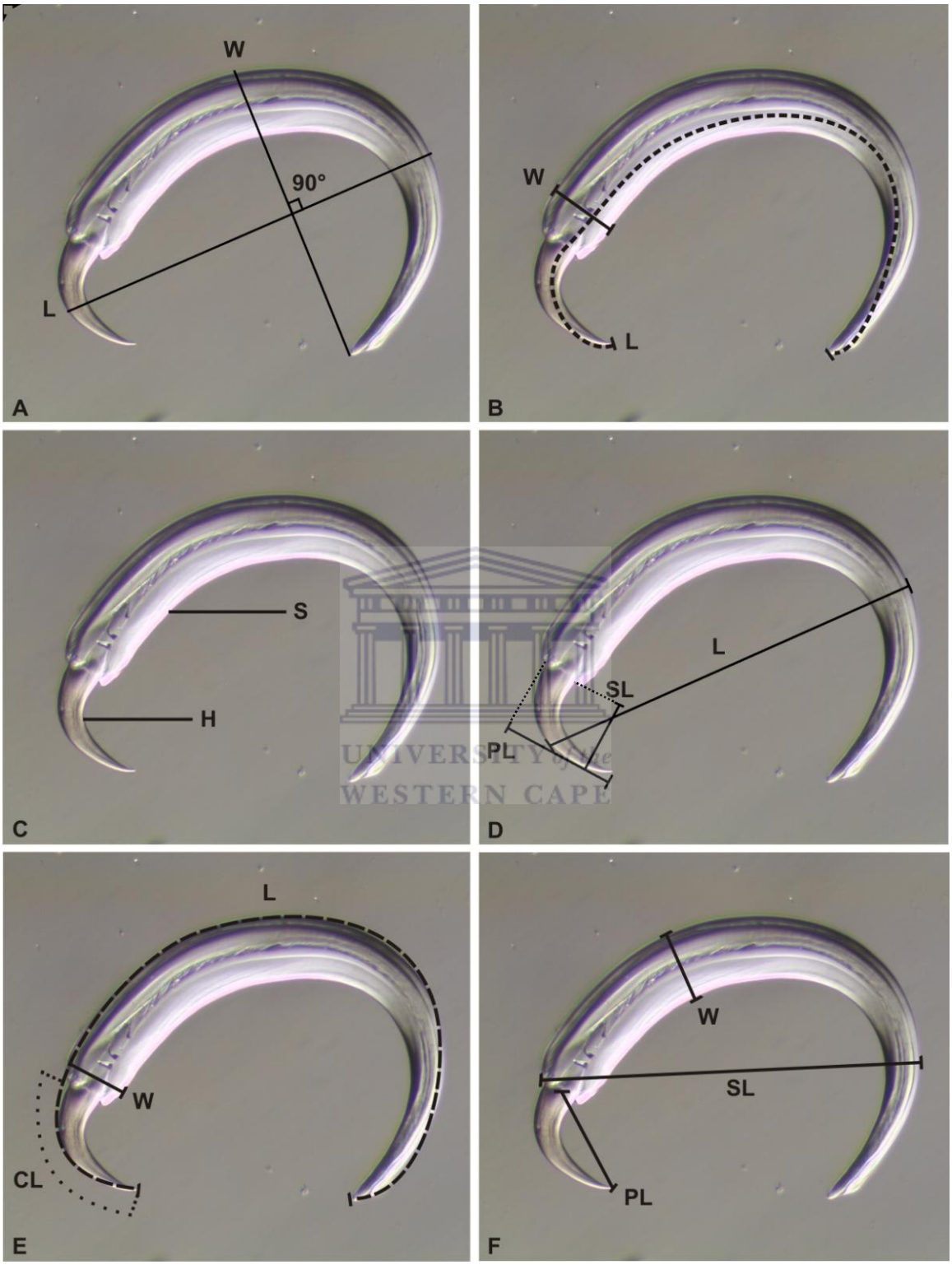

Fig. 1.1 Sclerite morphology and photomicrograph measurement overlays interpreted from: A. Dillon and Hargis (1968); B. Boeger and Kritsky (1989); C. Wiskin (1970); D. Boeger *et al.* (1989); E. Neifar *et al.* (2001); F. Glennon *et al.* (2005). Abbreviations: Sclerite length (L), sclerite width (W), shaft (S), hook (H), shaft length (SL), point length (PL), claw length (CL).

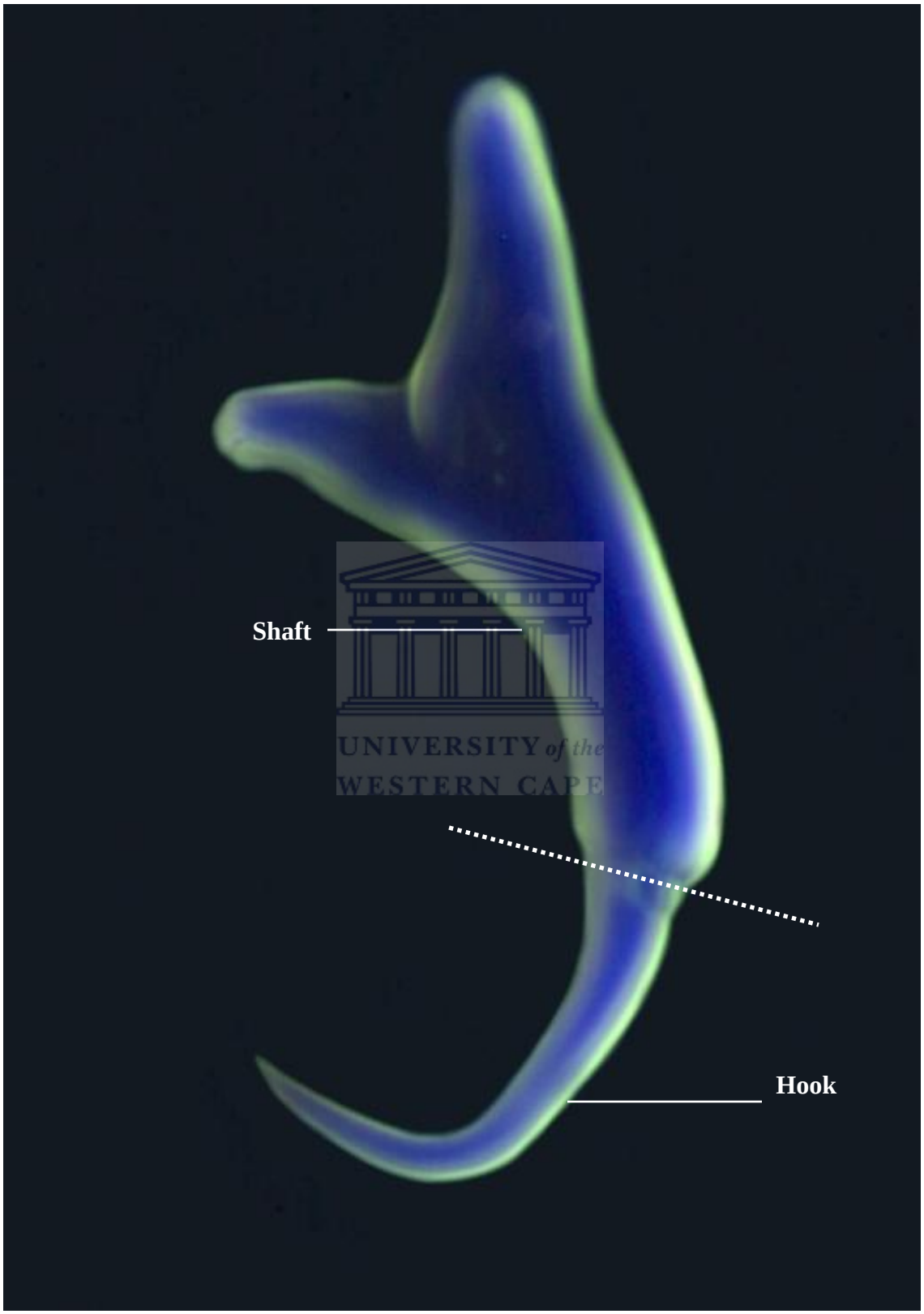

Fig. 1.2 Hamulus structures according to Wiskin (1970). A clear distinction is made between the hamulus shaft and the hamulus hook.

pair of hamuli. The exception is *Paraheteronchocotyle amazonensis*, the only known freshwater hexabothriid which loses the hamuli secondarily. Marginal hooklets may either be lost secondarily in the adult haptor or cryptically concealed within the musculature of the sclerite suckers of hexabothriids (*pers. comm*. Leslie A. Chisholm).

Little work has been done on the few oncomiracidia of hexabothriids and no emphasis has been given to the use of marginal hooklets as discriminating characters in this family although *B. octohamatus* is currently the only hexabothriid with an oncomiracidium which possesses 8 marginal hooklets (Glennon *et al*. 2005). The paucity of marginal hooklet reports in the hexabotrhriid literature and the slim chance of discovering them consistently in adult specimens limits their value as a discriminating character.

The lack of consensus for a single accepted measurement protocol for the sucker complex sclerites and hamuli in Hexabothriidae supports the call for a standard which will lead to subsequent stability. A new measurement protocol is therefore proposed as part of the dissertation, discussed in Chapter 3.

Only 2 hexabothriid species from separate genera have to date been reported from South Africa. *Callorhynchocotyle callorhynchi* and *Branchotenthes robinoverstreeti* Bullard and Dippenaar, 2003 were reported from the West and East coasts of South Africa respectively. Both respective genera are reviewed as part of this dissertation including redescriptions of the South African species using the new measurement protocol proposed.

1.3 Research aims / Hypotheses

The academic aims of the dissertation are:

1. To provide a critical review of the historical hexabothriid literature.
2. To propose a preliminary morphometric protocol for the discrimination of hexabothriids. Here, the hypothesis that the haptoral armature, represented by the sucker complex sclerites and hamuli, are robust characters for discriminating hexabothriid species, will be tested.
3. To investigate whether the novel use of morphometrics of the sucker complex sclerites and hamuli within the haptor can be used to support

robust discrimination of hexabothriid species, using representatives of *Callorhynchocotyle* as examples. The hypothesis will test whether statistical analyses of the characters of the haptoral armature will distinguish between representatives of *Callorhynchocotyle* from host families Callorhinchidae Garman, 1901 and Chimaeridae Bonaparte, 1831.

4. To review *Callorhynchocotyle* and *Branchotenthes*, providing new voucher material for members of *Callorhynchocotyle.*

CHAPTER 2
Material and methods

Callorhynchocotyle callorhynchi specimens were collected from the gills of their host *Callorhinchus capensis.* Host fishes were caught by the RV *Africana* as by-catch during annual surveys of the South African demersal hake (*Merluccius capensis* Castelnau, 1861 and *M. paradoxus* Franca, 1960) stocks between 2006 and 2008 (Figs 2.1 and 2.2).

Specimens of *Callorhynchocotyle amatoi* were provided by Prof. Venkatesh Byrappa, Principal Investigator for the Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Singapore. These specimens were collected as part of the molecular study currently being investigated for the host species *Callorhinchus milii* Bory de Saint-Vincent, 1823 off New Zealand and *C. capensis* off South Africa for which genetic material was collected and contributed as a reciprocal collaboration between both studies. Specimens of *C. sagamiensis* Kitamura, Ogawa, Taniuchi and Hirose, 2006 were provided by the Department of Aquatic Bioscience, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Japan. The *C. sagamiensis* material was collected in Tokyo Bay on board a trawler on 29 August 2008 from a female Ginzame, *Chimaera phantasma* Jordan and Snyder, 1900 measuring 1060 mm total length, 804 mm body length, and weighing 3980 g.

Specimens of *C. callorhynchi* were placed live without water into the inverted lid of a glass Petri dish and manipulated using a small paint brush before using the Petri dish, placed on top of the specimens to flat-fix them in absolute alcohol. All *C. amatoi* material was fixed with whole gill arches in absolute alcohol where specimens of *C. sagamiensis* were removed from the gills prior to fixation. All the voucher material collected for this study was preserved in absolute alcohol to facilitate proteolytic haptoral digests and future molecular investigation for which no previous work had been done on representatives of *Callorhynchocotyle*.

Fig. 2.1 The research vessel Africana operated by the South African Government Department of Environment and Tourism (DEAT), Marine and Coastal Management.

Fig. 2.2 A typical demersal trawl on board the research vessel Africana. Chondrichthyan fishes by-catch including *Callorhinchus capensis* can be seen in the foreground.

In addition to the new material collected as part of this study museum type and voucher material was accessed for all species of *Callorhynchocotyle* excluding *C. sagamiensis*, and *Branchotenthes*. However, museum specimens of *C. hydrolagi* Beverley-Burton and Chisholm, 1990 USNPC 080982.00 paratypes MT25-15A and B were of poor quality and could provide only supplemental information. USNPC 080982.00 paratype MT25-15B is mounted on its side and could not be used in any comparisons of internal structures. Other representatives of the type series were requested but could not be accessed. The type series of *C. sagamiensis*, although indicated as deposited in the Meguro Parasitological Museum in Japan (Kitamura *et al*. 2006) had not been deposited in the museum's collection at the time of this study and were not available for additional comparison.

2.1 Staining and mounting

Some of the voucher specimens collected for this study were hydrated in freshwater before being stained in Alum Carmine using the following recipe:

Stock solution

1) 1 g carminic acid
2) 10 g ammonium alum
3) 200 ml distilled water
4) 1 ml formalin (added after dissolving and filtering of the above)

Working solution

1) 5 ml stock solution
2) 0.4 ml glacial acetic-acid
3) 100 ml distilled water

The voucher material of the different species was stained for a period generally not exceeding 5 hours although some individual specimens were kept in the stain for up to 24 hours depending on the desired level of staining required. Freshly collected material

stained more evenly and more quickly than material that had been preserved and stored for up to 2 years.

After staining, the specimens were rinsed in freshwater and subsequently dehydrated in a graded alcohol series, cleared in cedar-wood oil and permanently mounted individually onto glass microscope slides in Canada balsam.

Remaining specimens were hydrated in freshwater and their haptors were removed with a scalpel blade. The haptors and corresponding bodies were given matching codes for processing. All haptors were digested following Harris (1999) using a Proteinase-K solution, modified, which was added to the correctly positioned haptor directly onto the microscope slide with a micro-pipette. The modified Proteinase-K solution recipe used in this study is the property of Dr. Andrew Shinn (Institute of Aquaculture, University of Stirling), and is unpublished. Bodies of corresponding haptor digests were stored in absolute alcohol for future molecular investigation.

The process of digestion was monitored for each individual haptor under an Olympus SZ60 Stereo zoom dissection microscope. The digestion process was controlled by either adding additional Proteinase-K solution heated to 55°C or cool distilled water to inhibit the process and re-hydrate crystalline enzyme during the procedure. Excess crystalline enzyme was re-hydrated and removed using paper towelling until only the sucker complex sclerites and hamuli remained. A small drop of molten glycerine jelly (see next page) was placed quickly on an inverted coverslip and slowly lowered onto the liberated sclerites and hamuli. Once the glycerine jelly had hardened the edges of the coverslips were sealed with clear nail varnish. The glycerine jelly recipe used here is a modification of that employed by Gussev (1983), and is presented below:

1) 7 g food-grade gelatine
2) 43 ml distilled water
3) 50 g A/R-grade glycerine
4) 0.5 g Phenol crystals

Inconsistencies in the hexabothriid literature and the lack of consensus for a single accepted measurement protocol for sucker complex sclerites and hamuli, calls for

stability in character nomenclature and the measurements thereof. The subsequently proposed measurement protocol for hexabothriid sclerites and hamuli follows the initial nomenclature of Wiskin (1970). However, of the traditional hamulus structures of Wiskin (1970), the hamulus hook is further separated into hamulus hook point and hamulus hook shank (after Shinn *et al*. 1996) in order to provide the necessary nomenclature to support the new variables proposed. Additionally, to ensure a high level of repeatability, all sucker complex sclerites and hamuli were first quadrangulated to anchor the same points of morphometric origin to minimise interpretive error and variability in the measurements of distances and angles (Brower and Veinus 1978). Character variable selection was based, in part on traditional hexabothriid literature, *Gyrodactylus* species morphometric literature examples (Shinn *et al.* 1996; 2001) and the traditional dactylogyrid hamulus morphometric example of N'Douba *et al*. (1997).

2.2 Morphometric analyses

All drawings and photomicrographs are original artwork and are not reproduced from existing literature or constructed by computer software. All drawings were done with the aid of an Olympus drawing tube. All measurements given in this study are given in micrometres as the mean ± standard deviation, followed by the range and number of structures measured, in parenthesis. Total body length, maximum body width, haptor length and haptor width were measured using an eye micrometer fitted to an Olympus SZ60 Stereo Zoom dissection microscope. All additional measurements were taken with Olympus AnalySIS5® analysis software registered to the Two Oceans Aquarium, calibrated to an Altra20 Olympus digital microscope camera fitted to an Olympus CX41 compound light microscope fitted with phase-contrast and dark-field condensers.

2.2.1 Morphometric measurement protocol

Fifteen separate measurements for each of the sclerites representing each sucker sclerite complex pair of Boeger and Kritsky (1989), and 13 separate measurements for the hamuli, are proposed (Figs 2.3–2.10). Each measurement is discussed in sequence beginning with the morphometrics of the sucker complex sclerites.

2.2.1.1 Sucker complex sclerite characters

Sclerite quadrangular orientation (Fig. 2.3A): Sclerites were first orientated into a rectangle (Brower and Veinus 1978) defined by their longest and widest points, with hook and shaft resting on the horizontal base of the rectangle.

1. Sclerite circumferential length (Fig. 2.3B): The outer circumferential distance between sclerite hook tip and the end of the shaft.
2. Sclerite total length (Fig. 2.3B): The furthest distance between ends of the sclerite, measured parallel with the rectangle horizons.
3. Sclerite total diameter (Fig. 2.3C): The furthest distance between ends of the sclerite, measured parallel with the vertical walls of the rectangle.
4. Sclerite width (Fig. 2.4A): A horizontal line was drawn, parallel with the horizons of the rectangle, meeting the point of inner-most curvature of the sclerite shaft. The maximum distance between this point and opposing point as defined by the horizon of the rectangle meeting the outer most curvature of the sclerite shaft, was measured.
5. Shaft length (Fig. 2.4B): The straight line distance between extremes of the shaft, as defined by the rectangle. Note that in some sclerites the points of this distance meet the vertical walls of the rectangle, and in others only one point meets one of the vertical walls.
6. Sclerite inner diameter (Fig. 2.4C): The vertical distance taken from the point of inner most shaft curvature and the base of the rectangle.
7. Sclerite aperture angle (Fig. 2.5A): The angle defined by the point of inner most shaft curvature, the hook tip and shaft base.
8. Sclerite aperture (Fig. 2.5B): The horizontal distance measured between hook tip and shaft base.
9. Sclerite hook-side curve length (Fig. 2.5C i): The 90° distance between the line defined by the aperture angle extending to the sclerite hook and the opposing inner most curve formed by the sclerite.

10. Sclerite shaft-side curve length (Fig. 2.5C ii): The 90° distance between the line defined by the aperture angle extending to the shaft base and the opposing inner most curve formed by the sclerite.
11. Sclerite hook length (Fig. 2.6A): The distance, bisecting the hook, measured as a straight line from the hook tip to the hook base.
12. Sclerite hook curve length (Fig. 2.6B): The 90° distance between the line defining the sclerite hook length and the inner most curve formed by the hook.
13. Sclerite hook aperture angle (Fig. 2.6C): The angle defined by the point of inner most hook curvature, the hook tip and hook base.
14. Sclerite hook aperture (Fig. 2.6D): The distance measured as a straight line from the hook tip to the hook base.
15. Sclerite hook base width (Fig. 2.6E): The distance between the points at the hook base defined by the hook length and hook aperture.

2.2.1.2 Hamulus character

Hamulus quadrangular orientation (Fig. 2.7A): The hamulus was first orientated into a rectangle (Brower and Veinus 1978) defined by its longest and widest points with outer root and shaft resting on the vertical side of the rectangle and hamulus hook resting on the horizontal base of the rectangle. Depending on the length of the inner-root, the hamulus hook point tip or inner root defined the opposite vertical side of the rectangle.

1. Hamulus total length (Fig. 2.7B): The furthest distance between ends of the hamulus, measured parallel with the rectangle vertical walls.
2. Hamulus total width (Fig. 2.7C): The distance between ends of the hamulus, measured parallel with the horizontal walls of the rectangle. The line was drawn to meet the inner most curve of the arc formed by both hamulus roots. This point was used as the origin for the root base angle.
3. Hamulus hook point length (Fig. 2.7D): The distance between the hook point tip and the point where the hook meets the horizontal base of the rectangle.

4. Hamulus hook shank length (Fig. 2.8A): The distance between the point where the hook meets the horizontal base of the rectangle, and the outer wall of the hook where the hook meets the shaft.
5. Hamulus distal hook point width (Fig. 2.8B): The vertical distance between the point where the hook point meets the horizontal base of the rectangle, drawn through the hook's width.
6. Hamulus outer aperture angle (Fig. 2.8C): The angle formed at the point where the hook meets the horizontal base of the rectangle, between hook point tip and the inner wall of the hook where the hook meets the shaft.
7. Hamulus inner aperture angle (Fig. 2.8D): The angle formed at the point on the inner wall of the hook defined by the distal hook point width, the hook point tip and the inner wall of the hook where the hook meets the shaft.
8. Hamulus hook aperture (Fig. 2.9A): The distance between the hook point tip and the inner wall of the hook where the hook meets the shaft.
9. Hamulus hook shank base width (Fig. 2.9B): The distance between the inner and outer walls of the hook where the hoot meets the shaft, as defined in 4, 6 and 7.
10. Hamulus outer root-shaft length (Fig. 2.9C): The distance from the inner wall of the hook where the hook meets the shaft, as defined in 6 and 7, and the point where the outer root meets the horizontal wall of the rectangle.
11. Hamulus inner root-shaft length (Fig. 2.9D): The distance from the inner wall of the hook where the hook meets the shaft, as defined in 6 and 7, and the dorsal-most point of the inner root.
12. Hamulus root base angle (Fig. 2.10A): The angle from the point defined in 2, between the outer and inner root opposing walls.
13. Hamulus root base width (Fig. 2.10B): The distance between points on each hamulus root, defined by the lines measuring both inner and outer root lengths.

2.2.2 Egg measurements

In the original description of *B. robinoverstreeti*, Bullard and Dippenaar 2003 included the tendrils of each egg pole in the total length measurement of the eggs. Not all hexabothriids produce eggs unattached to each other as in this species. All species within *Callorhynchocotyle* have eggs joined to each other by their tendrils in a long chain as does the second species in *Branchotenthes, B. octohama*tus. It is therefore impossible to differentiate between the total length measurements of joined eggs. In addition, the egg tendrils in some species are of considerable length and often twisted and looped. Therefore, for consistency, all hexabothriid eggs measured in this study were measured in total length of the furthest distance between inner walls of the egg capsule (Fig. 2.11). Total width was measured as the maximum width of the egg capsule bisecting the total length at 90° (Fig. 2.11).

2.3 Statistical analyses

The software Statistica 6 and 7 were used for all statistical analyses performed on transformed data. Raw data were all log and cosine (Cos) transformed. All linear measurements were log-transformed to compensate for increasing variance in increasing average sizes of characters (Shinn *et al.* 2001). All angular measurements were Cos-transformed to transform these angular data to a linear function (Shinn *et al.* 2001) and to provide a clear distinction between obtuse and acute angles of characters compared intra-specifically. These transformed data were further subjected to correlations between character variables and a surrogate variable selected to represent variance associated with age. All sucker complex sclerites were assumed to be influenced by age and were therefore considered allometric. Therefore, to reduce the variance associated with age upon individual character variables the age-dependant variables were ratio-transformed to the surrogate variable for age. The surrogate variable "circumferential length" was chosen because it best represented the direction of growth associated with age in hexabothriid sucker complex sclerites (see Wiskin 1970). The coefficient of variance (CV) representing the relative variability of each character variable was measured as a percentage of the mean ((standard deviation x 100) ÷ mean)). The CV of each variable was analysed after the ratio-transformation of the data. Any variables with high CV

values were disqualified from further analysis because high variance after compensating for age and log or Cos-transformation were considered the result of measurement error often experienced with very small structures (Shinn *et al*. 2001).

Transformed data for all character variables were used for univariate analysis to determine whether any single character variable could separate all the species from each other and the hexabothriid control taxon *Rajonchocotyle alba*. Univariate analyses were also used to indicate which character variables were significant in differentiating between the species. All data were subjected to a Levene test for homogeneity of variance to identify which data were normally distributed and therefore which variables required parametric or non-parametric testing. Character variables with parametric data were subjected to ANOVA using the Tukey HSD for unequal N (Spjotvoll/Stoline) post-hoc test. Non-parametric data were subjected to a standard Kruskal-Wallis ANOVA by ranks.

Transformed data were used for the multivariate analysis. The elimination of variance associated with age reduces the affects of allometry on the first principal component with positive coefficients for the variables where larger structures are associated with higher scores (Brower and Veinus 1978). Factors of the principal component analysis were selected using the Kaiser criterion (Kaiser 1960) selecting eigenvalues greater than 1 but only for factors consisting of the most significant factor loadings (those >0.7 both positive and negative). Only the first 2 or 3 factors, therefore, were used in the analysis allowing for a 2-dimensional or 3-dimensional representation of the data respectively. The breakdown of the factor analysis, eigenvalues and cumulative and total variances are tabulated in the appendices of the dissertation to provide the best representation of relevant information and to highlight character variables with the most influence on the analysis. Principal component analysis (PCA) is an exploratory technique and the disqualification of character variables possessing high CV values prior to this analysis allowed for a more refined final assessment of the characters tested. Currently, there are no strict rules governing the use of this technique, however it does allow for the best combinations of character variables to be explored and determined.

Power analysis of the data indicated that at least 30 specimens per species were required to make any meaningful statistical inferences. Unfortunately sample sizes for each species tested were too small (given the limitations of the number of specimens

borrowed) with the exception of *C. callorhynchi* for which 30 additional vouchers were collected for this study. As a result, the data for *C. callorhynchi* were used for the disqualification of character variables with high CV values associated with measurement error and correlation matrices to provide the resolution required for identifying age-dependant variables. The limitations of sample size compromised the sensitivity of the univariate tests and limited multivavriate analysis to the analysis of all the characters combined in a single PCA. Ideally, multivariate analyses should have been performed for each character separately and in various character combinations. However, preliminary results of these were inconclusive and are not reported on.

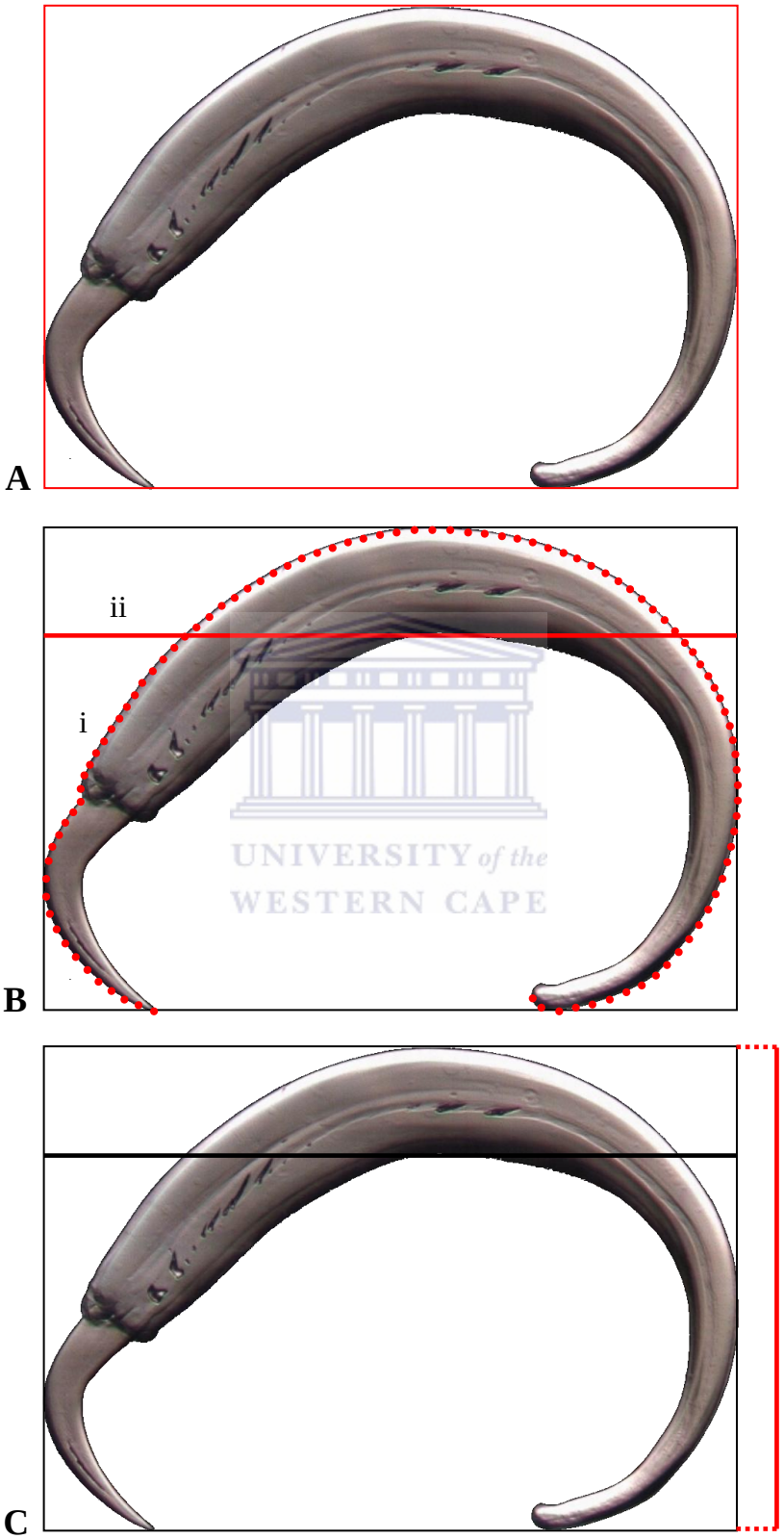

Fig. 2.3 Hexabothriid sucker complex sclerite measurement protocol. **A.** Orientation rectangle, **B.** sclerite circumferential (i) and total (ii) lengths, **C.** sclerite total diameter.

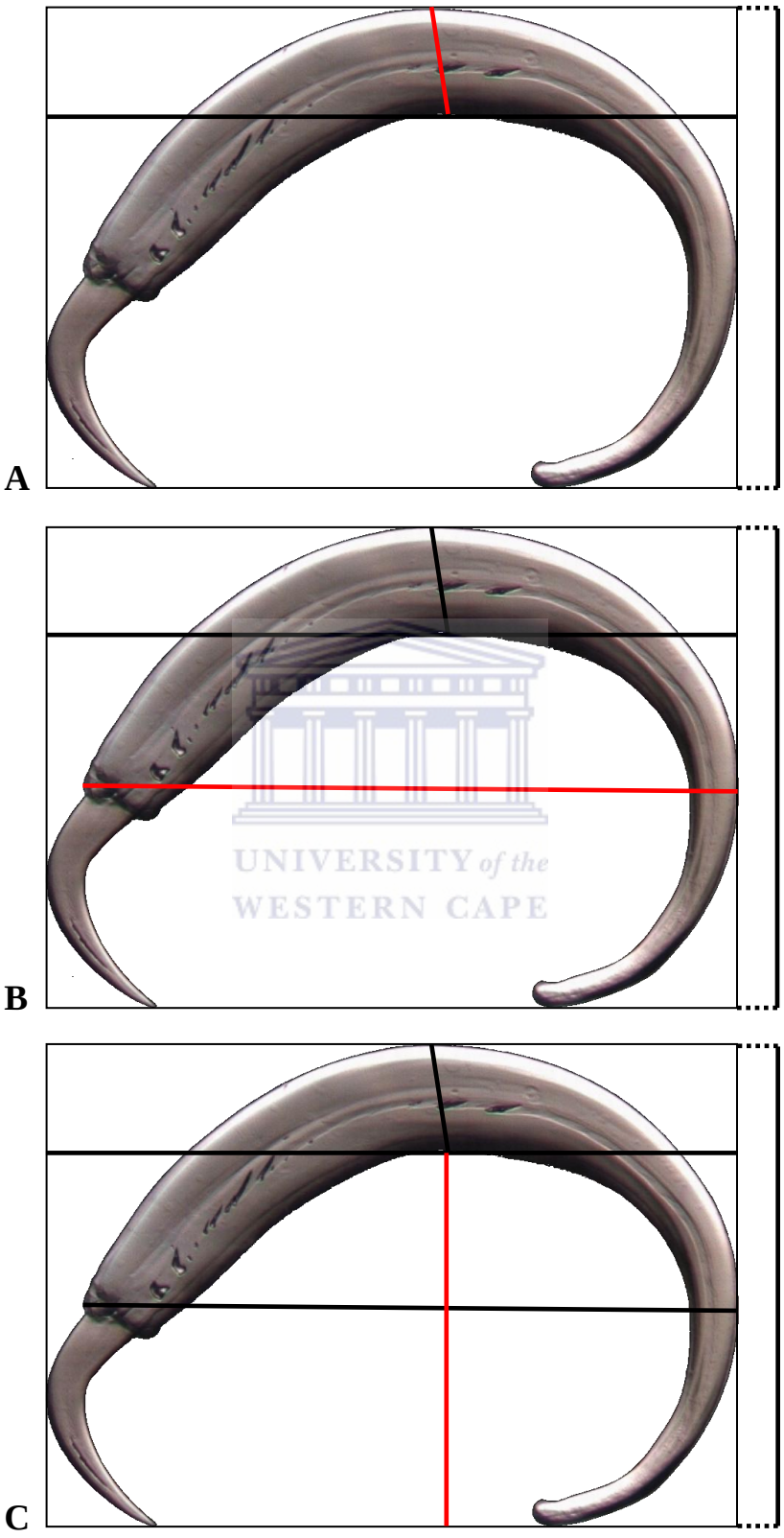

Fig. 2.4 Hexabothriid sucker complex sclerite measurement protocol continued. **A.** Sclerite width, **B.** sclerite shaft length, **C.** sclerite inner diameter.

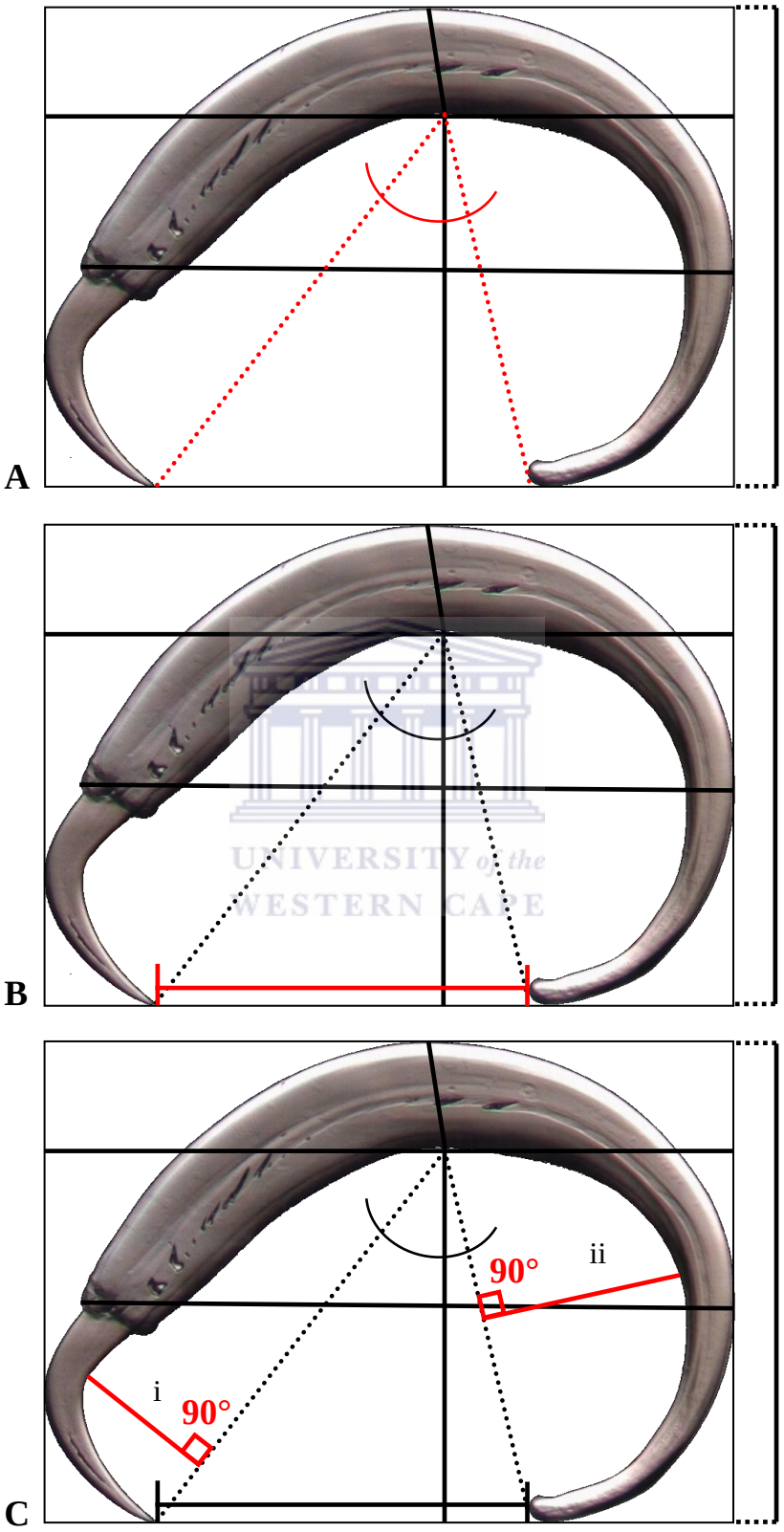

Fig. 2.5 Hexabothriid sucker complex sclerite measurement protocol continued. **A.** Sclerite aperture angle, **B.** sclerite aperture, **C.** sclerite hook (i) and shaft-side curve (ii) lengths.

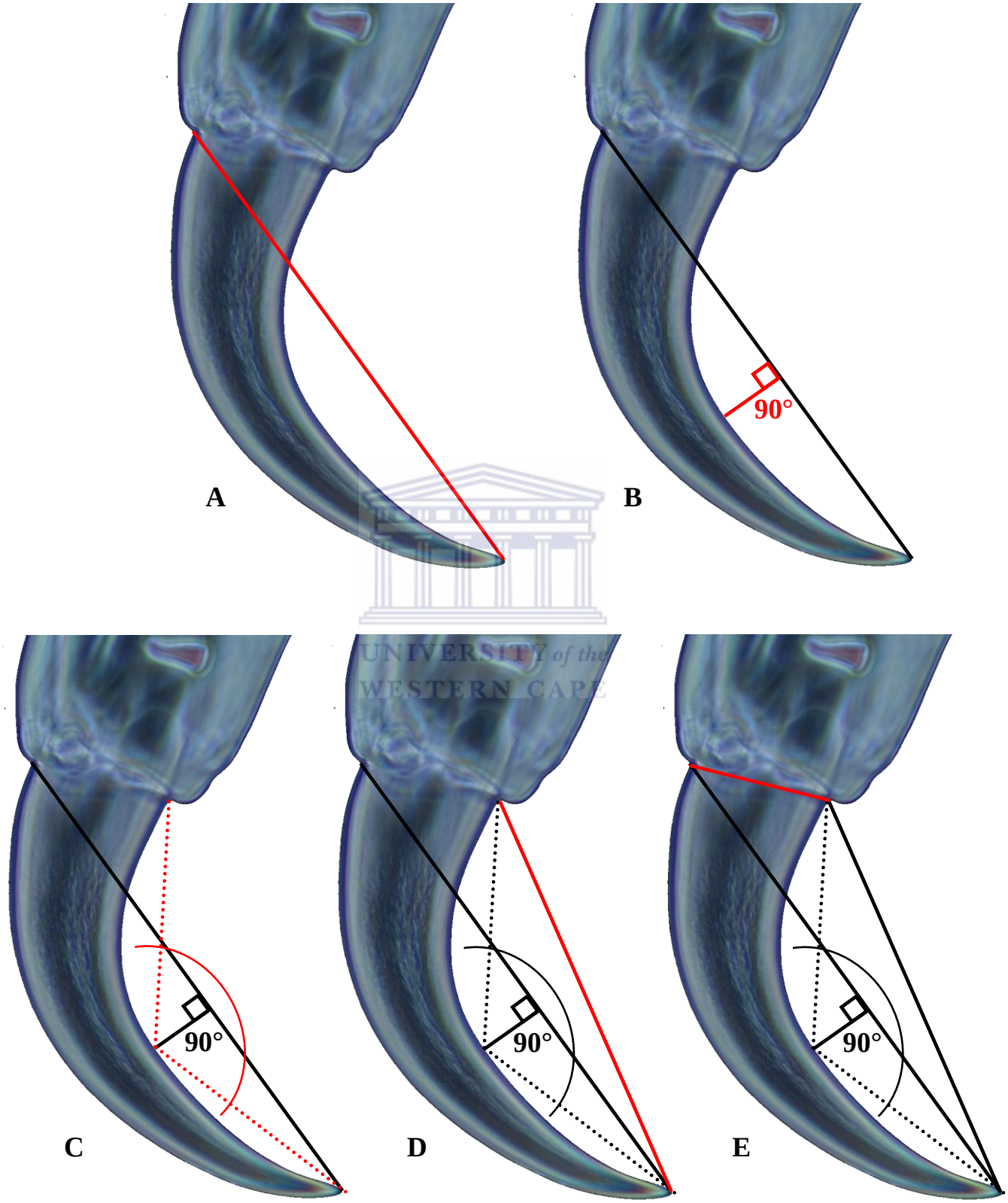

Fig. 2.6 Hexabothriid sucker complex sclerite measurement protocol continued. **A.** Sclerite hook length, **B.** sclerite hook curve length, **C.** sclerite hook aperture angle, **D.** sclerite hook aperture, **E.** sclerite hook base width.

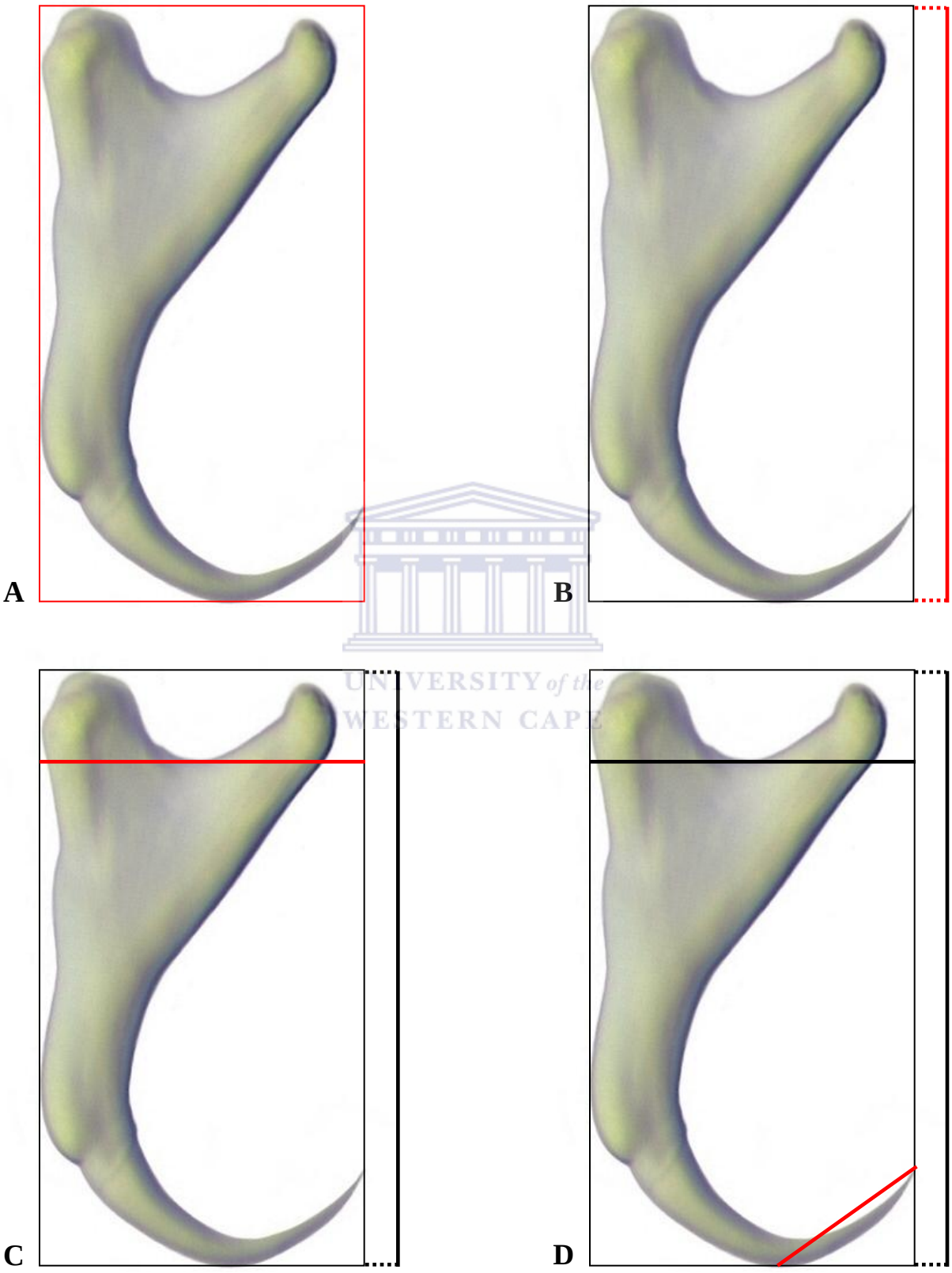

Fig. 2.7 Hexabothriid hamulus measurement protocol. **A.** Hamulus orientation rectangle, **B.** hamulus total length, **C.** hamulus total width, **D.** hamulus hook point length.

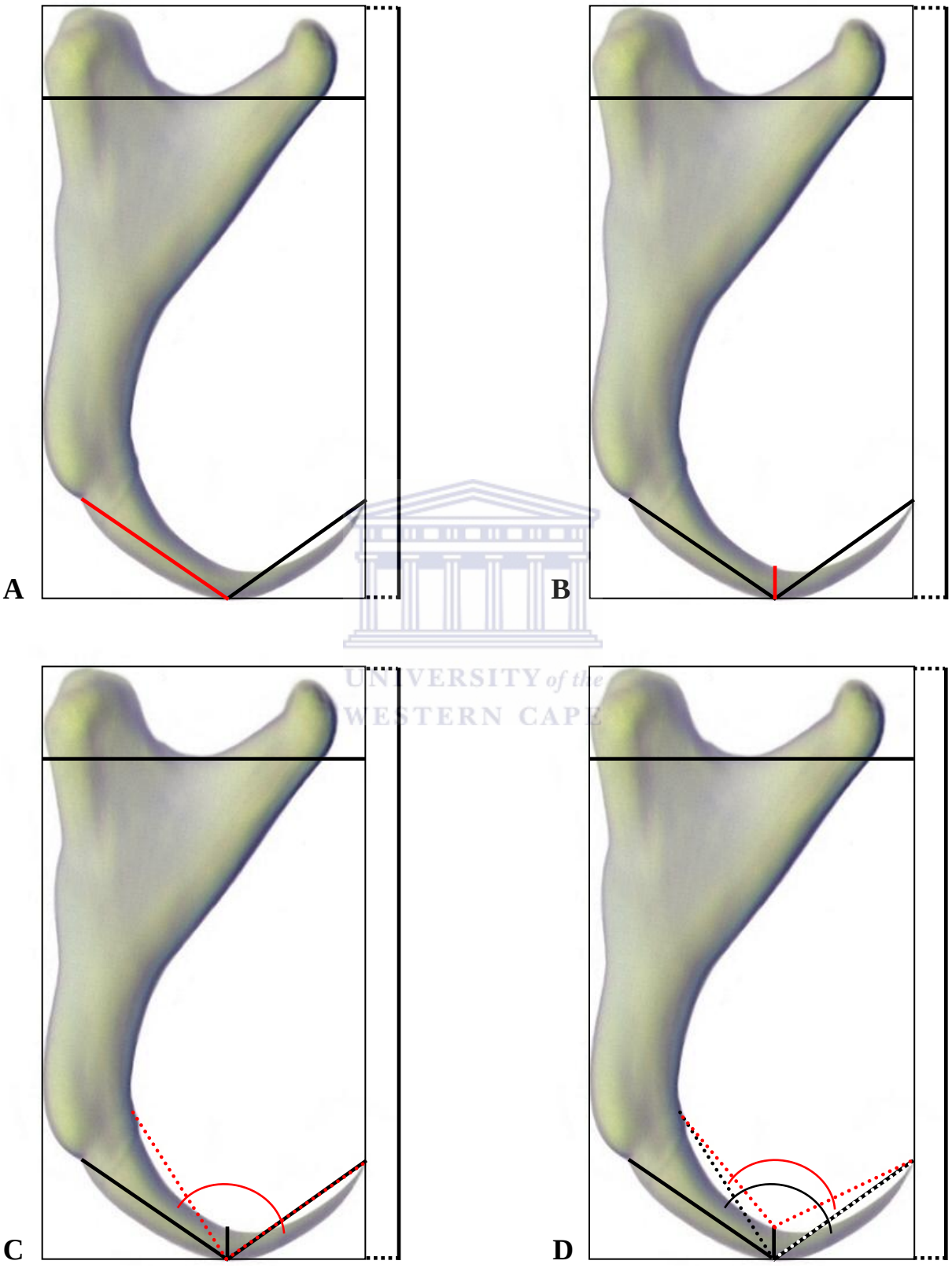

Fig. 2.8 Hexabothriid hamulus measurement protocol continued. **A.** Hamulus hook shank length, **B.** hamulus distal hook point width, **C.** hamulus outer aperture angle, **D.** hamulus inner aperture angle.

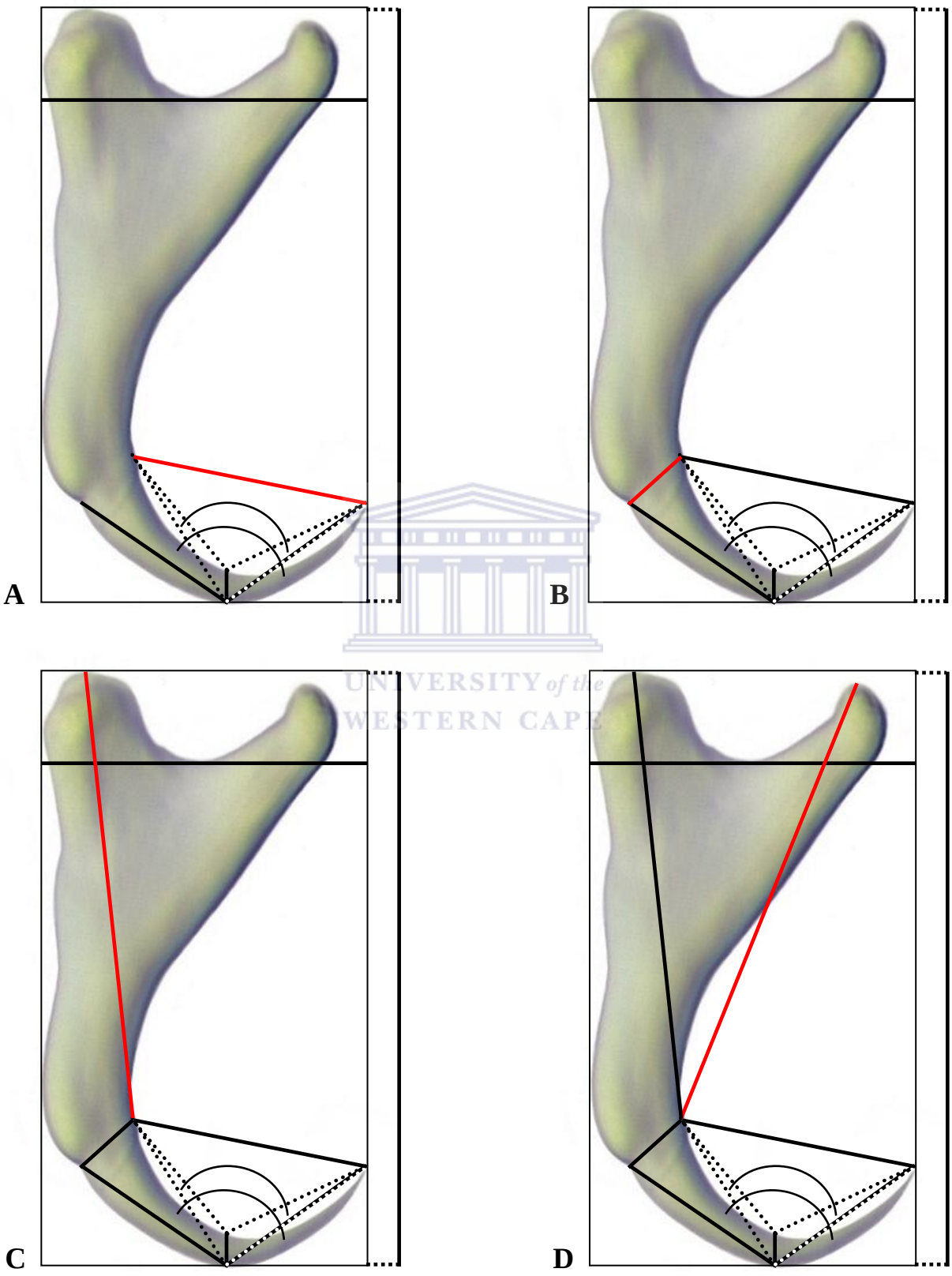

Fig. 2.9 Hexabothriid hamulus measurement protocol continued. **A.** Hamulus aperture, **B.** hamulus hook shank base width, **C.** hamulus outer root-shaft length, **D.** hamulus inner root-shaft length.

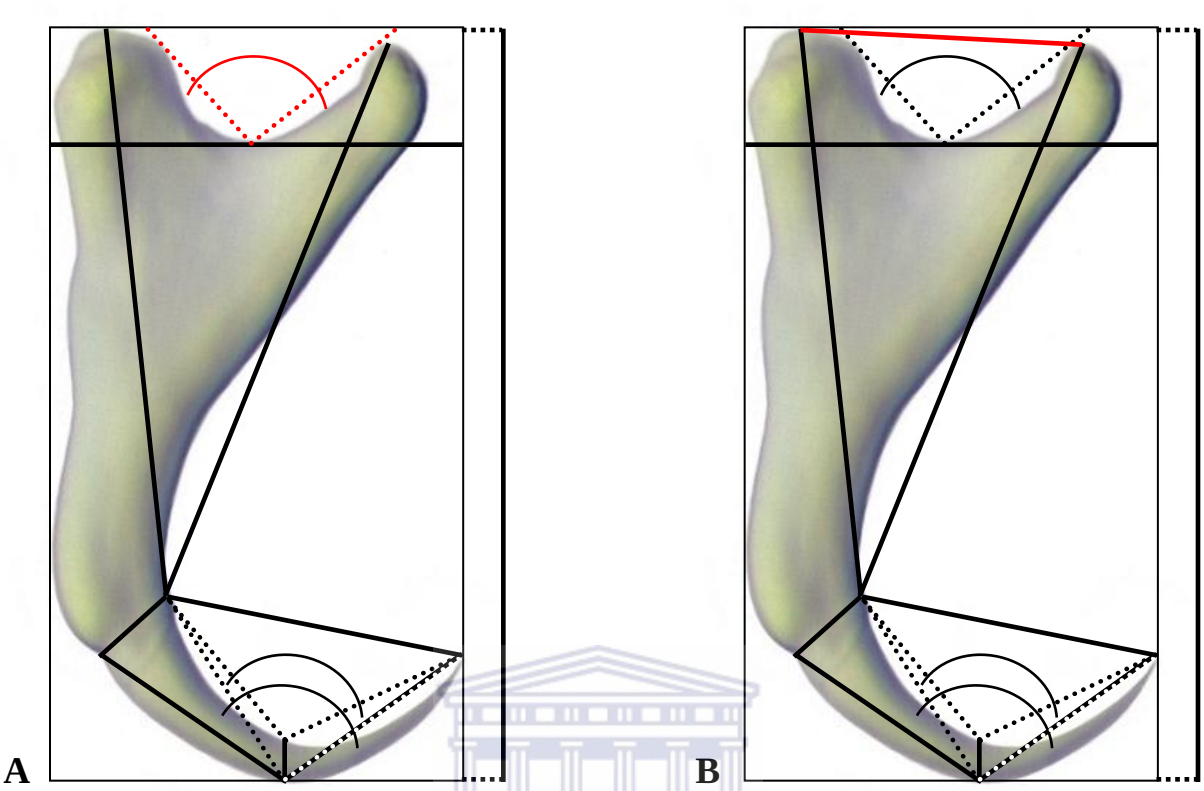

Fig. 2.10 Hexabothriid hamulus measurement protocol continued. **A.** Hamulus base angle, **B.** hamulus base width.

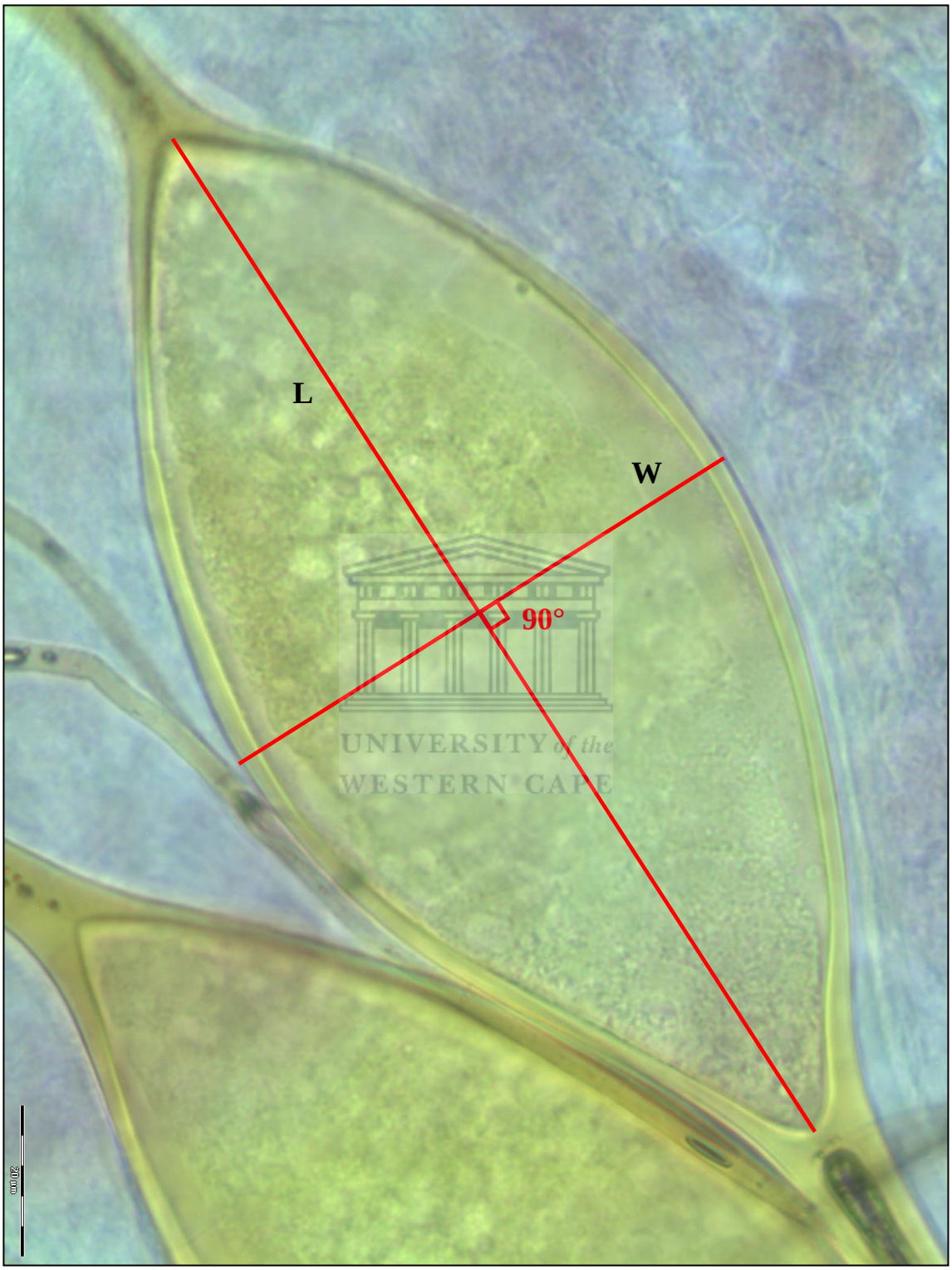

Fig. 2.11 Hexabothriid egg measurements used in the present study: **L.** total length, **W.** maximum width.

CHAPTER 3

Character variable selection, rationale and utility interrogation

The morphometric discrimination of hexabothriids has traditionally included a combination of both hard and soft characters. The resolution of soft characters in the hexabothriids is open to a certain amount of subjective interpretation since these characters are often affected by the fixation techniques employed by different taxonomists which results in high statistical variances around measures. However, the use of hard structures has not previously been subject to extensive testing and there is no standardised measurement protocol (see Chapter 1). The lack of consensus for a single accepted measurement protocol provides much ambiguity and possible error in both measurement and interpretation which is exacerbated by ambiguous nomenclature. Hard characters such as the sucker complex sclerites and the hamuli should produce results with lower variances as these structures if correctly prepared, should not be excessively affected by fixation methods.

Historically, measurement techniques for hexabothriid sucker complex sclerites have included only rudimentary morphometrics such as basic dimensions of length, width, circumference, and/or length-to-width ratio as an indication of "robustness" (Dillon and Hargis 1968; Boeger and Kritsky 1989; Boeger *et al.* 1989; Neifar *et al.* 2001; Glennon *et al.* 2005). Similarly, the morphometrics of the hamulus in hexabothriids is usually restricted to length, width (or base width) and root lengths (Kitamura *et al*. 2006). The use of hard characters however, has been shown to be important in the separation of morphologically similar hexabothriid species. Dillon and Hargis (1968) identified morphological differences between sucker complex sclerites of the hexabothriid *Callorhynchocotyle callorhynchi* from *Callorhinchus capensis* from South Africa and *Callorhinchus milii* from New Zealand. Boeger *et al.* (1989) subsequently described *Callorhynchocotyle amatoi* from the New Zealand host, separating it from *C. callorhynchi* by the differences in sucker complex sclerite morphology alone. Further evidence in support of the use of characters to separate similar species has been suggested by Beverley-Burton and Chisholm (1990), who provided obvious visual differences between the hamuli of both *C. callorhynchi* and *C. amatoi*. These authors did not however, discuss these differences in detail and only referred to differences in hamulus total length and base width measurements. The lack

of additional measuremens for hexabothriid sucker complex sclerites and hamuli reflects their often obscured angular position within the soft tissue of the haptoral complex in traditionally-fixed specimens. As a result Beverley-Burton and Chisholm (1990) raised concern over the measurement of these non-flat characters in the museum type series collections of *Callorhynchocotyle* species.

Previous studies on the diagnostic use of hard characters of monogeneans are provided by Shinn *et al*. (1996, 2001, 2004), Du Preez and Maritz (2006), Přikrylová *et al*. (2008). The studies by Shinn *et al*. (1996, 2001, 2004) concentrated on the discrimination of *Gyrodactylus* species infecting salmonids. Species in *Gyrodactylus* von Nordmann, 1832 share conserved overall morphology. However, the use of statistically supported morphometric analyses of hamuli and marginal hooklets has highlighted the robustness of each of these characters as valid species discriminators (Shinn *et al.* 2001; Přikrylová *et al*. 2008). Similarly, Du Preez and Maritz (2006) provided a revised protocol for the morphometric discrimination of species of *Polystoma* Zeder, 1800 using statistically supported variables of the marginal hooklet. Polystomatidae Carus, 1863 is more closely related to the Hexabothriidae than to Gyrodactylidae Van Beneden and Hesse, 1863. Hexabothriids however, lose their marginal hooklets secondarily as the haptor matures (Wiskin 1970) or they are cryptically concealed within the musculature of the sucker (*pers. comm*. Leslie A. Chisholm) and therefore these characters are unsuitable as discriminators in this family.

Current hexabothriid literature appears to have forgotten the pioneering work of Cerfontaine (1899) who used a basic digestion technique using an oxidising agent similar to sodium hypochlorite[3] in order to liberate the sclerites from the soft tissues of the haptor to allow their complete examination. Similarly, Wiskin (1970) dissected out sclerites and hamuli in her study on the ontogeny of the hexabothriid *Rajonchocotyle emarginata*. With the modification of the proteolytic digestion technique of Harris (1999) and Harris and Cable (2000) sucker complex sclerites and hamuli have been liberated from the soft tissue of the haptor of the new voucher material used in the current study to mitigate their non-flat nature in order to provide the framework necessary to test new measurement variables. Only completely flattened characters have been used for the morphometric analysis of existing type material borrowed from various museum collections.

[3]The original name of the oxidising agent used is lost in translation.

The lack of consensus for a single morphometric protocol for measuring hard characters in hexabothriids, ambiguity in nomenclature, their potential as discriminating characters and the mitigation of problems faced with non-flat hard characters through the use of proteolytic digestion of new material supports the proposal of a new morphometric measurement protocol tested preliminarily herein for representatives of the hexabothriid genus *Callorhynchocotyle*. Preliminary statistical analyses of characters and their variables following the rationale of Shinn *et al*. (1996, 2001, 2004) and Du Preez and Maritz (2006) were used to test the following research questions:

1. Can any single character variable discriminate between all the species tested?
2. Can any single character variable discriminate between *Callorhynchocotyle* species and *Rajonchocotyle alba*?
3. Could individual characters distinguish between *Callorhynchocotyle* species, and between *Callorhynchocotyle* species and *R. alba*?
4. Can the combination of characters be used to separate all the species tested?

All new measurements tested herein are extrapolated from existing hexabothriid literature, Shinn *et al*. (1996, 2001) and traditional hamulus morphometrics of true hamuli. True hamuli posses an inner and an outer root. All extrapolated measurement examples are combined, modified and in some instances corrected for by the quadrangular orientation of characters to return the same measurement points (or angles) of origin per character in order to reduce interpretive error and therefore unnecessary variance (see Chapter 2). Character variable abbreviations follow those defined in Chapter 2. As taxonomy is the study of classification, hard-character variables of the present study will be used in support of traditional soft-characters and not in isolation to them for the reviews which follow in Chapters 4 and 5.

3.1 Utility interrogation

Hexabothriid sucker complex sclerites develop in the immature haptor after the formation of the hamuli (Wiskin 1970). Growth of sucker sclerites begins with the

hook point, followed by the growth, in length, of the shaft (Wiskin 1970). Worms measured in this study are all of similar age and size and therefore age-dependent character variables may not necessarily indicate high variance. To account for the variability of worms of the same species but of different ages, correlations of age-dependent variables were calculated for all 3 sucker complex sclerites to determine which character variables change with the age of the worm. These variables were then ratio-transformed to reduce age-associated variance prior to multivariate analysis. The hamulus character was not included in the correlation because its variables are considered age-independent and its size and shape remains constant throughout the growth of the worm.

Character variables with a high coefficient of variance (CV) after accounting for age were disqualified for use in the multivariate analysis. High variability may be due to measurement error and/or the small size of the structures measured and therefore may reflect the limitations of the hardware used and the measurement software accuracy. The data for *C. callorhynchi* were used for the disqualification of character variables as data sets for the other species were too small to make conclusive inferences because of small samples sizes. Traditional hamulus measurements, in addition to the new measurements, were tested. Most traditional hamulus measurements expressed excessive CVs, possibly due to the size of measures and interpretive error. As a result these measurements are excluded from subsequent analyses. Sets of data for the 2 specimens of *C. hydrolagi* were incomplete because of the poor quality of the specimens. This species therefore is excluded from all the following analyses of this chapter.

3.1.1 Data transformation

To reduce variance expected to influence the measures of certain sucker complex sclerite variable as a function of age, a surrogate variable for age had to be selected. Correlation matrices were performed separately for each of these characters using the raw data for *C. callorhynchi* (n = 30) to select the most appropriate surrogate variable for age (Appendix 2). Circumferential length (CL) was selected as the most logical for all 3 sucker complex sclerites, given the ontogeny of these structures (Wiskin 1970). Character variables significantly correlated to the surrogate were considered age-dependant and were ratio-transformed using the surrogate to reduce variance associated with age.

Elimination of age as a factor influencing variance in the measurements of character variables would support the disqualification of variables with high CVs resulting from measurement error. To identify variables possibly affected by measurement error the raw data were log-transformed to compensate for increasing variance in increasing average sizes of characters (Shinn *et al.* 2001). The cosine (Cos) of all character variable angles was taken to transform these data to a linear function (Shinn *et al.* 2001). The CVs of all character variables were subsequently calculated and are represented as a histogram in Appendix 3.

The following character variables were disqualified due to high variance, considered the result of measurement error due to their small size after correcting for age and the standardising of measures through log or Cos-transformations: C1AA, C1HAA, C2HAA, C3HAA and HIAA.

3.1.2 Univariate approach

Univariate analyses were used to determine if there were any significant differences between species in all the character variables in their raw form and to identify any specific variables with the potential to discriminate all the species tested. Raw data were used for these analyses. All measurements for the character variables of each species were first subjected to a Levene test of homogeneity of variances to indicate whether they were suitable for either parametric or non-parametric univariate analyses (Appendix 1). Parametric data were subjected to ANOVA, using the Tukey HSD for unequal N (Spjotvoll/Stoline) post-hoc test of the Statistica 6 software. Non-parametric data were subjected to a standard Kruskal-Wallis ANOVA by ranks.

3.1.3 Results of univariate analysis

Complex 1 (Table 3.1): No single variable can be used to discriminate between all of the species tested. However, sclerite aperture (A) separated all the *Callorhynchocotyle* species from the control and therefore this character variable alone has the strength to distinguish between representatives of *Callorhynchocotyle* and *Rajonchocotyle*.

The control was significantly separated from *C. callorhynchi* on the following variables: CL, TL, TD, SW, SL, ID, A, HSCL, HCL and HBW; from *C. amatoi* on A only; from *C. marplatensis* on A and HCL; from *C. sagamiensis* on TL, SL, A and HA.

Callorhynchocotyle callorhynchi was significantly separated from *C. sagamiensis* on SW and HBW. However, there were no significant differences in any variables between *C. callorhynchi* and *C. amatoi* or *C. marplatensis*; *C. amatoi* and *C. marplatensis* or *C. sagamiensis*; *C. marplatensis* and *C. sagamiensis*.

Variables SSCL and HL performed weakest and produced insignificant differences through all the species tested.

Table 3.1. Univariate analysis displaying significant *P*-values of complex 1 sucker sclerites compared between all species

	C. callorhynchi vs				*C. amatoi vs*			*C. marplatensis vs*		*C. sagamiensis vs*
variables	***C. a***	***C. m***	***C. s***	**Cont**	***C. m***	***C. s***	**Cont**	***C. s***	**Cont**	**Cont**
C1CL	1.000	1.000	0.213	**0.000**	1.000	0.632	0.087	1.000	1.000	1.000
C1TL	0.967	0.992	0.636	**0.003**	0.999	0.329	0.304	0.376	0.452	**0.010**
C1TD	1.000	1.000	1.000	**0.000**	1.000	1.000	0.623	1.000	1.000	1.000
C1SW	1.000	1.000	**0.048**	**0.004**	1.000	0.926	1.000	1.000	1.000	1.000
C1SL	0.973	0.992	0.559	**0.002**	0.999	0.282	0.261	0.310	0.425	**0.006**
C1ID	1.000	1.000	1.000	**0.000**	1.000	1.000	0.513	1.000	1.000	0.555
C1A	0.999	0.985	0.998	**0.000**	0.993	0.994	**0.000**	0.925	**0.003**	**0.000**
C1HSCL	1.000	1.000	0.224	**0.000**	1.000	0.781	0.126	1.000	1.000	1.000
C1SSCL	0.989	0.999	0.956	0.702	0.999	0.998	0.683	0.988	0.894	0.643
C1HL	1.000	1.000	0.168	1.000	1.000	0.655	1.000	1.000	1.000	0.342
C1HCL	1.000	0.826	1.000	**0.001**	0.331	1.000	1.000	1.000	**0.002**	0.073
C1HA	1.000	1.000	0.314	0.365	1.000	1.000	0.505	1.000	1.000	**0.020**
C1HBW	1.000	1.000	**0.029**	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000

P-values were significant at ≤ **0.05**. *Callorhynchocotyle hydrolagi* is excluded for lack of data. Abbreviations: *C. a* – *Callorhynchocotyle amatoi*; *C. m* – *C. marplatensis*; *C. s* – *C. sagamiensis*; Cont – control; C1 – complex 1; CL – circumferential length; TL – total length; TD – total diameter; SW – shaft width; SL – shaft length; ID – internal diameter; AA – aperture angle; A – aperture; HSCL – hook-side curve length; SSCL – shaft-side curve length; HL – hook length; HCL – hook curve length; HAA – hook aperture angle; HA – hook aperture; HBW – hook base width.

Complex 2 (Table 3.2): As with complex 1 sucker complex sclerites, no single variable can be used to discriminate between all of the species tested. However, sclerite aperture angle (AA) and sclerite aperture (A) separated all the *Callorhynchocotyle* species from the control and therefore these variables alone have the strength to distinguish between representatives of *Callorhynchocotyle* and *Rajonchocotyle*.

The control was significantly separated from *C. callorhynchi* on the following variables: SW, AA, A, HL, HCL, HA and HBW; from *C. amatoi* on TL, SW, AA and A; from *C. marplatensis* on AA and A; from *C. sagamiensis* on SW, SL, AA, A and HBW. *Callorhynchocotyle callorhynchi*, *C. amatoi* and *C. sagamiensis* were

significantly different to the control in the measures of SW, however *C. marplatensis* was not.

There were insignificant differences in the measures of all variables between all the *Callorhynchocotyle* species. Variables CL, TD, SL, ID, HSCL and SSCL performed weakest and provided insignificant differences between all species tested.

Table 3.2. Univariate analysis displaying significant *P*-values of complex 2 sucker sclerites compared between all species

	C. callorhynchi vs				*C. amatoi vs*			*C. marplatensis vs*		*C. sagamiensis vs*
variables	***C. a***	***C. m***	***C. s***	**Cont**	***C. m***	***C. s***	**Cont**	***C. s***	**Cont**	**Cont**
C2CL	1.000	1.000	1.000	1.000	1.000	1.000	0.581	1.000	1.000	1.000
C2TL	0.153	1.000	1.000	1.000	1.000	1.000	**0.043**	1.000	1.000	1.000
C2TD	1.000	0.692	1.000	1.000	0.526	1.000	1.000	0.316	0.164	1.000
C2SW	1.000	1.000	1.000	**0.003**	0.866	1.000	**0.033**	0.253	1.000	**0.007**
C2SL	1.000	1.000	0.692	0.494	1.000	0.866	1.000	1.000	1.000	1.000
C2ID	1.000	0.892	1.000	1.000	1.000	1.000	1.000	1.000	0.225	1.000
C2AA	0.564	0.983	0.532	**0.000**	0.955	0.997	**0.000**	0.842	**0.001**	**0.000**
C2A	0.137	0.989	0.708	**0.000**	0.573	0.956	**0.000**	0.928	**0.004**	**0.000**
C2HSCL	0.091	1.000	1.000	0.003	1.000	1.000	1.000	1.000	0.945	1.000
C2SSCL	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.369	1.000
C2HL	0.493	1.000	1.000	**0.000**	1.000	1.000	1.000	1.000	0.430	1.000
C2HCL	0.151	1.000	0.434	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000
C2HA	0.473	1.000	1.000	**0.000**	1.000	1.000	1.000	1.000	0.489	1.000
C2HBW	1.000	1.000	0.956	**0.000**	1.000	1.000	0.419	1.000	0.629	**0.003**

P-values were significant at **≤ 0.05**. Abbreviation: C2 – complex 2.

Complex 3 (Table 3.3): Similarly for complex 1 and 2 sucker complex sclerites, no single variable can be used to discriminate between all of the species tested. However, sclerite aperture angle (AA) and sclerite aperture (A) separated all the *Callorhynchocotyle* species from the control and therefore these variables alone have the strength to distinguish between representatives of *Callorhynchocotyle* and *Rajonchocotyle*.

The control was significantly separated from *C. callorhynchi* on the following variables: SW, AA, A, HSCL, HL, HCL, HA and HBW; from *C. amatoi* on SW, SL, AA and A; from *C. marplatensis* on AA, A, HL and HA; from *C. sagamiensis* on SW, AA, A and HBW. *Callorhynchocotyle callorhynchi*, *C. amatoi* and *C. sagamiensis* were significantly different to the control in the measures of SW, however *C. marplatensis* was not. The variables HL and HA provided significant differences in the measures between *C. callorhynchi* and *C. marplatensis* with the control, while HBW provided significant differences between *C. callorhynchi* and *C. sagamiensis* with the control. There were significant differences in the measures of the variable

HSCL between *C. callorhynchi* and *C. amatoi*, and *C. amatoi* and the control. *Callorhynchocotyle amatoi* was also significantly different to the control in SL.

The following variables performed weakest with insignificant differences between all species tested: CL, TL, TD, ID and SSCL.

Table 3.3. Univariate analysis displaying significant *P*-values of complex 3 sucker sclerites compared between all species

	C. callorhynchi vs				*C. amatoi vs*			*C. marplatensis vs*		*C. sagamiensis vs*
variables	***C. a***	***C. m***	***C. s***	**Cont**	***C. m***	***C. s***	**Cont**	***C. s***	**Cont**	**Cont**
C3CL	1.000	1.000	1.000	1.000	1.000	0.373	0.204	1.000	1.000	1.000
C3TL	0.098	1.000	0.407	1.000	1.000	1.000	0.162	1.000	1.000	0.480
C3TD	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.814	1.000
C3SW	1.000	1.000	0.564	**0.001**	1.000	1.000	**0.017**	0.253	1.000	**0.001**
C3SL	1.000	1.000	1.000	0.065	0.679	1.000	**0.019**	1.000	1.000	1.000
C3ID	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.489	1.000
C3AA	0.136	1.000	1.000	**0.007**	1.000	1.000	**0.000**	1.000	**0.037**	**0.015**
C3A	**0.000**	0.884	0.351	**0.000**	**0.010**	0.097	**0.000**	0.877	**0.000**	**0.000**
C3HSCL	**0.048**	1.000	1.000	**0.026**	0.271	1.000	1.000	1.000	0.489	1.000
C3SSCL	0.836	0.528	1.000	1.000	1.000	1.000	0.239	1.000	0.164	1.000
C3HL	0.785	1.000	1.000	**0.000**	0.507	1.000	1.000	0.721	**0.015**	1.000
C3HCL	0.301	1.000	0.571	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000
C3HA	0.476	1.000	1.000	**0.000**	0.388	1.000	1.000	1.000	**0.018**	1.000
C3HBW	0.860	1.000	1.000	**0.000**	1.000	0.837	1.000	1.000	0.073	**0.044**

P-values were significant at ≤ **0.05**. Abbreviations: C3 – complex 3.

Hamulus (Table 3.4): No single variable can be used to discriminate between all of the species tested. However, the measures of the hamulus hook point length (HPL) were significantly different between the control and all the *Callorhynchocotyle* species. Therefore, this variable has the potential to be used to separate representatives of *Callorhynchocotyle* and *Rajonchocotyle*. The control was significantly different from *C. callorhynchi* in almost all variables, excluding A, IRL and RBA; *C. amatoi* in HPL, ORL and RBA; *C. marplatensis* in HPL and DHPW; *C. sagamiensis* in TL, HPL, HSL and ORL. Measures of the variable ORL were significantly different between the control and all *Callorhynchocotyle* species except *C. marplatensis*.

Callorhynchocotyle callorhynchi and *C. marplatensis* were significantly different in the measures of TW and HPL. *Callorhynchocotyle marplatensis* differed significantly in the measures of HPL to C. sagamiensis. *Callorhynchocotyle amatoi* differed significantly to *C. sagamiensis* in the measures of A, and from the control in RBA. The only variable which performed poorly with insignificant differences between all the species tested was IRL.

Table 3.4. Univariate analysis displaying significant *P*-values of the hamulus compared between all species

	C. callorhynchi vs				*C. amatoi vs*			*C. marplatensis vs*	*C. sagamiensis vs*
variables	***C. a***	***C. m***	***C. s***	**Cont**	***C. m***	***C. s***	**Cont**	***C. s***	**Cont**	**Cont**
HTL	0.089	1.000	1.000	**0.000**	1.000	0.142	1.000	0.687	1.000	**0.033**
HTW	0.600	**0.031**	0.904	**0.009**	0.340	0.997	0.903	0.203	0.765	0.841
HHPL	0.386	**0.001**	0.999	**0.000**	0.066	0.733	**0.000**	**0.003**	**0.042**	**0.000**
HDHPW	0.412	1.000	1.000	**0.000**	0.124	1.000	1.000	1.000	**0.004**	0.498
HOAA	0.240	1.000	1.000	**0.000**	1.000	1.000	1.000	1.000	1.000	0.118
HA	0.660	0.761	0.111	0.775	0.173	**0.008**	0.239	0.675	0.979	0.339
HHSL	1.000	1.000	0.657	**0.000**	1.000	1.000	0.076	0.178	1.000	**0.001**
HHSBW	0.690	1.000	1.000	**0.000**	1.000	0.990	1.000	1.000	1.000	0.060
HORL	0.936	0.215	1.000	**0.000**	0.545	0.965	**0.000**	0.208	0.072	**0.000**
HIRL	0.285	0.150	0.324	0.081	0.948	0.998	0.998	0.991	0.878	0.986
HRBA	0.209	1.000	1.000	1.000	1.000	1.000	**0.027**	1.000	0.281	0.480
HBW	0.870	1.000	1.000	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000

P-values were significant at ≤ **0.05**. Abbreviations: H – hamulus; TW – total width; HPL – hook point length; DHPW – distal hook point width; OAA – outer aperture angle; IAA – inner aperture angle; A – aperture; HSL – hook shank length; HSBW – hook shank base width; ORL – outer root-shaft length; IRL – inner root-shaft length; RBA – root base angle; BW – base width.

3.1.4 Multivariate approach

Multivariate analysis was performed on the combination of the sucker complex sclerites and hamulus characters (excluding disqualified variables) using the ratio-transformed data subjected to principal component analysis. Multivariate analysis on the combination of characters was performed to provide clarity on all species tested together, given the limitations of sample size. Therefore multivariate analyses of all of the characters in isolation, and additional combinations thereof, are excluded.

3.1.5 Results of multivariate analysis

3.1.5.1 All characters combined

Eight factors were produced by the PCA, of which only the first 3 contained significant factor loadings (see Appendix 4). All factors accounted for a cumulative variance of 89.4% and the first 3 factors accounted for 71.4% of the cumulative variance. Factor 1 (46.1%) *vs* Factor 3 (9.7%) was used for the graphic representation of the PCA in Fig. 3.1. Visual representations of Factor 1 *vs* Factor 2 (15.4%) and Factor 1 *vs* Factor 2 *vs* Factor 3 (3-dimentional representation of data) are included in Appendix 5 for additional reference.

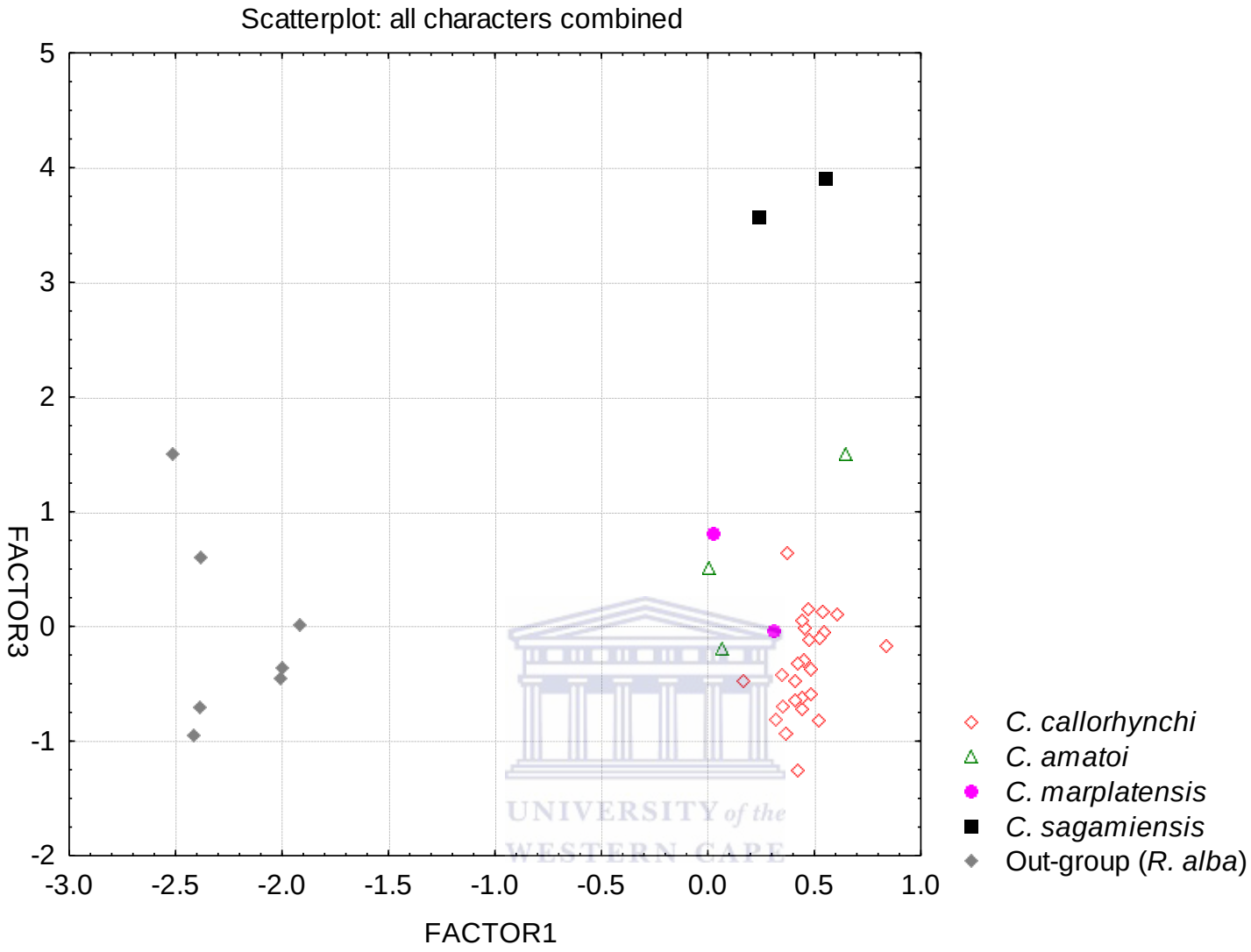

Fig. 3.1 Scatterplot of all characters combined from the PCA using factor 1 *versus* factor 3.

Callorhynchocotyle callorhynchi, *C. amatoi* and *C. marplatensis* could not be separated from each other on Factors 1, 2 or 3. However, these species were separated from the control taxon on Factor 1 by the majority of variables including C1CL, C1TD, C1ID, C1A, C1HSCL, C1HCL, C2AA, C2A, C2HL, C2HCL, C2HA, C2HBW, C3SW, C3AA, C3A, C3HBW, HTL, HHPL, HHSL, HDHPW, HOAA, HHSBW, HORL and HBW. *Callorhynchocotyle sagamiensis* was separated from *C. callorhynchi*, *C. amatoi* and *C. marplatensis* on Factor 3 by C1SW, C1HL, C1HA and C1HBW. *Callorhynchocotyle sagamiensis* could not be separated from *C. callorhynchi* or *C. amatoi* on Factor 2, but was separated from *C. marplatensis* on Factor 2 (Appendix 5) by C2TL, C2TD, C2SSL, C2ID, C3TL, C3TD and C3ID.

3.2 Discussion

Results of any classical statistical test are dependant upon sample size (Quinn and Keough 2002). Statistically significant results are more likely to be obtained from larger sample sizes giving greater degrees of freedom. Sample sizes should therefore be large enough that statistical analyses can detect a desired effect in a population (Quinn and Keough 2002). As a result only preliminary results could be obtained from the various analyses performed in the present study as the sample sizes of all species except *C. callorhynchi* (n = 30) were too small. Data for *C. callorhynchi* were therefore used for the selection and ratio-transformation of age-dependant character variables (Appendix 2) and the disqualification of variables with high variance associated with measurement error (Appendix 3).

Sucker complex sclerites are affected by the age of the worm, however variation in the measures of character due to age is not considered in the historic or current hexabothriid literature. The correlation matrices of the 3 sucker complex sclerites revealed several age-dependant character variables. Variation in the measures of age-dependant variables can be reduced by ratio-transforming these data to a surrogate variable for age. Wiskin (1970), in her explanation of the ontogeny of *R. emarginata*, observed that the sucker-complex sclerites originate at the hook tip and grow into the characteristic shape following the extension of the shaft. In the present study, the circumferential length of these characters measured from hook tip to shaft tip was selected as the surrogate for age given the ontogenic development of these characters.

No single character variable can be used in separating *Callorhynchocotyle* species from each other. The variables C1A, C2AA, C2A, C3AA, C3A and HHPL in isolation could significantly separate all the *Callorhynchocotyle* species from the control taxon *R. alba* in the univariate analyses. However, the sensitivity of the univariate analyses was compromised by the small samples sizes of all the species except *C. callorhynchi* and therefore there may be true differences between species which were not considered significant by the analyses i.e. total length, total width and total diameter of complex 1 sucker sclerites of *C. sagamiensis* and the other *Callorhynchocotyle* species.

Callorhynchocotyle species are separated into 2 groups representing those species found on chimaerid hosts including *C. sagamiensis* and *C. hydrolagi*, and callorhynchid hosts including all the other known species. These groups of species

can be distinguished from each other effectively by the relationship in size of the complex 1 sucker sclerite to the other 2 sucker complex sclerites. In those species representing the former group all sucker complex sclerites are similar in size. In the latter group the second and third sucker complex sclerites are of similar size while the first sucker complex sclerites are smaller. This difference is highlighted in the multivariate analysis with *C. sagamiensis* differing significantly to all other *Callorhynchocotyle* species and the control taxon. *Callorhynchocotyle hydrolagi* was expected to have performed similarly. However, insufficient data were collected from this species due to the poor quality of the 2 specimens examined.

Characters in combination have the potential to separate *Callorhynchocotyle* species from each other and from other hexabothriid genera. Separation of *C. sagamiensis* and the control taxon (*R. alba*) in the multivariate analysis indicates that the current method has merit, given the limitations of sample size. Although the multivariate analysis in the present study was limited to the use of all characters in combination, the addition of data from more specimens could possibly provide better resolution between *Callorhynchocotyle* species. Furthermore, with the addition of specimens, characters tested in isolation to each other and in various combinations may also provide species separations especially considering the complex 1 sucker sclerite and hamulus variables which appear to provide the most differences between species.

The hamulus character has in the past not been considered an important discriminator of *Callorhynchocotyle* species. This is due in part to the difficulty in obtaining accurate measurements from hamuli that are often not correctly flattened in type and voucher material (Beverley-Burton and Chisholm 1990). The factor loadings for the character variables used in combination for multivariate analysis provided evidence that 8 of 12 hamulus variables influenced the overall analysis. The protocol used in the present study for measuring hamuli presents a new combination of novel parameters facilitated by the liberation of the hamulus from the surrounding soft tissue of the haptoral appendix. The potential of the hamulus as a species discriminator may originate from its independence of age because of its early complete development in the developing haptor before the development of sucker complex sclerites. Following this rationale, the hamulus was not subjected to ratio-transformation of any of its variables.

The preliminary results of the present study warrants further exploratory investigation using data from additional specimens. Few specimens however are available for comparison from museum-deposited type series of *Callorhynchocotyle* species. For future investigations to be completed accurately, new voucher material needs to be collected and deposited into the museum collections. It is also suggested that care be taken when preparing the haptoral armature of additional vouchers since many existing type specimens (e.g. *C. hydrolagi* and some *C. callorhynchi*) are mounted poorly and therefore some of their characters cannot be used. All voucher material collected for the present study is deposited in either the IZIKO South African Museum, or the Australian Helminth Collection at the South Australian Museum in Adelaide, Australia.

CHAPTER 4

Review of *Callorhynchocotyle* Suriano and Incorvaia, 1982

4.1 Introduction

Fifteen valid genera of Hexabothriidae Price, 1942 are currently accepted (Boeger and Kritsky 1989) of which *Callorhynchocotyle* is unique in the family. All other hexabothriid genera are gill parasites of elasmobranchs while the members of *Callorhynchocotyle* are exclusively parasitic on the gills of holocephalans.

Manter (1955) described *Squalonchocotyle callorhynchi* (Manter, 1955) (junior synonym for *C. callorhynchi*) from the holocephalan host *Callorhinchus capensis* from South Africa, and from *C. milii* from New Zealand. *Squalonchocotyle callorhynchi* was subsequently redescribed by Dillon and Hargis (1968) as *Erpocotyle callorhynchi* (Manter, 1955) (junior synonym for *C. amatoi*) from new material collected off the South Island of New Zealand from *C. milii*.

Erpocotyle callorhynchi (junior synonym for *C. callorhynchi*) was collected by Lebedev and Parukhin (1969) from *C. capensis* off Walvis Bay, South West Africa (Namibia), but in 1970 *S. callorhynci* was identified as the hexabothriid collected from a third host, *Callorhinchus callorhynchus* (Linnaeus, 1758) (junior synonym *C. antarcticus* Fleming, 1822) off Pategonia by Kuznetsova (1970). *Callorhynchocotyle* was subsequently proposed by Suriano and Incorvaia (1982) for the same hexabothriid collected off Mar del Plata (Argentina) from the gills of *C. callorhynchus,* which they named *C. marplatensis* Suriano and Incorvaia, 1982.

Boeger *et al*. (1989) revised *Callorhynchocotyle,* redescribing *S. callorhynci* and transferring the species to *Callorhynchocotyle*. In addition they erected a new species, *C. amatoi,* for all the hexabothriid material previously collected from *C. milii* from New Zealand.

The fourth species, *C. hydrolagi* was described from preserved reference material of *Hydrolagus ogilbyi* (Waite, 1898) from Australia, as well as from donated specimens. More recently *C. sagamiensis* was described from *Chimaera phantasma* collected in Sagami Bay on Japan's Pacific coast. Both *C. hydrolagi* and *C. sagamiensis* are parasites

of the host family Chimaeridae, while all the other species are parasites of Callorhynchidae.

As part of the present study, new voucher material for *C. callorhynchi*, *C. amatoi* and C. *sagamiensis* was collected off the West coast of South Africa, New Zealand and from Japan respectively. All *Callorhynchocotyle* species except *C. hydrolagi* are redescribed using additional character variables for the sucker sclerites and hamuli. Supplemental data are provided for *C. hydrolagi*.

4.2 Callorhynchocotyle Suriano and Incorvaia, 1982

4.2.1 Amended diagnosis

Body elongate, robust with smooth tegument. Haptor asymmetrical containing 3 sucker sclerite complex pairs (*sensu* Boeger and Kritsky 1989). Complex 1 sucker sclerites smaller than or similar to those of complex 2 and 3. Haptoral longitudinal axis at an angle to that of body proper. Oral and haptoral suckers papillate or non-papillate. Pair of hamuli present between pair of terminal suckers of the dorsal appendix. Hamulus root base angle obtuse. Branched intestinal caeca unite posterior to testes and extends into haptor. Testes numerous, irregular in shape. Vas deferens dorsal to uterus, sinuous, glandular for the majority of its length. Single loop of vas deferens proximal to base of cirrus complex, present or absent. Unarmed cirrus muscular with or without bulbous distal region, without prostatic region. Genital pore at level of or immediately posterior to initial intestinal bifurcation. Ovary anteriorly lobate or branched, situated sinistral or dextral to body proper midpoint. Descending ovarian branch coiled or sinuous, narrowing proximally to form oviduct. Oviduct branches to form reduced sack-like seminal receptacle before receiving thin descending branch of vitelline duct, thereafter expands to form smooth oötype. Uterus dorsal to ovary, initially narrow, widening anteriorly. Ovate eggs chain-linked by elongate tendril forming at each end. Vaginal pores muscular, opening ventrally, lateral to cirrus. Parallel vaginal ducts with glandulo-muscular distal region, narrowing thin-walled proximal region running with intestinal caeca, but often obscured by vitellarium. Follicular vitellarium extending from region immediately level with or posterior to vaginal pores, forming bi-lateral bands up to but not including the haptor. Excretory pores anterior to vaginal pores, opening laterally at margin of body proper. Oncomiracidia unciliated, blind with 10 marginal hooklets. Ventral rostrum

associated with anterior part. Parasites of the Subclass Holocephali, families Callorhynchidae and Chimaeridae.

4.2.2 Callorhynchocotyle marplatensis Suriano and Incorvaia, 1982

Type host: Callorhinchus callorhynchus (Linnaeus, 1758) (Callorhynchidae, Holocephali).

Type locality: Mar Del Plata coastal region, Argentina, South America (38°S; 57°W).

Additional locality: Uruguay coastal region, South America (Boeger *et al.* 1989).

Site on host: Gills.

Material examined: USNPC 080279.00: vouchers M1496-1, 6, 7 and 10.

Redescription (Figs. 4.1–4.3, Table 4.1.)

Total body length (excluding haptor) (Fig. 4.1) 9750 ± 1021.43(8300–10600, n = 4), maximum body width 917 ± 171.63(683–1095, n = 4). Oral sucker non-papillate, diameter 335 ± 36.57(290–365, n = 4). Pharynx 75 ± 5.83(67–81, n = 4) long, 79 ± 5.33(72–84, n = 4) wide. Branched intestinal caeca unite after testes and extend into haptor (see Fig. 1). Asymmetrical haptor 2930 ± 995.75(1933–3920, n = 4) long, 1225 ± 646.51(277–1640, n = 4) wide with 3 paired sucker sclerite complexes *sensu* Boeger *et al.* (1989). Haptoral suckers non-papillate.

Sclerites of sucker complex 1 (Fig. 4.2A) smaller than similarly sized sclerites of complex 2 and 3 with circumferential length 922 ± 30.70(896–966, n = 4); total length 418 ± 11.62(401–423, n = 4); total diameter 262 ± 20.13(233–280, n = 4); width 42 ± 5.35(35–47, n = 4); shaft length 420 ± 9.22(408–427, n = 4); inner diameter 222 ± 15.37(199–232, n = 4); aperture angle 59° ± 5.36(53°–65°, n = 4); aperture 270 ± 18.72(256–297, n = 4); hook-side curve length 69 ± 3.64(64–72, n = 7) and shaft-side curve length 101 ± 14.08(88–114, n = 4). Complex 1 sucker sclerite hook length 63 ± 5.94(54–72, n = 7); hook curve length 15 ± 1.13(13–16, n = 4); aperture angle 103° ± 5.04(93°–109°, n = 7); aperture 47 ± 5.33(40–53, n = 4) and base-width 16 ± 1.31(15–19, n = 7).

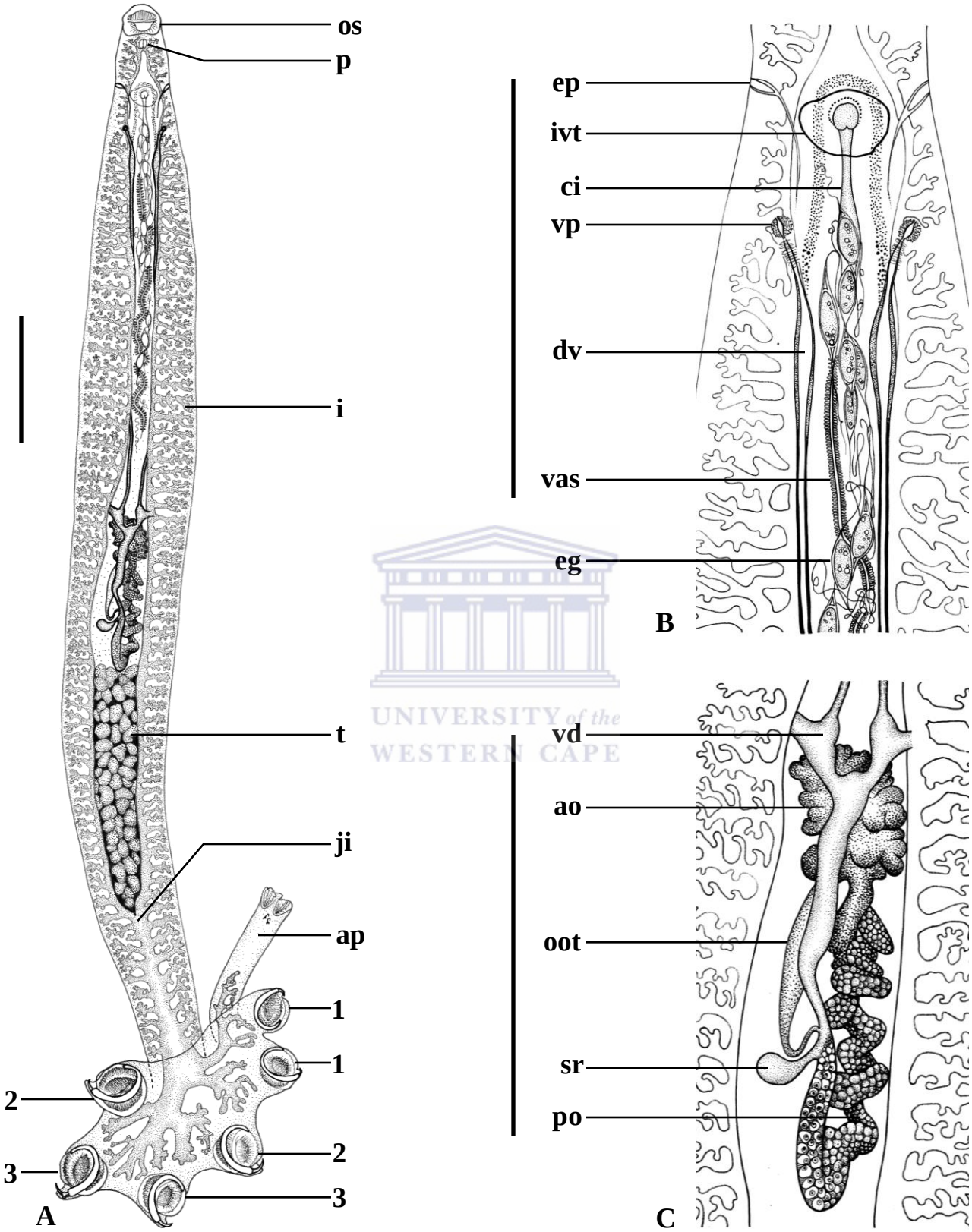

Fig. 4.1 *Callorhynchocotyle marplatensis*: **A.** Whole mount; **B.** Enlarged anterior section of whole mount; **C.** Enlarged mid-section of whole mount. **Abbreviations:** ao – anterior section of ovary; ap – appendix; ci – cirrus; dv – distal portion of vagina; eg – egg; ep – excretory pore; i – intestinal caecum; ivt – indentation of ventral tegument surrounding ovate distal cirrus; ji – junction of caeca posterior to testes; oot – oötype; os – oral sucker; p – pharynx; po – posterior section of ovary; sr – seminal receptacle; t – testes; vas – vas deferens; vd – vitteline duct; vp – vaginal pore; 1–3 – sucker-sclerite complexes 1–3. Scale bars = 1000µm.

Sclerites of sucker complex 2 (Fig. 4.2B): circumferential length 1244 ± 43.93(1206–1306, n = 4); total length 541 ± 22.71(511–566, n = 4); total diameter 344 ± 22.89(320–374, n = 4); width 66 ± 8.20(59–78, n = 4); shaft length 523 ± 26.03(498–559, n = 4); inner diameter 280 ± 14.02(265–299, n = 4); aperture angle 54° ± 2.53(51°–57°, n = 4); aperture 303 ± 19.09(278–381, n = 4); hook-side curve length 115 ± 13.02(99–134, n = 8) and shaft-side curve length 140 ± 5.53(135–147, n = 4). Complex 2 sucker sclerite hook length 153 ± 12.04(145–171, n = 4); hook curve length 30 ± 3.37(28–35, n = 4); aperture angle 109° ± 5.40(102°–115°, n = 4); aperture 121 ± 9.98(110–134, n = 4) and base width 38 ± 3.52(35–43, n = 4).

Sclerites of sucker complex 3 (Fig. 4.2C): circumferential length 1197 ± 42.98(1149–1249, n = 4); total length 535 ± 27.21(503–562, n = 4); total diameter 336 ± 17.03(314–355, n = 4); width 69 ± 6.50(60–76, n = 4); shaft length 506 ± 21.66(480–528, n = 4); inner diameter 269 ± 11.17(255–280, n = 4); aperture angle 56° ± 4.57(50°–61°, n = 4); aperture 302 ± 25.80(278–332, n = 4); hook-side curve length 116 ± 3.30(113–120, n = 4) and shaft-side curve length 134 ± 6.35(123–141, n = 4). Complex 3 sucker sclerite hook length 172 ± 5.35(168–180, n = 4); hook curve length 30 ± 7.71(23–41, n = 4); aperture angle 110° ± 9.53(96°–118°, n = 4); aperture 138 ± 3.70(134–142, n = 4) and base width 46 ± 5.00(41–53, n = 4).

Dorsal haptoral appendix 1655 ± 124.74(1514–1773, n = 4) long, 286 ± 48.42(223–337, n = 4) wide. Terminal suckers of appendix 232 ± 28.45(193–264, n = 6) long, 141 ± 20.09(120–167, n = 6) wide. Pair of hamuli between appendix terminal suckers (see Fig.1). Hamulus (Fig. 4.2D) total length 58 ± 3.33(56–61, n = 2); hook point length 14 ± 0.34, n = 2; hook shank length 17 ± 0.09, n = 2; total width 26 ± 2.07(25–28, n = 2); distal hook point width 4 ± 0.29, n = 2; outer aperture angle 17° ± 0.09(n = 2); inner aperture angle 67° ± 18.28(54°–80°, n = 2); aperture 88 ± 19.63(74–102, n = 2); hook shank base width 6 ± 0.63, n = 2; inner root-shaft length 45 ± 1.79(44–46, n = 2); outer root-shaft length 41 ± 3.96(38–44, n = 2); root base angle 105° ± 4.48(102°–108°, n = 2), and root base width 23 ± 0.97(23–24, n = 2).

Testes irregular in shape, 83 ± 18.67(65–107, n = 4) in number; 95 ± 9.16(83–109, n = 10) wide. Vas deferens sinuous, surrounded by small gland cells along the majority of its length (see Fig. 4.1B).

Presence of vas deferens loop proximal to entrance into cirrus obscured in some specimens (see Fig. 4.1B). Unarmed muscular cirrus total length 410 ± 29.68(374–447, n = 4); maximum width 38 ± 4.06(34–44, n = 4); distal bulb length 67 ± 2.65(65–70, n = 4), and distal bulb width 62 ± 3.08(58–66, n = 4). Area of ventral tegument surrounding distal portion of cirrus, weakly indented (Figs. 4.1B, 4.3).

Ovary (dextral = 2, sinistral = 2) 1422 ± 141.93(1265–1530, n = 4) long, anteriorly lobate, coiled posteriorly, ascending to oviduct, branching to sack-like, reduced seminal receptacle (see Fig. 4.1C). Oötype smooth, leading to uterus, dorsal to ovary, ventral to vas deferens. Ovate eggs chain-linked by tendrils at each pole. Eggs (*in utero*) 154 ± 5.25(146–165, n = 13) long, 67 ± 7.58(57–83, n = 13) wide. Parallel vaginal ducts with glandulo-muscular distal portion and thin-walled proximal portion. Ventral vaginal pores muscular, lateral to proximal portion of cirrus. Follicular vitellarium originates posterior to vaginal pores. Excretory pores marginal and anterior to vaginal pores (see Fig. 4.1).

Remarks

Comparative measurements for *C. marplatensis* are represented in Table 4.1. A discrepancy exists in the total length measurements between all 3 sucker complex sclerites of Suriano and Incorvaia (1982), those of Boeger *et al.* (1989) and the present study. It is unclear how the total length measurements of the sucker complex sclerites were measured in Suriano and Incorvaia (1982). However it is likely these measurements are erroneous as they are more than twice the length of those for Boeger *et al*. (1989) who reviewed the Holotype and nearly twice the length of those measured in the present study. The scale bar given for sclerites of *C. marplatensis* by Suriano and Incorvaia is 0.05mm (50µm). The scale bar is drawn to equate to sucker complex sclerite length but is inaccurate. It is likely that the sucker complex sclerite measurements of Suriano and Incorvaia (1982) were miscalculated.

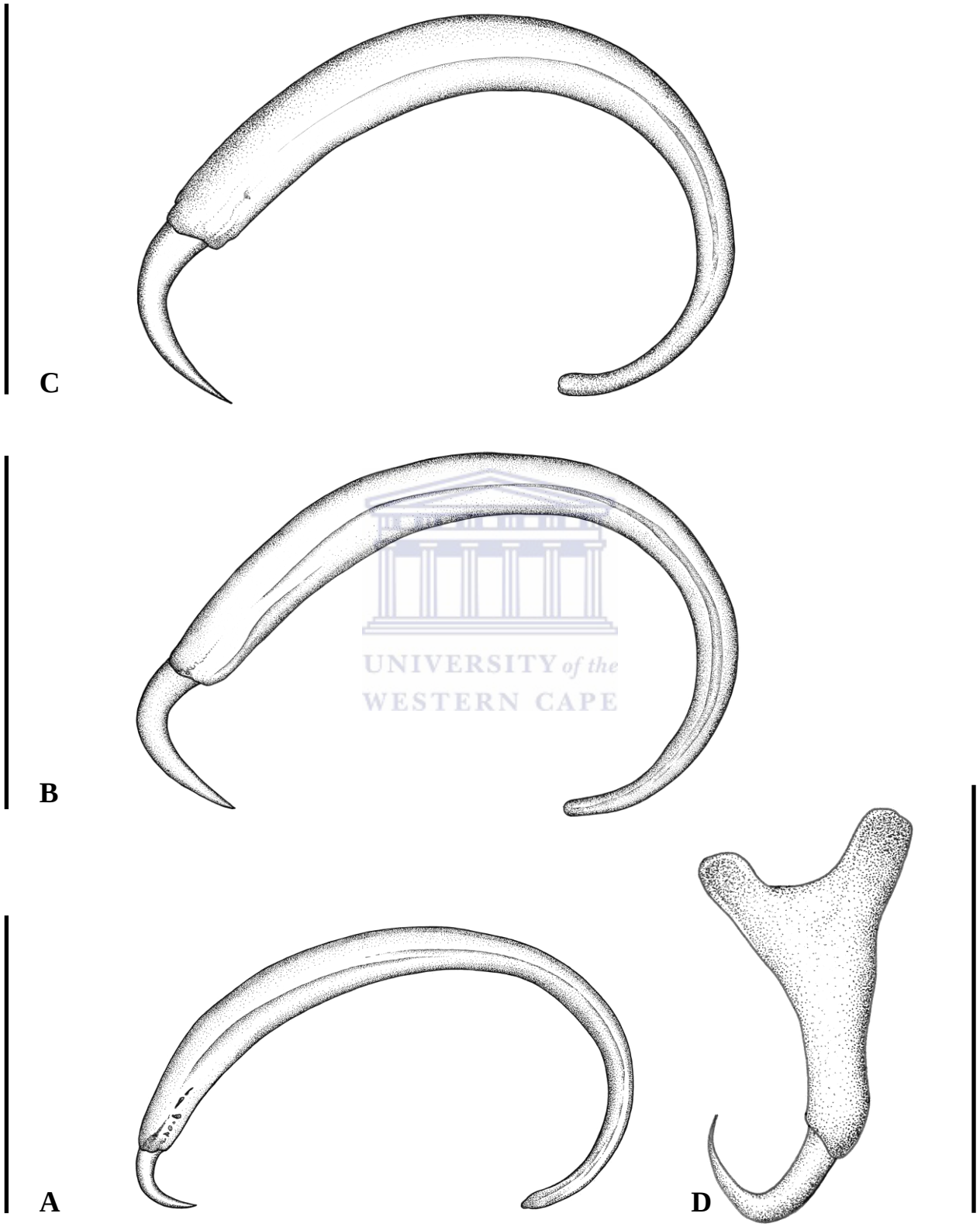

Fig. 4.2 *Callorhynchocotyle marplatensis* sclerites of sucker complexes 1 (**A**), 2 (**B**) and 3(**C**), and hamulus (**D**). Scale bars = 260µm, 340µm, 340µm and 60µm respectively.

Table 4.1 Comparative measurements for *Callorhynchocotyle marplatensis* Suriano and Incorvaia, 1982

	Suriano and Incorvaia (1982)	Boeger *et al.* (1989)	Beverley-Burton and Chisholm (1990)	**Present study**[1]
Total body length	7140 (5750–8820, n = 45)	9989 (7200–12430, n = 17)*	-	**9750 ± 1021.4(8300–10600, n = 4)**
Maximum body width	1300 (1080–1690, n = 45)	939 (649–1217, n = 17)	-	**917 ± 171.6(683–1095, n = 4)**
Oral sucker diameter	442 (320–458), n = 45	302 (231–362, n = 17)	-	**335 ± 36.6(290 – 365, n = 4)**
Pharynx length	-	71 (51–83, n = 17)	-	**75 ± 5.8(67–81, n = 4)**
Pharynx width	95 (88–100, n = 45)	71 (60–78, n = 17)	-	**79 ± 5.3(72–84, n = 4)**
Haptor length	2800 (2160–3060, n = 45)	-	-	**2930 ± 995.8(1933–3920, n = 4)**
Haptor width	-	-	-	**1225 ± 646.5(277–1640, n = 4)**
Appendix length	1330 (900–1800, n = 45)	1397 (1275–1637, n = 17)	-	**1655 ± 124.7(1514–1773, n = 4)**
Appendix width	-	256 (149–350, n = 17)	-	**286 ± 48.4(223 – 337, n = 4)**
Terminal appendix sucker length	-	181 (164–204, n = 17)	-	**232 ± 28.5(193–264, n = 6)**
Terminal appendix sucker width	-	195 (154–190, n = 17)	-	**142 ± 20.1(120–167, n = 6)**
No. of testes	>60, n = 45		-	**83 ± 18.7(65–107, n = 4)**
Testes width	-	-	-	**95 ± 9.2(83–109, n = 10)**
Cirrus total length	-	-	360 (298–417)	**410 ± 29.3(374–447, n = 4)**
Cirrus maximum width	-	-	36 (31–45)	**38 ± 4.1(34–44, n = 4)**
Distal bulb length	-	-	111 (91–135)	**67 ± 2.7(65–70, n = 4)**
Distal bulb width	-	-	72 (58–86)	**62 ± 3.1(58–66, n = 4)**
Ovary length	-	1147 (816–1505, n = 17)	-	**1422 ± 141.9(1265–1530, n = 4)**
Egg length	75	145 (125–193)	-	**154 ± 5.3(146–165, n = 13)**
Egg width	25	59 (52–66)	-	**67 ± 7.6(57–83, n = 13)**
Complex 1 sclerite Circumferential length	-	-	-	**922 ± 30.7(896–966, n = 4)**
Total length	740 (630–840, n = 45); 800 (650–1120, n = 45)	353 (289–385)	-	**418 ± 11.6(401–423, n = 4)**
Shaft length	-	33 (27–44)	-	**420 ± 9.2(408–427, n = 4)**
Hook-side curve length	-	-	-	**68 ± 4.0(64–72, n = 4)**
Shaft-side curve length	-	-	-	**101 ± 14.0(88–114, n = 4)**
Total diameter	-	-	-	**262 ± 20.1(233–280, n = 4)**
Inner diameter	-	-	-	**222 ± 15.3(199–232, n = 4)**
Aperture angle	-	-	-	**59° ± 5.3(53°–65°, n = 4)**
Aperture	-	-	-	**270 ± 18.7(256–297, n = 4)**
Width	-	-	-	**42 ± 5.3(35–47, n = 4)**
Hook length	-	50 (42–57)	-	**59 ± 4.4(54–64, n = 4)**
Hook curve length	-	-	-	**15 ± 1.1(13–16, n = 4)**
Hook aperture angle	-	-	-	**100° ± 5.1(93°–104°, n = 4)**
Hook aperture	-	-	-	**47 ± 5.3(40–53, n = 4)**
Hook base width	-	-	-	**16 ± 0.8(15–17, n = 4)**
Complex 2 sclerite Circumferential length	-	-	-	**1244 ± 43.9(1206–1306, n = 4)**

Table 4.1 cont

Total length	810 (630–990, n = 45); 830 (580–1090, n = 45)	460 (362–499)	-	**541 ± 22.7(511–566, n = 4)**
Shaft length	-	76 (59–92)	-	**523 ± 26.0(498–559, n = 4)**
Hook-side curve length	-	-	-	**105 ± 9.5(99–119, n = 4)**
Shaft-side curve length	-	-	-	**140 ± 9.5(99–119, n = 4)**
Total diameter	-	-	-	**344 ± 22.8(320–374, n = 4)**
Inner diameter	-	-	-	**280 ± 14.0(265–299, n = 4)**
Aperture angle	-	-	-	**54° ± 2.5(51°–57°, n = 4)**
Aperture	-	-	-	**303 ± 19.0(278–318, n = 4)**
Width	-	-	-	**66 ± 8.2(59–78, n = 4)**
Hook length	-	117 (95–141)	-	**153 ± 12.0(145–171, n = 4)**
Hook curve length	-	-	-	**30 ± 3.3(28–35, n = 4)**
Hook aperture angle	-	-	-	**109° ± 5.4(102°–115°, n = 4)**
Hook aperture	-	-	-	**121 ± 9.9(110–134, n = 4)**
Hook base width	-	-	-	**38 ± 3.5(35–43, n = 4)**
Complex 3 sclerite	-	-	-	**1197 ± 42.9(1149–1249, n = 4)**
Circumferential length				
Total length	930 (850–1030, n = 45); 930 (630–1040, n = 45)	454 (382–512)	-	**535 ± 27.2(503–562, n = 4)**
Shaft length	-	80 (65–92)	-	**506 ± 21.6(480–528, n = 4)**
Hook-side curve length	-	-	-	**116 ± 5.3(113–120, n = 4)**
Shaft-side curve length	-	-	-	**134 ± 6.3(126–141, n = 4)**
Total diameter	-	-	-	**336 ± 17.0(314–355, n = 4)**
Inner diameter	-	-	-	**269 ± 11.1(255–280, n = 4)**
Aperture angle	-	-	-	**56° ± 4.5(50°–61°, n = 4)**
Aperture	-	-	-	**302 ± 25.8(278–332, n = 4)**
Width	-	-	-	**69 ± 6.5(60–76, n = 4)**
Hook length	-	129 (99–145)	-	**172 ± 5.3(168–180, n = 4)**
Hook curve length	-	-	-	**30 ± 7.7(23–41, n = 4)**
Hook aperture angle	-	-	-	**110° ± 9.5(96°–118°, n = 4)**
Hook aperture	-	-	-	**138 ± 3.7(134–142, n = 4)**
Hook base width	-	-	-	**46 ± 5.0(41–53, n = 4)**
Hamulus total length	76.5 (70–86)	190 (144–210)	58 (53–64)	**58 ± 3.3(56–61, n = 2)**
Hook point length	-	-	-	**14 ± 0.3, n = 2**
Hook shank length	-	-	-	**17 ± 0.1, n = 2**
Total width	-	-	-	**26 ± 2.1(25–28, n = 2)**
Distal hook point width	-	-	-	**4 ± 0.3, n = 2**
Outer aperture angle	-	-	-	**17° (n = 2)**
Inner aperture angle	-	-	-	**67° ± 18.2(54°–80°, n = 2)**
Aperture	-	-	-	**88 ± 19.6(74–102, n = 2)**
Hook shank base width	-	-	-	**6 ± 0.7, n = 2**
Outer root-shaft length	-	-	-	**41 ± 3.9(38–44, n = 2)**
Inner root-shaft length	-	-	-	**45 ± 1.8(44–46, n = 2)**
Root base width	-	87 (72–98)	27 (25–28)	**23 ± 1.0(23–24, n = 2)**
Root base angle	-	-	-	**105° ± 4.5(102°–108°, n = 2)**

[1]**Bold script =** all measurements of the present study: USNPC 080279.00: Vouchers M1496-1, 6, 7 and 10.
*Boeger *et al*. (1989) included the haptor into the total body length of *C. marplatensis*.

Boeger *et al*. (1989) redescribed *C. marplatensis* adding additional voucher material collected off Uruguay and Argentina from the type host *Callorhinchus callorhynchus*. They amended the original description of Suriano and Incorvaia (1982) to include the presence of a "weak genital sucker" and the lack of papillae in both the oral and haptoral sclerite complex suckers. Beverley-Burton and Chisholm (1990) disputed the existence of this genital sucker after examination of the same voucher material used for the present redescription (USNPC 080279.00) adding that since it was lacking this feature was questionable as a diagnostic character. In the present study, examination of voucher M1496-10 revealed the presence of the "weak genital sucker" of Boeger *et al*. (1989) (see Fig. 4.3). However, its function as a true sucker is questionable. The structure surrounds the position of the distal portion of the cirrus and is likely a weak indentation of the ventral tegument in this region. As a result, this feature is not added to the description as a separate diagnostic character.

Suriano and Incorvaia (1982) indicated the presence of many small cuticular tubercles (papillae) on the inner surface of the oral sucker in the original description of *C. marplatensis*. Boeger *et al*. (1989) redescribed *C. marplatensis* with a non-papillate oral sucker which is confirmed in the voucher specimens examined for the present study.

Callorhynchocotyle marplatensis can be distinguished from all of the other species of by the lack of papillae in the suckers of all the sucker sclerite pair complexes and oral sucker and is found exclusively on the host *Callorhinchus callorhynchus*.

Boeger *et al*. (1989) in their redescription of *C. marplatensis* included the synonyms *C. callorhynchi* and *C. callorhyncy*. Both synonyms are invalid since they are discussed in error in the original description of Suriano and Incorvaia (1989). Maintaining them as synonyms only adds to the confusion in nomenclature and as such they are omitted from the redescription of the present study.

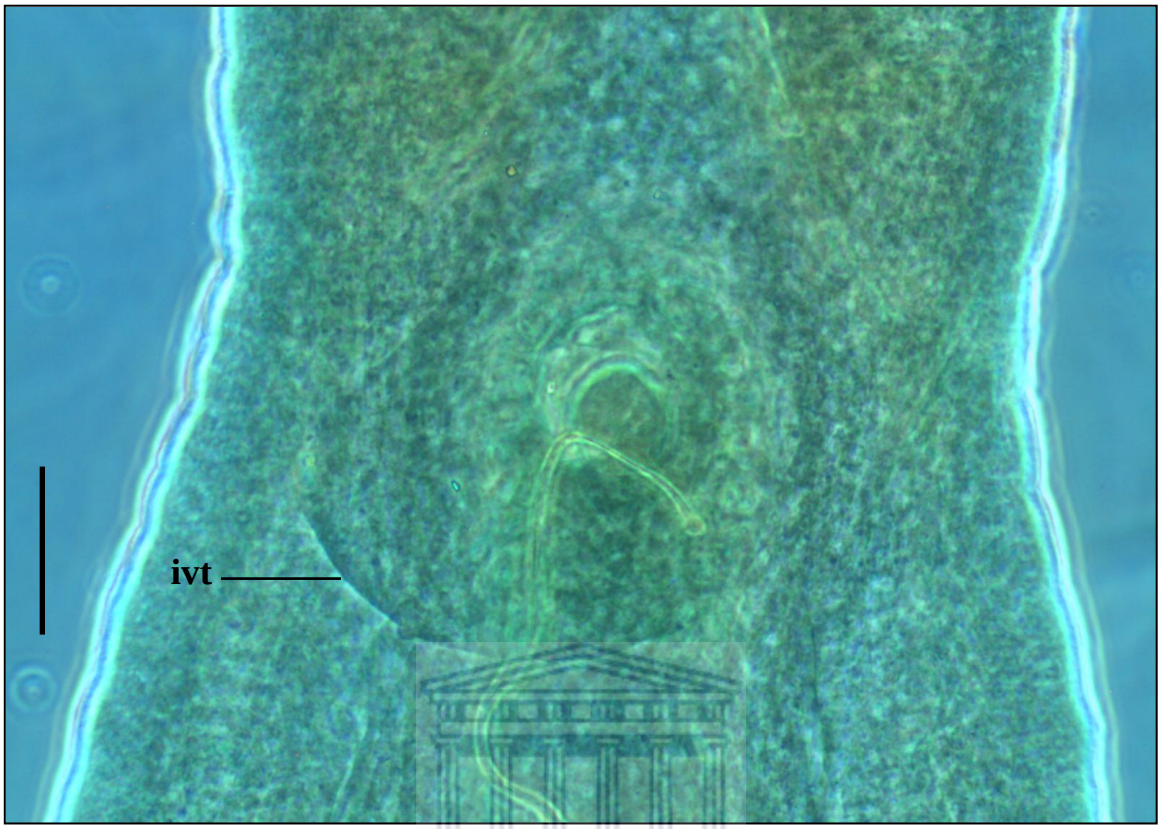

Fig. 4.3 Area of weakly indented ventral tegument (ivt) surrounding the distal portion of the cirrus in *Callorhynchocotyle marplatensis* (USNPC 80279 voucher M1496-10). Scale bar = 100µm.

4.2.3 Callorhynchocotyle callorhynchi (Manter, 1955)

Synonyms: Squalonchocotyle callorhynchi Manter, 1955; *Erpocotyle callorhynchi* Dillon and Hargis, 1968.

Type host: Callorhinchus capensis (Duméril, 1865) (Callorhynchidae, Holocephali).

Type locality: Cape Town, South Africa.

Additional locality: Walvis Bay, South West Africa (Namibia) (Lebedev and Parukhin (1969); West and South coasts of South Africa (present study).

Site on host: Gills.

Material examined: USNPC 037447.00: paratype 399-17; USNPC 080984.00: vouchers M1523-7, 9 and 10; GL 10475-80; vouchers SAMCTA 29465 (15 whole mounts), SAMCTA 29466 (10 haptor digests); vouchers AHC 29747 (10 whole mounts) AHC 29478 (5 haptor digests).

Redescription (Figs. 4.4–4.6, Table 4.2.)

Total body length (Fig. 4.4A) 6885 ± 1214.59(5500–11100, n = 24), maximum body width 1700 ± 161.65(1425–2100, n = 24). Oral sucker internally papillate, diameter 360 ± 34.26(269–411, n = 24). Pharynx 91 ± 9.65(68–113, n = 24) long, 89 ± 7.05(75–100, n = 24) wide. Branched intestinal caeca unite after testes and extend into the haptor. Asymmetrical haptor 2494 ± 362.89(2025–3640, n = 24) long, 1595 ± 169.75(1275–1960, n = 24) wide with 3 paired sucker sclerite complexes. Haptoral suckers papillate.

Sclerites of sucker complex 1 (Fig. 4.5A) smaller than similarly sized sucker sclerites of complex 2 and 3 with circumferential length 858 ± 44.49(758–953, n = 30); total length 382 ± 19.55(347–428, n = 30); total diameter 246 ± 15.07(219–271, n = 30); width 40 ± 4.95(29–49, n = 30); shaft length 384 ± 19.66(347–432, n = 30); inner diameter 209 ± 14.10(183–235, n = 30); aperture angle 60° ± 5.71(49°–70°, n = 30); aperture 253 ± 5.71(49–70, n = 30); hook-side curve length 61 ± 5.72(50–71, n = 30) and shaft-side curve length 89 ± 11.68(74–111, n = 30). Complex 1 sucker sclerite hook length 56 ± 4.88(44–65, n = 30); hook curve length 12 ± 1.84(7–16, n = 30); aperture angle 105° ± 7.70(91°–124°, n = 35); aperture 45 ± 4.90(32–54, n = 30) and base-width 14 ± 1.28(11–18, n = 30).

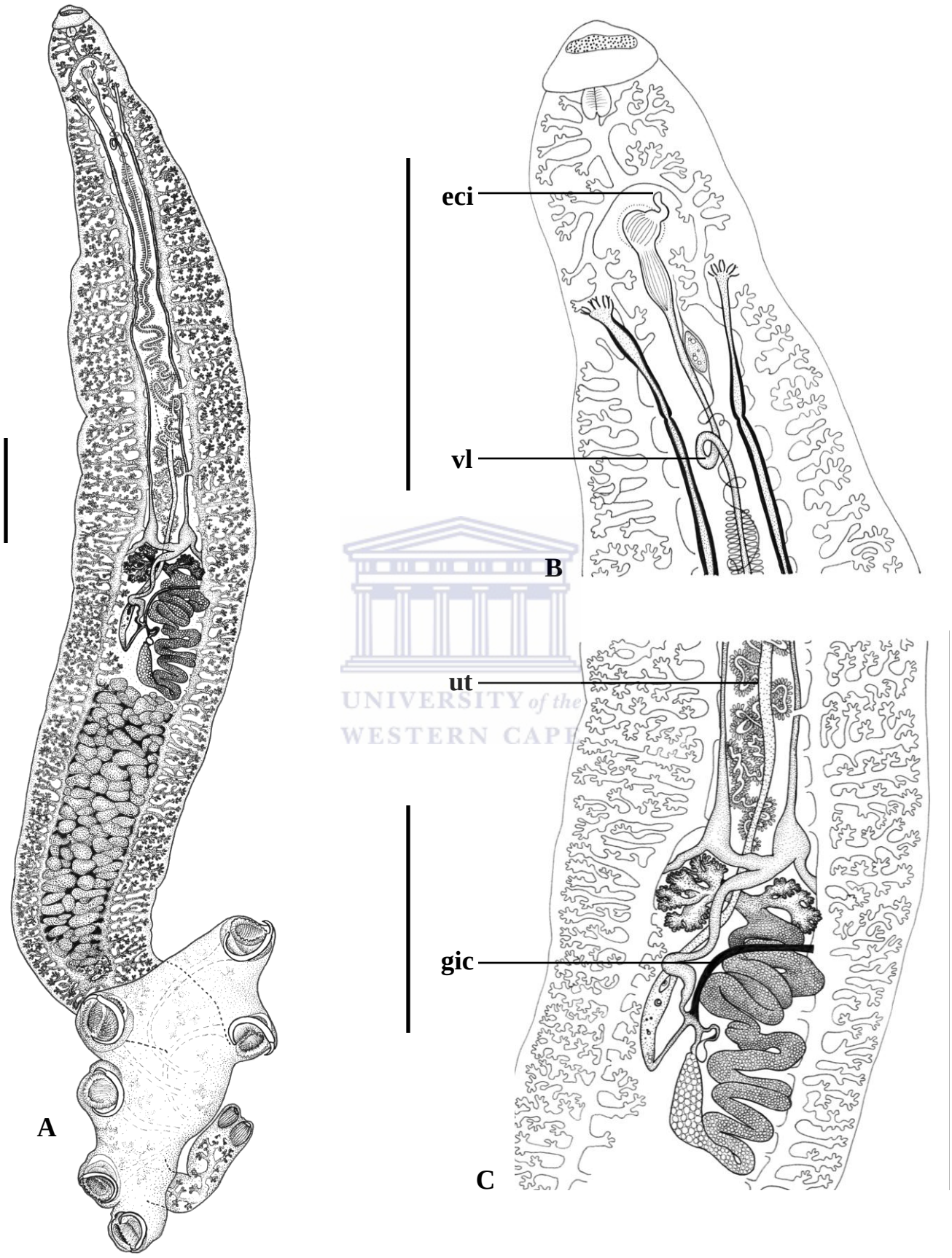

Fig. 4.4 *Callorhynchocotyle callorhynchi*. **A.** Whole mount; **B.** Enlarged anterior section of whole mount; **C.** Enlarged mid-section of whole mount. **Abbreviations:** eci – everted portion of cirrus; gic – genito-intestinal canal; ut – uterus; vl – loop of vas deferens. Scale bars = 1000µm.

Sclerites of sucker complex 2 (Fig. 4.5B): circumferential length 1191 ± 59.85(1076–1292, n = 30); total length 525 ± 19.44(484–558, n = 30); total diameter 351 ± 15.77(323–381, n = 30); width 66 ± 7.69(54–83, n = 30); shaft length 482 ± 19.57(441–520, n = 30); inner diameter 285 ± 13.51(255–310, n = 30); aperture angle 55° ± 4.45(45°–67°, n = 30); aperture 305 ± 23.75(249 – 348, n = 30); hook-side curve length 110 ± 12.44(81–127, n = 30) and shaft-side curve length 123 ± 13.42(89–145, n = 30). Complex 2 sucker sclerite hook length 162 ± 19.29(118–191, n = 30); hook curve length 35 ± 4.28(28–41, n = 30); aperture angle 107° ± 6.86(89°–118°, n = 30); aperture 132 ± 17.35(93–157, n = 30) and base width 40 ± 3.02(32–46, n = 30).

Sclerites of sucker complex 3 (Fig. 4.5C): circumferential length 1179 ± 57.83(1070–1320, n = 30); total length 530 ± 20.42(488–585, n = 30); total diameter 345 ± 16.56(310–374, n = 30); width 70 ± 5.77(61–81, n = 30); shaft length 476 ± 20.95(431–532, n = 30); inner diameter 277 ± 14.92(236–310, n = 30); aperture angle 57° ± 4.33(48°–68°, n = 30); aperture 311 ± 24.70(257–357, n = 30); hook-side curve length 113 ± 9.15(96–132, n = 30) and shaft-side curve length 119 ± 12.06(90–140, n = 30). Complex 3 sucker sclerite hook length 168 ± 16.26(138–205, n = 30); hook curve length 37 ± 4.24(31–48, n = 30); aperture angle 104° ± 6.44(89°–119°, n = 30); aperture 135 ± 15.51(106–178, n = 30) and base width 43 ± 3.72(35–50, n = 30).

Dorsal haptoral appendix 1223 ± 183.72(918–1668, n = 20) long, 423 ± 62.52(248–509, n = 23) wide. Terminal suckers of appendix 293 ± 30.95(243–364, n = 44) long, 146 ± 15.67(118–188, n = 44) wide. Single pair of hamuli present before terminal suckers. Hamulus (Fig. 4.5D) total length 63 ± 2.45(56–67, n = 25); hook point length 15 ± 0.81(13–16, n = 25); hook shank length 20 ± 1.37(16–22, n = 25) total width 31 ± 1.34(28–33, n = 25); distal hook point width 3 ± 0.31(3–4, n = 25); outer aperture angle 20° ± 1.37(16°–22°, n = 25); inner aperture angle 78° ± 3.72(67°–85°, n = 25); aperture 94 ± 5.63(82–105, n = 25); hook shank base width 7 ± 1.00(5–9, n = 25); inner root-shaft length 37 ± 2.24(33–43, n = 25); outer root-shaft length 47 ± 1.99(43–50, n = 25); root base angle 115° ± 14.13(86°–139°, n = 25), and root base width 26 ± 1.98(22–30, n = 25).

Testes irregular in shape, 88 ± 12.46(57–111, n = 22) in number; 82 ± 13.00(62–109, n = 18) wide. Vas deferens sinuous, surrounded by small gland cells along the

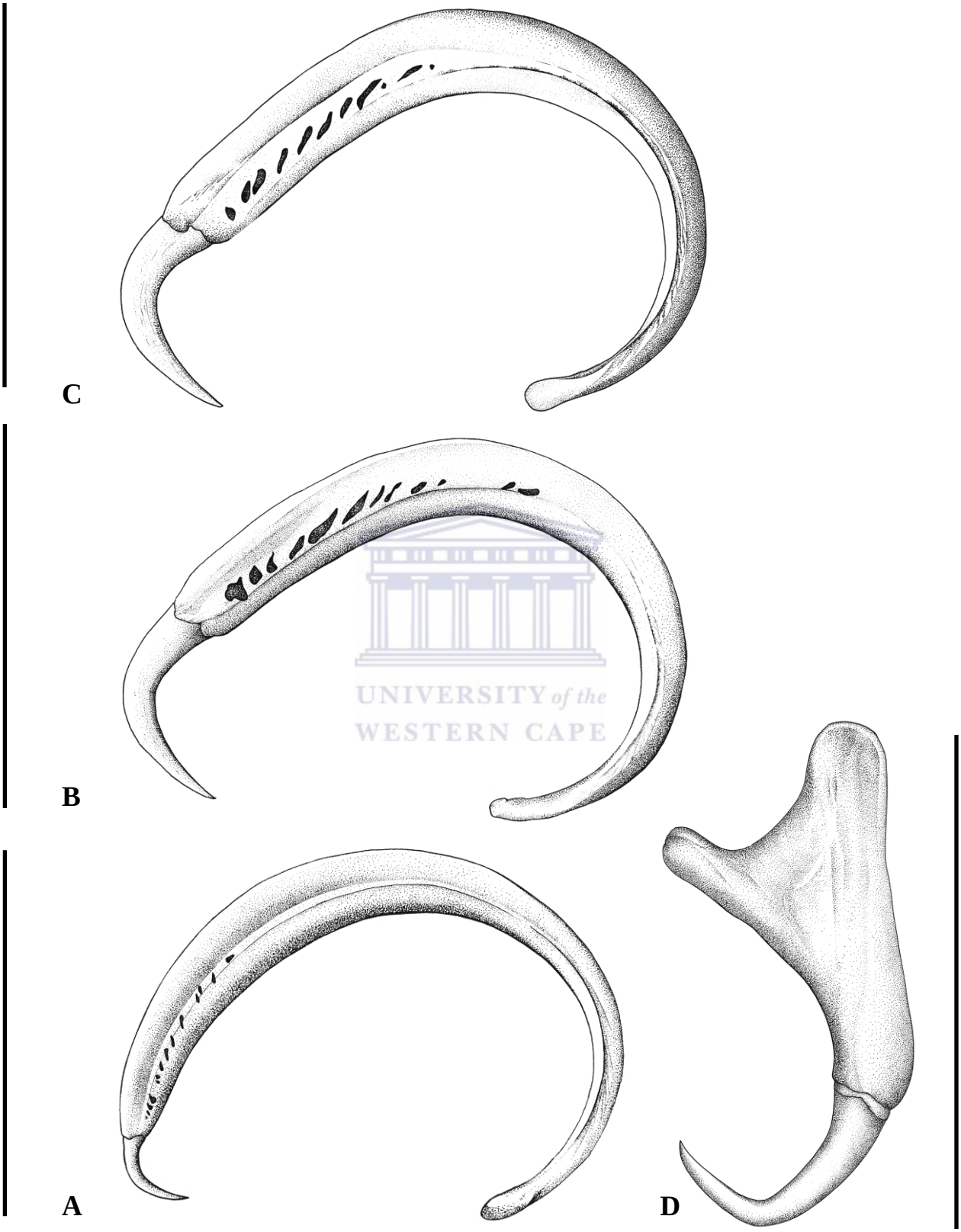

Fig. 4.5 *Callorhynchocotyle callorhynchi* sclerites of sucker complexes 1 (**A**), 2 (**B**) and 3(**C**), and hamulus (**D**). Scale bars = 260µm, 350µm, 350µm and 60µm respectively.

majority of its length (Fig. 4.4B). Vas deferens loop proximal to entrance into cirrus absent in some specimens. Unarmed muscular cirrus total length 472 ± 67.23(356–623, n = 20); maximum width 74 ± 7.87(49–85, n = 20); distal bulb length 125 ± 21.85(76–175, n = 20), and distal bulb width 113 ± 7.98(97–131, n = 20).

Ovary (dextral = 17, sinistral = 7) 1110 ± 176.21(845–1527, n = 19) long, anteriorly branched, coiled posteriorly, ascending to oviduct, branching to sack-like, reduced seminal receptacle (Fig. 4.4C). Oötype smooth, leading to uterus, dorsal to ovary, ventral to vas deferens. Ovate eggs chain-linked by tendrils at each pole. Eggs (*in utero*) 173 ± 9.94(158–189, n = 9) long, 72 ± 4.07(65–77, n = 9) wide. Parallel vaginal

ducts with glandulo-muscular distal portion and thin-walled proximal portion. Ventral vaginal pores muscular, lateral to proximal portion of cirrus. Follicular vitellarium originates posterior to vaginal pores. Excretory pores not observed.

Remarks

Comparative measurements for *C. callorhynchi* are provided in Table 4.2. The cirrus measurements of *C. callorhynchi* of Beverley-Burton and Chisholm (1990) did not agree with those taken for the voucher material examined for the present study. Subsequent discussions with L.A. Chisholm have revealed that their measurements were erroneous, and they should therefore not be used in future comparisons. However, these measurements were re-measured correctly for the same type specimens in the present study. Manter (1955) differentiated *S. callorhynchi* (junior synonym of *C. callorhynchi*) from other members of *Squalonchocotyle* Cerfontaine, 1899 partly on the lack of a seminal receptacle in his specimens. *Callorhynchocotyle callorhynchi* does posses a small, smooth, reduced seminal receptacle, as do all members of the genus (Boeger *et al*. 1989).

Table 4.2 Comparative measurements for *Callorhynchocotyle callorhynchi* Manter, 1955

	Manter (1955)[1]	Boeger *et al.* (1989)	Beverley-Burton and Chisholm (1990)	**Present study[2] (additional vouchers)**
Total body length	*4662–8170, n = 9;* **4733, n = 1**	6052 (4995–7109), n = 2)*	5169 (3810–6104, n = 16); **5237 ± 826.7(3867–6333, n = 9)**	**6885 ± 1214.6(5500–11100, n = 24)**
Maximum body width	*774–1505, n = 9;* **778, n = 1**	845 (669–1021, n = 2)	1131 (847–1330, n = 16); **1102 ± 146.6(841–1286, n = 9)**	**1700 ± 161.7(1425–2100, n = 24)**

Table 4.2 cont

Oral sucker diameter	*210–366, n = 9;* **288, n = 1**	284 (248–322, n = 2)	231 (205–265, n = 9); **366 ± 81.5(296–560, n = 8)**	**360 ± 34.3(269–411, n = 24)**
Pharynx length	**85, n = 1**	72, n = 2	99 (78–123, n = 6); **78 ± 8.3(65–86, n = 5)**	**91 ± 9.7(68–113, n = 24)**
Pharynx width	*78–98;* **85, n = 1**	64 (56–72, n = 2)	72 (63–83, n = 6); **68 ± 4.8(63–74, n = 5)**	**89 ± 7.1(75–100, n = 24)**
Haptor length	**1900, n = 1**	-	1881 (1398–2252, n = 15); **2088 ± 444.5(1320–2733, n = 9)**	**2494 ± 362.9(2025–3640, n = 24)**
Haptor width	**880, n = 1**	-	1068 (682–1305, n = 15); **1155 ± 140.4(905–1267, n = 9)**	**1595 ± 169.8(1275–1960, n = 24)**
Appendix length	*702–1720, n = 9;* **724, n = 1**	2104 (1824–2363, n = 2)	941 (703–1142, n = 4); **905 ± 140.4(760–1132, n = 7)**	**1223 ± 183.7(918–1668, n = 20)**
Appendix width	**362, n = 1**	997 (972–10.21, n = 2)	361 (243–504, n = 13); **378 ± 58.6(292–479, n = 7)**	**423 ± 62.5(248–509, n = 23)**
Terminal appendix sucker length	*156–351;* **243 ± 15.9(231–254, n = 2)**	289, n = 2	249 (182–315, n = 27); **364 ± 44.7(282–424, n = 13)**	**293 ± 30.9(243–364, n = 44)**
Terminal appendix sucker width	**114 ± 18.3(101–127, n = 2)**	109, n = 2	118 (82–149, n = 27); **142 ± 24.6(101–190, n = 13)**	**146 ± 15.7(118–188, n = 44)**
No. of testes	*50–65;* **107, n = 1**	-	**83 ± 7.8(67–94, n = 9)**	**88 ± 12.5(57–111, n = 22)**
Testes width	**47 ± 5.9(38–56, n = 10)**	-	**74 ± 11.8 (54–98, n = 10)**	**82 ± 13.0(62–109, n = 18)**
Cirrus total length	-	-	23; **501, n = 1**	**472 ± 67.2(356–623, n = 20)**
Cirrus maximum width	-	-	56; **87, n = 1**	**74 ± 7.9(49–85, n = 20)**
Distal bulb length	**105, n = 1**	-	126; **96 ± 11.2(80–113, n = 7)**	**125 ± 21.8(76–175, n = 20)**
Distal bulb width	**83, n = 1**	-	93; **86 ± 3.6(81–90, n = 7)**	**113 ± 7.9(97–131, n = 20)**
Ovary length	**614, n = 1**	639 (459–818, n = 2)	**619 ± 149.6(315–745, n = 9)**	**1110 ± 176.2(845–1527, n = 19)**
Egg length	*148–203;* **151, n = 1**	152 (136–168, n = 2)	181 (158–216, n = 20); **181 ± 13.5(170–210, n = 7)**	**173 ± 9.9(158–189, n = 9)**
Egg width	*56–74;* **68, n = 1**	58 (53–62, n = 2)	60 (47–67, n = 20); **68 ± 5.7(62–73, n = 7)**	**72 ± 4.1(65–77, n = 9)**
Complex 1 sclerite				
Circumferential length	-	-	-	**858 ± 44.4(758–953, n = 30)**
Total length	*319–444;* **322, n = 1**	283 (281–285, n = 2)	381 (238–450, n = 17); **406 ± 21.9(377–425, n = 4)**	**381 ± 19.5(347–428, n = 30)**
Shaft length	**325, n = 1**	31–32, n = 2	39 (26–46, n = 13); **410 ± 21.6(381–427, n = 4)**	**384 ± 19.6(347–432, n = 30)**
Hook-side curve length	**56, n = 1**	-	**65 ± 13.1(47–76, n = 4)**	**61 ± 5.7(50–71, n = 30)**
Shaft-side curve length	-	-	-	**89 ± 11.6(74–111, n = 30)**
Total diameter	**221, n = 1**	-	**271 ± 18.2(254–297, n = 4)**	**246 ± 15.0(219–271, n = 30)**
Inner diameter	**187, n = 1**	-	**231 ± 16.1(217–254, n = 4)**	**209 ± 14.1(183–235, n = 30)**
Aperture angle	-	-	-	**60° ± 5.7(49°–70°, n = 30)**
Aperture	-	-	-	**253 ± 25.6(199–306, n = 30)**
Width	**36, n = 1**	-	**42 ± 3.8(38–47, n = 4)**	**40 ± 4.9(29–49, n = 30)**
Hook length	-	42 (39–45, n = 2)	44 (31–59, n = 13); **59 ± 9.7(49–69, n = 5)**	**56 ± 4.8(44–65, n = 30)**

Table 4.2 cont

Hook curve length	-	-	-	**12 ± 1.8(7–16, n = 30)**
Hook aperture angle	-	-	**109° ± 10.9(96°–125°, n = 5)**	**105° ± 7.7(91°–124°, n = 30)**
Hook aperture	-	-	-	**45 ± 4.9(32–54, n = 30)**
Hook base width	-	-	**16 ± 1.3(14–17, n = 5)**	**14 ± 1.2(11–18, n = 30)**
Complex 2 sclerite				
Circumferential length	-	-	-	**1191 ± 59.8(1076–1292, n = 30)**
Total length	*343–538*	362, n = 2	488 (350–549, n = 8); **529 ± 7.8(525–538, n = 3)**	**525 ± 19.4(484–558, n = 30)**
Shaft length	-	88, n = 2	98 (81–117, n = 6); **513 ± 3.0(509–515, n = 3)**	**482 ± 19.5(441–520, n = 30)**
Hook-side curve length	-	-	**94 ± 12.8(83–108, n = 3)**	**110 ± 12.4(81–127, n = 30)**
Shaft-side curve length	-	-	-	**123 ± 13.4(89–145, n = 30)**
Total diameter	-	-	**351 ± 9.7(341–360, n = 3)**	**351 ± 15.7(323–381, n = 30)**
Inner diameter	-	-	**288 ± 3.0(286–291, n = 3)**	**285 ± 13.5(255–310, n = 30)**
Aperture angle	-	-	-	**55° ± 4.4(45°–67°, n = 30)**
Aperture	-	-	-	**305 ± 23.7(249 – 348, n = 30)**
Width	-	-	**67 ± 7.7(58–72, n = 3)**	**66 ± 7.6(54–83, n = 30)**
Hook length	**154 ± 28.5(133–174, n = 2)**	106, n = 2	122 (92–136, n = 6); **165 ± 18.9(143–177, n = 3)**	**162 ± 19.2(118–191, n = 30)**
Hook curve length	-	-	-	**35 ± 4.2(28–41, n = 30)**
Hook aperture angle	**119° ± 1.11(118°–119°, n = 2)**	-	**112° ± 8.8(102°–118°, n = 3)**	**107° ± 6.8(89°–118°, n = 30)**
Hook aperture	-	-	-	**132 ± 17.3(93–157, n = 30)**
Hook base width	**35 ± 7.2(30–40, n = 2)**	-	**40 ± 1.7(38–41, n = 3)**	**40 ± 3.0(32–46, n = 30)**
Complex 3 sclerite				
Circumferential length	-	-	-	**1179 ± 57.8(1070–1320, n = 30)**
Total length	-	351, n = 2	468 (402–538, n = 10); **504, n = 1**	**530 ± 20.4(488–585, n = 30)**
Shaft length	-	84 (83–86, n = 2)	97 (73–111, n = 7); **471, n = 1**	**476 ± 20.9(431–532, n = 30)**
Hook-side curve length	-	-	**116, n = 1**	**113 ± 9.1(96–132, n = 30)**
Shaft-side curve length	-	-	-	**119 ± 12.0(90–140, n = 30)**
Total diameter	-	-	**347, n = 1**	**345 ± 16.5(310–374, n = 30)**
Inner diameter	-	-	**281, n = 1**	**277 ± 14.9(236–310, n = 30)**
Aperture angle	-	-	-	**57° ± 4.33(48°–68°, n = 30)**
Aperture	-	-	-	**311 ± 24.70(257–357, n = 30)**
Width	-	-	**69, n = 1**	**70 ± 5.7(61–81, n = 30)**
Hook length	**174, n = 1**	123–124, n = 2	133 (105–162, n = 7); **177 ± 6.2(172–186, n = 4)**	**168 ± 16.2(138–205, n = 30)**
Hook curve length	-	-	-	**37 ± 4.2(31–48, n = 30)**
Hook aperture angle	**122°, n = 1**	-	**123° ± 8.8(116°–136°, n = 4)**	**104° ± 6.4(89°–119°, n = 30)**
Hook aperture	-	-	-	**135 ± 15.5(106–178, n = 30)**
Hook base width	**40, n = 1**	-	**41 ± 1.6(40–43, n = 4)**	**43 ± 3.7(35–50, n = 30)**
Hamulus total length	*57–61*	194 (186–203, n = 2)	60 (54–71); **60, n = 1**	**63 ± 2.5(56–67, n = 25)**
Hook point length	-	-	**15, n = 1**	**15 ± 0.8(13–16, n = 25)**
Shaft length	-	-	**18, n = 1**	**20 ± 1.4(16–22, n = 25)**
Total width	-	-	**28, n = 1**	**31 ± 1.3(28–33, n = 25)**
Distal hook point width	-	-	**3, n = 1**	**3 ± 0.3(3–4, n = 25)**

Table 4.2 cont

Outer aperture angle	-	-	-	**20° ± 1.3(16°–22°, n = 25)**
Inner aperture angle	-	-	-	**78° ± 3.7(67°–85°, n = 25)**
Aperture	-	-	-	**94 ± 5.6(82–105, n = 25)**
Hook shank base width	-	-	**6, n = 1**	**7 ± 1.0(5–9, n = 25)**
Outer root-shaft length	-	-	**47, n = 1**	**47 ± 1.9(43–50, n = 25)**
Inner root-shaft length	-	-	**40, n = 1**	**37 ± 2.2(33–43, n = 25)**
Root base width	-	-	26 (20–31); **21, n = 1**	**26 ± 1.9(22–30, n = 25)**
Root base angle	-	-	**90°, n = 1**	**115° ± 14.1(86°–139°, n = 25)**

[1]Manter (1955) combined both *C. callorhynchi* and *C. amatoi* measurements in the original description, represented by the italicised measurement sets. These measurements are not used in comparison, but rather as a reference to the historical data.
[2]**Bold script =** all measurements of the present study: Manter (1955): USNPC 037447.00: paratype 399-17; Beverley-Burton and Chisholm (1990): USNPC 080984.00: vouchers M1523-7, 9 and 10; Queensland Museum GL 10475-80.
*Note that Boeger *et al.* (1989) measured the same paratype and included the haptor in the total length measurements of both specimens.

Dillon and Hargis (1968) reported that the sucker complex sclerite hooks of the type material from South Africa differed considerably in length to those of the additional material of *E. callorhynchi* (junior synonym of *C. callorhynchi*) they collected off New Zealand's South Island. Boeger *et al*. (1989) subsequently erected a separate species, *C. amatoi* Boeger *et al*. (1989) to accommodate all the New Zealand material based on differences in sucker complex sclerite morphology. In addition, Dillon and Hargis (1968) referred to the sucker complex sclerites of *E. callorhynchi* as having spines along the lateral margins, a feature repeated for other members of the family in the same publication. This feature is erroneous and a misinterpretation of the lateral sucker complex sclerite pits and indentations ("parallel ridges" of Manter 1955) detailed in Figs. 4.5 and 4.6.

The sclerites of sucker complexes 1, 2 and 3 in *C. callorhynchi* are shorter in total and shaft length than those of *C. marplatensis*. The hook aperture angle of the complex 1 sucker sclerite is more obtuse than that of *C. marplatensis*. The hamulus total width, hook point and hook shank lengths are greater than that of *C. marplatensis*. However, the inner root-shaft length of *C. callorhynchi* is comparatively shorter. *Callorhynchocotyle callorhynchi* is differentiated from *C. marplatensis* in possessing papillate oral and haptoral sclerite suckers, and is found exclusively on the gills of *Callorhinchus capensis* found off Southern Africa.

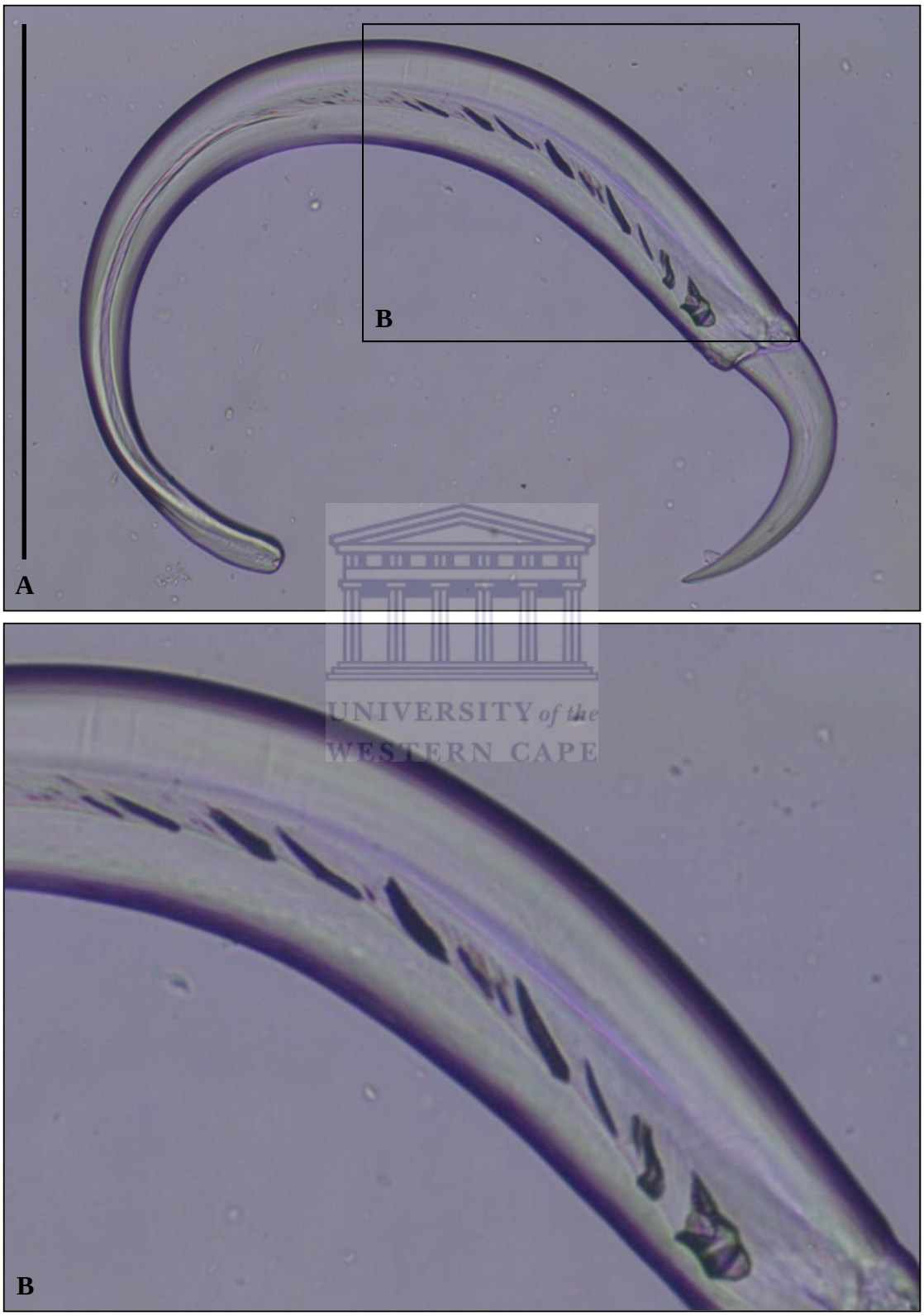

Fig. 4.6A-B *Callorhynchocotyle callorhynchi* sucker complex 3 sclerite digest highlighting the lateral pits and indentations ("parallel ridges" of Manter 1955). Scale bar = 350µm.

4.2.4 Callorhynchocotyle amatoi Boeger, Kritsky and Pereira, 1989

Synonyms: Squalonchocotyle callorhynchi Manter, 1955; *Erpocotyle callorhynchi* Dillon and Hargis, 1968.

Type host: Callorhinchus milii Bory de Saint-Vincent, 1823 (Callorhynchidae, Holocephali).

Type locality: Coast of New Zealand.

Site on host: Gills.

Material examined: USNPC 071197.02 paratypes M1015-16 and 17; USNPC 080983.00 vouchers M1523-3, 4 and 5; vouchers SAMCTA 29467 (2 whole mounts and 2 haptor digests); vouchers AHC 29749 (1 whole mount) and AHC 29750 (1 haptor digest).

Redescription (Figs. 4.7–4.9, Table 4.3)

Total body length (Fig. 4.7A) 6892 ± 359.10(6625–7300, n = 3), maximum body width 1349 ± 114.46(1254–1476, n = 3). Oral sucker internally papillate, diameter 366 ± 35.58(338–406, n = 3). Pharynx 109 ± 9.64(103–120, n = 3) long, 91 ± 4.03(88–96, n = 3) wide. Branched intestinal caeca unite after testes and extend into the haptor. Asymmetrical haptor 2750 ± 534.11(2200–3267, n = 3) long, 1138 ± 282.31(937–1460, n = 3) wide with 3 paired sucker sclerite complexes. Haptoral suckers papillate.

Sclerites of sucker complex 1 (Fig. 4.8A) smaller than similarly sized sucker sclerites of complex 2 and 3 with circumferential length 863 ± 27.80(837–892, n = 3); total length 401 ± 69.96(323–458, n = 3); total diameter 257 ± 17.09(237–267, n = 3); width 43 ± 4.06(38–46, n = 3); shaft length 402 ± 66.29(328–456, n = 3); inner diameter 217 ± 21.63(192–232, n = 3); aperture angle 64° ± 28.61(34°–92°, n = 3); aperture 280 ± 130.12(142–400, n = 3); hook-side curve length 61 ± 10.37(49–69, n = 3); and shaft-side curve length 86 ± 20.97(62–99, n = 3). Complex 1 sucker sclerite hook length 56 ± 0.57(56–57, n = 3); hook curve length 10 ± 0.76(10–11, n = 3); aperture angle 112° ± 2.69(109°–114°, n = 3); aperture 47 ± 0.75(46–48, n = 3) and base-width 15 ± 0.62(15–16, n = 3).

Sclerites of sucker complex 2 (Fig. 4.8B): circumferential length 1111 ± 98.34(998–1172, n = 3); total length 451 ± 37.10(410–481, n = 3); total diameter 336 ± 32.42(299–357, n = 3); width 73 ± 3.77(69–76, n = 3); shaft length 451 ± 35.82(411–479, n = 3); inner diameter 264 ± 28.28(232–282, n = 3); aperture angle 48° ± 15.10(31°–59°,

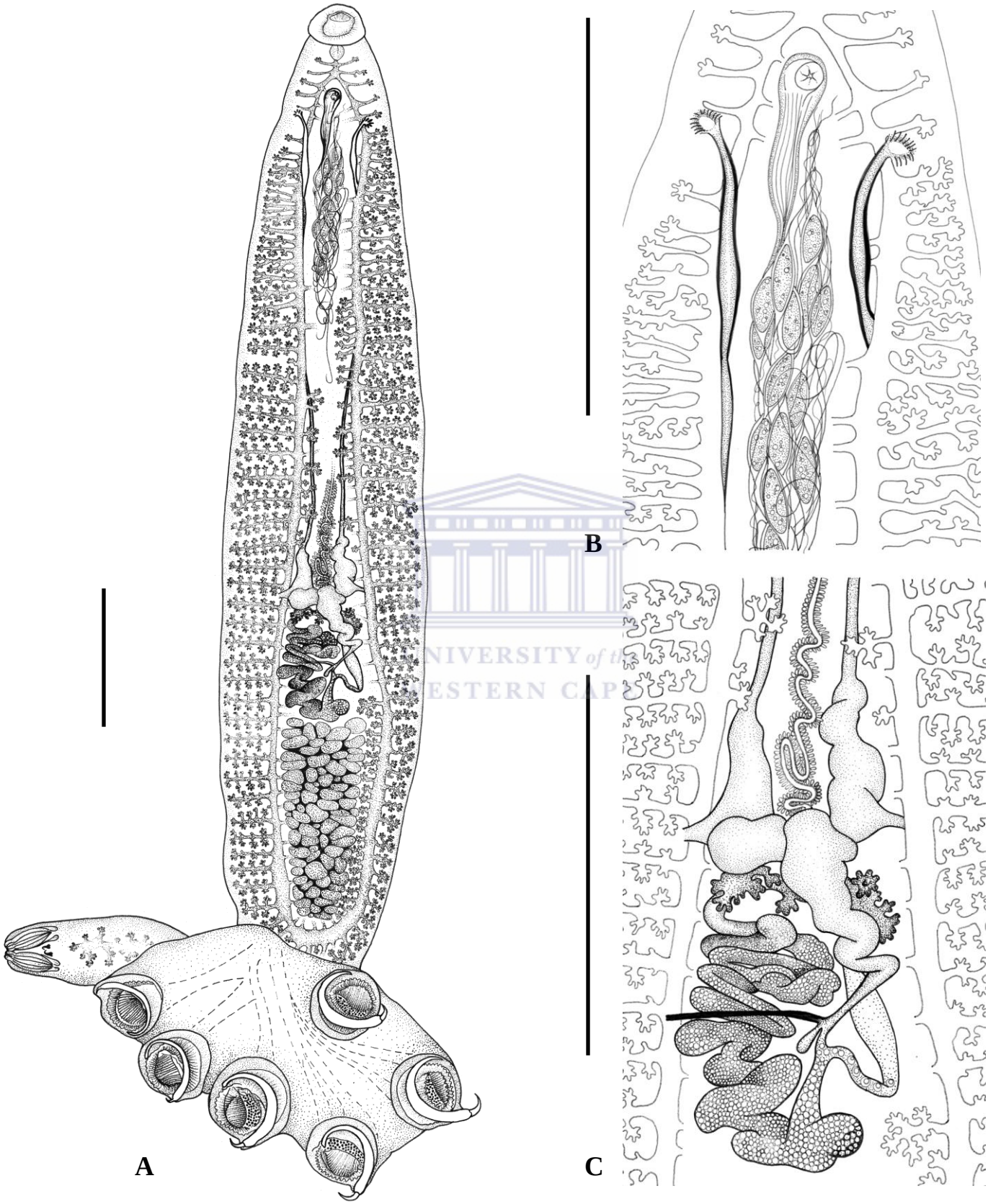

Fig. 4.7 *Callorhynchocotyle amatoi*. **A.** Whole mount; **B.** Enlarged anterior section of whole mount; **C.** Enlarged mid-section of whole mount. Scale bars = 1000µm.

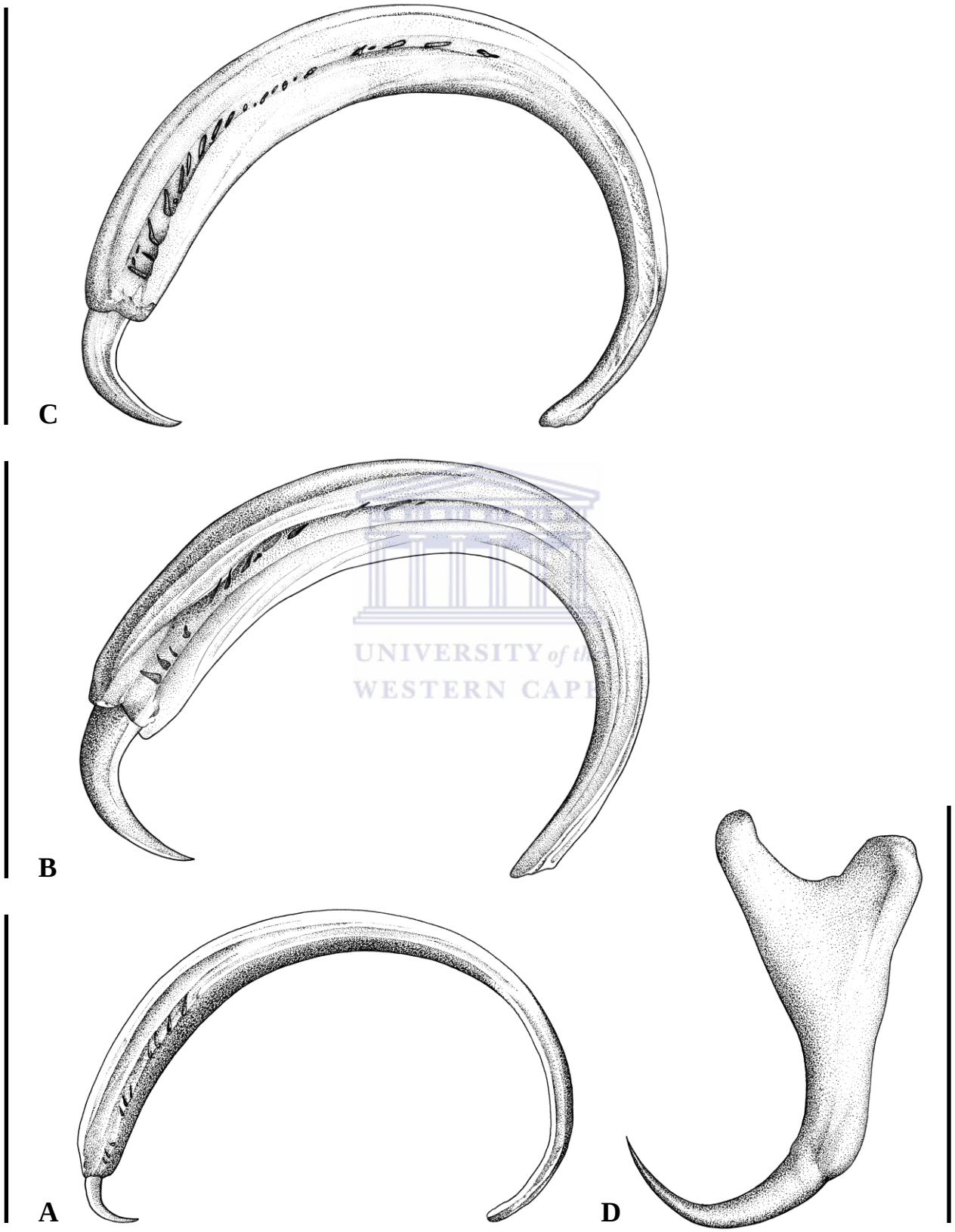

Fig. 4.8 *Callorhynchocotyle amatoi* sclerites of sucker complexes 1 (**A**), 2 (**B**) and 3(**C**), and hamulus (**D**). Scale bars = 260µm, 340µm, 340µm and 50µm respectively.

n = 3); aperture 248 ± 97.12(137–311, n = 3); hook-side curve length 80 ± 3.60(78–84, n = 3) and shaft-side curve length 116 ± 20.65(99–139, n = 3). Complex 2 sucker sclerite hook length 126 ± 7.70(120–135, n = 3); hook curve length 18 ± 0.30(17–18, n = 3); aperture angle 120° ± 5.53(113°–123°, n = 3); aperture 102 ± 7.14(96–110, n = 3) and base width 37 ± 3.28(35–41, n = 3).

Sclerites of sucker complex 3 (Fig. 4.8C): circumferential length 1092 ± 96.43(989–1181, n = 3); total length 428 ± 12.01(415–436, n = 3); total diameter 319 ± 15.91(304–336, n = 3); width 71 ± 2.85(68–74, n = 3); shaft length 421 ± 13.35(407–433, n = 3); inner diameter 249 ± 14.29(237–265, n = 3); aperture angle 40° ± 5.96(35°–46°, n = 3); aperture 191 ± 31.55(158–221, n = 3); hook-side curve length 77 ± 1.89(75–79, n = 3) and shaft-side curve length 130 ± 10.73(118–138, n = 3). Complex 3 sucker sclerite hook length 138 ± 5.22(134–144, n = 3); hook curve length 18 ± 2.83(15–21, n = 3); aperture angle 121° ± 4.79(117°–127°, n = 3); aperture 109 ± 4.42(106–114, n = 3) and base width 40 ± 0.43(39–40, n = 3).

Dorsal haptoral appendix 1016 ± 223.91(810–1254, n = 3) long, 434 ± 18.33(413 – 444, n = 3) wide. Terminal suckers of appendix 287 ± 28.06(257–330, n = 6) long, 142 ± 6.53(135–154, n = 3) wide. Single pair of hamuli present before terminal suckers.

Hamulus (Figs. 4.8D, 4.9) total length 54 ± 1.36(53–56, n = 3); hook point length 15 ± 0.30(15–16, n = 3); hook shank length 16 ± 0.84(15–17, n = 3) total width 29 ± 0.79(28–30, n = 3); distal hook point width 3 ± 0.19, n = 3; outer aperture angle 17° ± 0.32(16°–17°, n = 3); inner aperture angle 86° ± 3.84(83°–90°, n = 3); aperture 102 ± 4.50(98–107, n = 3); hook shank base width 6 ± 0.58(5–7, n = 3); inner root-shaft length 42 ± 1.29(40–44, n = 3); outer root-shaft length 44 ± 2.94(41–49, n = 3); root base angle 105° ± 10.74(93°–118°, n = 3), and root base width 23 ± 1.40(20–24, n = 3).

Testes irregular in shape, 103 ± 7.76(94–109, n = 8) in number; 86 ± 9.96(74–103, n = 17) wide. Vas deferens sinuous, surrounded by small gland cells along the majority of its length (Fig. 4.7B). Vas deferens loop proximal to entrance into cirrus absent in some specimens. Unarmed muscular cirrus total length 429, n = 1; maximum width 70, n = 1; distal bulb length 85, n = 1, and distal bulb width 85, n = 1.

Ovary (dextral = 2, sinistral = 1) 763 ± 59.16(694–799, n = 3) long, anteriorly branched, coiled posteriorly, ascending to oviduct, branching to sack-like, reduced seminal receptacle (Fig. 4.7C). Oötype smooth, leading to uterus, dorsal to ovary, ventral

to vas deferens. Ovate eggs chain-linked by tendrils at each pole. Eggs (*in utero*) 163 ± 16.10(151–186, n = 4) long, 82 ± 12.55(70–100, n = 4) wide. Parallel vaginal ducts with glandulo-muscular distal portion and thin-walled proximal portion. Ventral vaginal pores muscular, lateral to median portion of cirrus. Follicular vitellarium originates posterior to vaginal pores. Excretory pores not observed.

Remarks

Comparative measurements for *C. amatoi* are represented in Table 4.3.

Boeger *et al*. (1989) erected *C. amatoi* for all the New Zealand material of *C. callorhynchi* from *Callorhinchus milii*, separating *C. amatoi* from *C. callorhynchi* on sucker complex sclerite morphology alone. Complex 2 and 3 sucker sclerites are smaller than those of *C. marplatensis* and *C. callorhynchi* in total and hook-side curve lengths. Complex 2 sucker sclerite shaft lengths are less than those of *C. callorhynchi* and *C. marplatensis*. Complex 3 sucker sclerite shaft length and total and inner diameters are shorter than that *C. marplatensis* and *C. callorhynchi*. The hook of complex 2 and 3 sucker sclerites are shorter in hook length than both those of *C. marplatensis* and *C. callorhynchi*. The hook aperture angle of complex 1 sclerite hooks is more obtuse than those of both *C. callorhynchi* and *C. marplatensis*. The base widths of complex 2 and 3 sclerite hooks are narrower than those of *C. callorhynchi*.

Callorhynchocotyle amatoi is differentiated from *C. callorhynchi* by the shape of the hamulus (Fig. 4.9). The hamulus of *C. amatoi* is shorter in total and hook shank lengths, narrower in total, hook shank base and base widths. The inner root-shaft length is greater than that of *C. callorhynchi*. Hamulus hook point length is longer than that of than *C. marplatensis*, and distal hook point width less than both those of *C. marplatensis*. *Callorhynchocotyle amatoi* is differentiated from *C. marplatensis* in possessing papillate oral and haptoral sclerite suckers and is only found on the gills of *Callorhinchus milii*.

Beverley-Burton and Chisholm (1990) presented a simple key to the *Callorhynchocotyle* species wherein *C. amatoi* is separated from *C. callorhynchi* by the presence of an expanded proximal portion of the cirrus complex, absent in the former. The additional voucher material collected and examined for the present study has revealed that *C. callorhynchi* also possesses an expansion of the proximal cirrus portion, and therefore this character is invalid as a diagnostic character.

Table 4.3 Comparative measurements for *Callorhynchocotyle amatoi* Boeger *et al*., 1989

	Manter (1955)[1]	Boeger *et al.* (1989)	Beverley-Burton and Chisholm (1990)	**Present study[2] (additional vouchers)**
Total body length	*4662–8170, n = 9*	7091 (5329–8474, n = 8); **5792 ± 1473.1(4750–6833, n = 2)**	**5960 ± 925.0(4750–6950, n = 5)**	**6892 ± 359.1(6625–7300, n = 3)**
Maximum body width	*774–1505, n = 9*	989 (706–1316, n = 8); **944 ± 33.7(921–968, n = 2)**	**1083 ± 170.7(921–1317, n = 5)**	**1349 ± 114.5(1254–1476, n = 3)**
Oral sucker diameter	*210–366, n = 9*	252 (191–356, n = 8); **295 ± 1.3(294–296, n = 2)**	**313 ± 30.5(294–365, n = 5)**	**366 ± 35.6(338–406, n = 3)**
Pharynx length	-	79 (69–91, n = 8); **82 ± 7.9(77–88, n = 2)**	**84 ± 7.3(77–95, n = 5)**	**109 ± 9.6(103–120, n = 3)**
Pharynx width	*78–98*	70 (62–89, n = 8); **73 ± 5.9(69–77, n = 2)**	**75 ± 5.6(69–83, n = 5)**	**91 ± 4.0(88–96, n = 3)**
Haptor length	-	1959 (1451–2482, n = 8); **2350 ± 777.8(1800–2900, n = 2)**	**2343 ± 524.3(1800–2900, n = 5)**	**2750 ± 534.1(2200–3267, n = 3)**
Haptor width	-	1201 (755–1639, n = 8); **817 ± 33.7(794–841, n = 2)**	**1044 ± 10.2(794–1302, n = 5)**	**1138 ± 282.3(937–1460, n = 3)**
Appendix length	*702–1720, n = 9*	1108 (674–1429, n = 8); **900 ± 364.9(642–1158, n = 2)**	**907 ± 226.2(642–1158, n = 5)**	**1016 ± 223.9(810–1254, n = 3)**
Appendix width	-	850 (541–1206, n = 8); **243 ± 13.2(233–253, n = 2)**	**332 ± 85.7(233–434, n = 5)**	**434 ± 18.3(413 – 444, n = 3)**
Terminal appendix sucker length	*156–351*	272 (236–320, n = 8); **294 ± 12.4(285–302, n = 2)**	**294 ± 31.5(253–343, n = 5)**	**287 ± 28.1(257–330, n = 6)**
Terminal appendix sucker width	-	144 (102–185, n = 8); **127 ± 16.9(115–139, n = 2)**	**123 ± 14.3(106–149, n = 5)**	**142 ± 6.5(135–154, n = 3)**
No. of testes	*50–65*	**64 ± 1.4(63–65, n = 2)**	**68 ± 5.4(63–77, n = 5)**	**103 ± 7.8(94–109, n = 8)**
Testes width	-	**97 ± 12.3(76–113, n = 10)**	**97 ± 12.3(76 –113, n = 10)**	**86 ± 9.9(74–103, n = 17)**
Cirrus total length	-	**271, n = 1**	307 (273–360, n = 15); **351 ± 69.2(271–392, n = 3)**	**429, n = 1**
Cirrus maximum width	-	**52, n = 1**	73 (68–81, n = 15); **69 ± 14.1(52–78, n = 3)**	**70, n = 1**
Distal bulb length	-	**111, n = 1**	108 (99–121, n = 15); **109 ± 8.3(100–116, n = 3)**	**85, n = 1**
Distal bulb width	-	**103, n = 1**	92 (77–109, n = 15); **103 ± 7.1(96–110, n = 3)**	**85, n = 1**
Ovary length	-	859 (651–971, n = 8); **634 ± 51.8(597–671, n = 2)**	**748 ± 148.7(597–953, n = 5)**	**763 ± 59.2(694–799, n = 3)**
Egg length	*148–203*	156 (144–169); **167 ± 13.6(157–183, n = 4)**	**178 ± 25.1(157–230, n = 7)**	**163 ± 16.1(151–186, n = 4)**
Egg width	*56–74*	54 (48–60); **66 ± 7.5(58–72, n = 4)**	**64 ± 6.3(58–72, n = 7)**	**82 ± 12.6(70–100, n = 4)**
Complex 1 sclerite				
Circumferential length	-	-	-	**863 ± 27.8(837–892, n = 3)**

Table 4.3 cont

Total length	*319–444*	297 (239–355, n = 8);	**356, n = 1**	**401 ± 69.9(323–458, n = 3)**
Shaft length	-	20 (18–22, n = 8)	**356, n = 1**	**402 ± 66.2(328–456, n = 3)**
Hook-side curve length	-	-	**65, n = 1**	**61 ± 10.3(49–69, n = 3)**
Shaft-side curve length	-	-	-	**86 ± 20.9(62–99, n = 3)**
Total diameter	-	-	**233, n = 1**	**257 ± 17.0(237–267, n = 3)**
Inner diameter	-	-	**192, n = 1**	**217 ± 21.6(192–232, n = 3)**
Aperture angle	-	-	-	**64° ± 28.6(34°–92°, n = 3)**
Aperture	-	-	-	**47 ± 0.7(46–48, n = 3)**
Width	-	-	**43, n = 1**	**43 ± 4.0(38–46, n = 3)**
Hook length	-	37 (36–39, n = 8)	**59, n = 1**	**56 ± 0.5(56–57, n = 3)**
Hook curve length	-	-	-	**10 ± 0.7(10–11, n = 3)**
Hook aperture angle	-	-	**115°, n = 1**	**112° ± 2.6(109°–114°, n = 3)**
Hook aperture	-	-	-	**47 ± 0.7(46–48, n = 3)**
Hook base width	-	-	**15, n = 1**	**15 ± 0.6(15–16, n = 3)**
Complex 2 sclerite				
Circumferential length	-	-	-	**1111 ± 98.3(998–1172, n = 3)**
Total length	*343–538*	412 (347–452, n = 8); **484 ± 7.7(479–490, n = 2)**	**483 ± 17.2(450–496, n = 6)**	**451 ± 37.1(410–481, n = 3)**
Shaft length	-	54 (46–70, n = 8); **483 ± 10.6(475–490, n = 2)**	**467 ± 27.5(423–499, n = 6)**	**451 ± 35.8(411–479, n = 3)**
Hook-side curve length	-	**92 ± 18.0(79–105, n = 2)**	**93 ± 8.6(79 – 105, n = 6)**	**80 ± 3.6(78–84, n = 3)**
Shaft-side curve length	-	-	-	**116 ± 20.6(99–139, n = 3)**
Total diameter	-	**328 ± 2.4(326–329, n = 2)**	**336 ± 21.7(303–364, n = 6)**	**336 ± 32.4(299–357, n = 3)**
Inner diameter	-	**252 ± 2.7(250–254, n = 2)**	**264 ± 17.5(244–287, n = 6)**	**264 ± 28.2(232–282, n = 3)**
Aperture angle	-	-	-	**48° ± 15.1(31°–59°, n = 3)**
Aperture	-	-	-	**248 ± 97.1(137–311, n = 3)**
Width	-	**77 ± 0.3, n = 2**	**74 ± 6.3(61–78, n = 6)**	**73 ± 3.7(69–76, n = 3)**
Hook length	-	83 (70–98, n = 8); **128 ± 19.5(114–142, n = 2)**	**131 ± 11.9(114–143, n = 6)**	**126 ± 7.7(120–135, n = 3)**
Hook curve length	-	-	-	**18 ± 0.3(17–18, n = 3)**
Hook aperture angle	-	**109° ± 4.5(106°–113°, n = 2)**	**109° ± 3.8(103°–113°, n = 6)**	**120° ± 5.5(113°–123°, n = 3)**
Hook aperture	-	-	-	**102 ± 7.1(96–110, n = 3)**
Hook base width	-	**37 ± 5.7(33–41, n = 2)**	**38 ± 3.7(33–41, n = 6)**	**37 ± 3.2(35–41, n = 3)**
Complex 3 sclerite				
Circumferential length	-	-	-	**1092 ± 96.4(989–1181, n = 3)**
total length	-	408 (332–469, n = 8); **482, n = 1**	**483 ± 26.9(439–512, n = 5)**	**428 ± 12.0(415–436, n = 3)**
Shaft length	-	56 (45–66, n = 8); **473, n = 1**	**470 ± 35.1(409–498, n = 5)**	**421 ± 13.3(407–433, n = 3)**
Hook-side curve length	-	**82, n = 1**	**87 ± 5.4(82–96, n = 5)**	**77 ± 1.8(75–79, n = 3)**
Shaft-side curve length	-	-	-	**130 ± 10.7(118–138, n = 3)**
Total diameter	-	**312, n = 1**	**331 ± 22.1(299–353, n = 5)**	**319 ± 15.9(304–336, n = 3)**
Inner diameter	-	**241, n = 1**	**256 ± 20.7(228–275, n = 5)**	**249 ± 14.2(237–265, n = 3)**
Aperture angle	-	-	-	**40° ± 5.9(35°–46°, n = 3)**
Aperture	-	-	-	**191 ± 31.5(158–221, n = 3)**
Width	-	**82, n = 1**	**77 ± 4.7(70–82, n = 5)**	**71 ± 2.8(68–74, n = 3)**
Hook length	-	92 (86–98, n = 8); **133, n = 1**	**136 ± 9.0(128–150, n = 5)**	**138 ± 5.2(134–144, n = 3)**
Hook curve length	-	-	-	**18 ± 2.8(15–21, n = 3)**

Table 4.3 cont

Hook aperture angle	-	**106°, n = 1**	**106° ± 4.4(103°–114°, n = 5)**	**121° ± 4.7(117°–127°, n = 3)**
Hook aperture	-	-	-	**109 ± 4.4(106–114, n = 3)**
Hook base width	-	**45, n = 1**	**41 ± 2.9(37–45, n = 5)**	**40 ± 0.4(39–40, n = 3)**
Hamulus total length	*57–61*	182 (172–195, n = 8)	54 (44–62, n = 15)	**54 ± 1.6(53–56, n = 3)**
Hook point length	-	-	-	**15 ± 0.3(15–16, n = 3)**
Hook shank length	-	-	-	**16 ± 0.8(15–17, n = 3)**
Total width	-	-	-	**29 ± 0.8(28–30, n = 3)**
Distal hook point width	-	-	-	**3 ± 0.2, n = 3**
Outer aperture angle	-	-	-	**17° ± 0.3(16°–17°, n = 3)**
Inner aperture angle	-	-	-	**86° ± 3.8(83°–90°, n = 3)**
Aperture	-	-	-	**102 ± 4.5(98–107, n = 3)**
Hook shank base width	-	-	-	**6 ± 0.6(5–7, n = 3)**
Outer root-shaft length	-	-	-	**44 ± 2.9(41–49, n = 3)**
Inner root-shaft length	-	-	-	**42 ± 1.3(40–44, n = 3)**
Root base width	-	85 (83–87, n = 8)	22 (18–25, n = 15)	**23 ± 1.4(20–24, n = 3)**
Root base angle	-	-	-	**105° ± 10.7(93°–118°, n = 3)**

[1]Manter (1955) combined both *C. amatoi* and *C. callorhynchi* measurements in the original description of *C. callorhynchi*, represented by the italicised measurement sets. These measurements are not used in comparison, but rather as a reference to the historical data. [2]**Bold script =** all measurements of the present study: Boeger *et al*. (1989): USNPC 71197 paratypes M1015-16 and 17; Beverley-Burton and Chisholm (1990): paratypes, and USNPC 80983 vouchers M1523-3, 4 and 5.

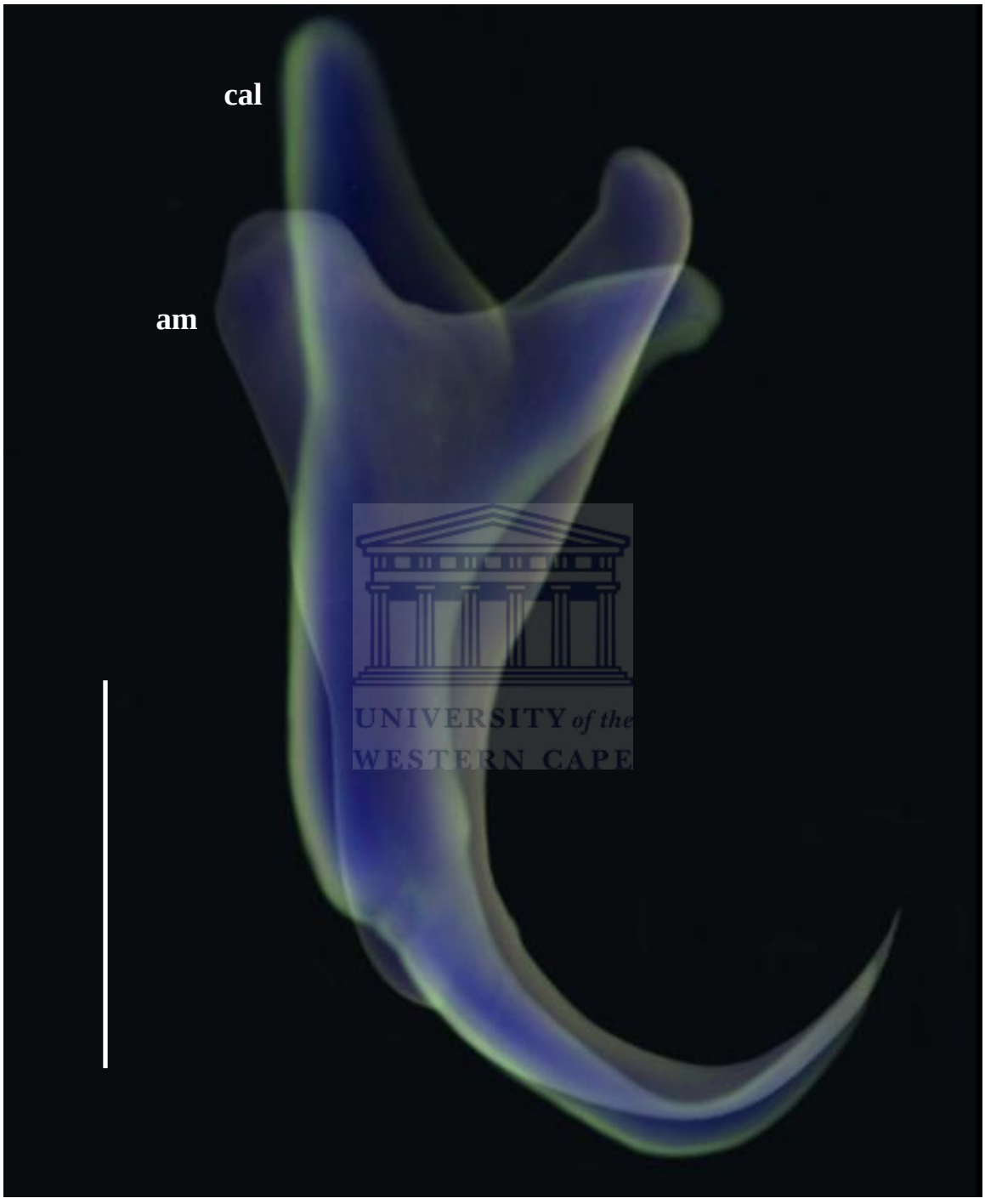

Fig. 4.9 Hamulus digest photomicrograph overlay: am – *Callorhynchocotyle amatoi* (voucher SAMCTA 29467), cal – *C. callorhynchi* (voucher SAMCTA 29466). Scale bar = 20µm.

4.2.5 ***Callorhynchocotyle hydrolagi*** **Beverley-Burton and Chisholm, 1990**

Type host: Hydrolagus ogilbyi (Waite, 1898) (Chimaeridae, Holocephali).

Type locality: Offshore waters, Coffs Harbour, New South Whales, Australia.

Site on host: Gills.

Material examined: USNPC 080982.00 paratypes MT25-15A and B.

Supplemental information (Figs. 4.10 and 4.11, Table 4.4.)

Total body length (Fig. 4.10A) 4450 ± 212.13(4300–4600, n = 2), maximum body width 897 ± 78.57(841–952, n = 2). Oral sucker internally papillate, diameter 922 ± 397.74(340–903, n = 2). Pharynx 85 ± 1.37(84–85, n = 2) long, 63 ± 10.35(55–70, n = 2) wide. Branched intestinal caeca unite after testes and extend into the haptor. Asymmetrical haptor 1990 ± 70.71(1940–2040, n = 2) long, 1076 ± 175.09(952–1200, n = 2) wide with 3 paired sucker sclerite complexes. Haptoral suckers papillate.

Sclerites of sucker complex 1 similarly sized to those of complex 2 and 3. Complex 3 sucker sclerite (Fig. 4.11A) circumferential length 1048, n = 1; total length 450 ± 6.53(445–454, n = 2); total diameter 303 ± 7.42(298–308, n = 2); width 73 ± 0.26(72–73, n = 2); shaft length 452 ± 7.62(446–457, n = 2); inner diameter 234 ± 7.72(228–239, n = 2); aperture angle 56° ± 6.72(51°–61°, n = 2); aperture 245 ± 28.8(224–265, n = 2); hook-side curve length 90 ± 8.46(84–96, n = 2) and shaft-side curve length 95 ± 3.48(92–97, n = 2). Complex 3 sclerite hook length 114 ± 10.40(106–121, n = 2); hook curve length 19 ± 0.87(18–19, n = 2); aperture angle 117° ± 16.40(106–129°, n = 2); aperture 81 ± 10.87(74–89, n = 2) and base-width 34 ± 1.97(32–35, n = 2).

Dorsal haptoral appendix 739, n = 1 long, 320, n = 1 wide. Single pair of hamuli present before terminal suckers. Hamulus (Fig. 4.11B) total length 67, n = 1; hook point length 13, n = 1; shaft length 30, n = 1; total width 37, n = 1; distal hook point width 3, n = 1; outer aperture angle 30°, n = 1; inner aperture angle 58°, n = 1; aperture 73, n = 1; hook shank base width 7, n = 1; inner root-shaft length 42, n = 1; outer root-shaft length 40, n = 1; root base angle 100°, n = 1, and root base width 33, n = 1).

Testes irregular in shape, 65, n = 1 in number; 57 ± 5.60(48–66, n = 10) wide. Vas deferens sinuous, surrounded by small gland cells along the majority of its length. Vas deferens loop proximal to entrance into cirrus, absent. Unarmed muscular cirrus total

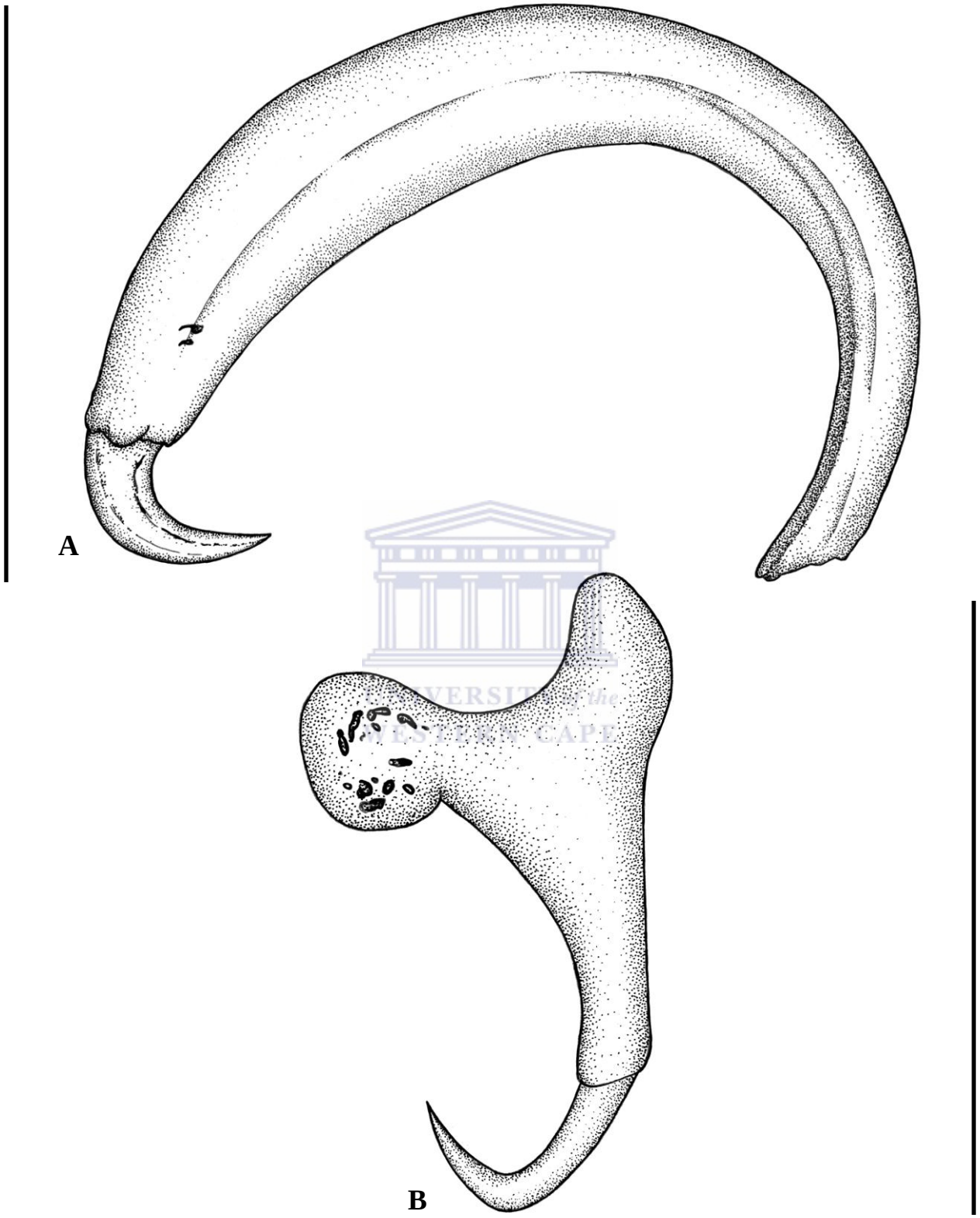

Fig. 4.10 *Callorhynchocotyle hydrolagi* sclerite of sucker complexes 3 (**A**), and hamulus (B). Scale bars = 300µm and 60µm respectively.

length 319, n = 1; maximum width 86, n = 1; distal bulb length 175, n = 1, and distal bulb width 148, n = 1.

Ovary (sinistral = 1) 754, n = 1, anteriorly lobed, coiled posteriorly, ascending to oviduct, branching to sack-like, reduced seminal receptacle.

Oötype smooth, leading to uterus, dorsal to ovary, ventral to vas deferens. Parallel vaginal ducts with glandulo-muscular distal portion and thin-walled proximal portion. Ventral vaginal pores muscular, lateral to median portion of cirrus. Follicular vitellarium originates posterior to vaginal pores. Excretory pores not observed.

Remarks

Callorhynchocotyle hydrolagi was the first of the genus to be recorded from the holocephalan host family Chimaeridae from *Hydrolagus ogilbyi*. It was the first *Callorhynchocotyle* species to be described with haptoral sclerites all of similar size.

Comparative measurements for *C. hydrolagi* are represented in Table 4.4. Beverley-Burton and Chisholm (1990) indicated that the sucker complex sclerite width of all 3 sucker complex sclerites of *C. hydrolagi* were thicker than those of *C. marplatensis*, *C. callorhynchi* and *C. amatoi*. Although the limitations of the 2 paratypes examined in the present study prevented the collection of a complete set of data for all 3 sucker complex sclerites, complex 3 sucker sclerite measurements from paratype USNPC 080982.00 MT25-15A overlap in width with those of *C. marplatensis* and *C. callorhynchi*. The appearance of being thicker is likely the result of the reduced sucker complex sclerite total and inner diameters in *C. hydrolagi* which are narrower than those of *C. marplatensis*, *C. callorhynchi* and *C. amatoi*. Additionally, total and shaft lengths and hook-side curve length are shorter than those of *C. callorhynchi* and *C. marplatensis*. *Callorhynchocotyle hydrolagi* can be distinguished from *C. marplatensis* by the presence of papillae in the oral and haptoral sclerite suckers, but from *C. marplatensis*, *C. callorhynchi* and *C. amatoi* by the comparatively thick cirrus, similarity in sclerite sizes of all 3 complex sucker sclerite pairs and the unique shape of the hamulus.

The hamulus of *C. hydrolagi* is greater in total length, total width, hook shank length and base width, yet the hook point length is less than those of *C. marplatensis*, *C. callorhynchi* and *C. amatoi*. Hamulus hook shank base width is wider than that of *C. amatoi*. The distal hook point width is wider than those of *C. callorhynchi* and *C. amatoi*.

The outer root-shaft length is shorter than those of *C. callorhynchi* and *C. amato*i, but the inner root-shaft is longer than that of *C. callorhynchi* yet shorter than that of *C. marplatensis*. The root base angle is more acute than that of *C. callorhynchi*.

Table 4.4 Comparative measurements for *Callorhynchocotyle hydrolagi* Beverley-Burton and Chisholm, 1990

	Beverley-Burton and Chisholm (1990)	**Present study[1]**
Total body length	4149 (3283–4702, n = 24)	**4450 ± 212.1(4300–4600, n = 2)**
Maximum body width	1061 (579–1349, n = 24)	**897 ± 78.6(841–952, n = 2)**
Oral sucker diameter	271 (202–307, n = 20)	**922 ± 397.7(340–903, n = 2)**
Pharynx length	89 (79–104, n = 13)	**85 ± 1.4(84–85, n = 2)**
Pharynx width	59 (51–66, n = 13)	**63 ± 10.4(55–70, n = 2)**
Haptor length	2260 (1666–2672, n = 20)	**1990 ± 70.7(1940–2040, n = 2)**
Haptor width	934 (656–1206, n = 20)	**1076 ± 175.1(952–1200, n = 2)**
Appendix length	767 (657–948, n = 13)	**739, n = 1**
Appendix width	327 (237–410, n = 13)	**320, n = 1**
Terminal appendix sucker length	330 (263–470, n = 13)	**Not measured**
Terminal appendix sucker width	-	**Not measured**
No. of testes	88 (61–104, n = 6)	**65, n = 1**
Testes width	65 (48–87)	**57 ± 5.6(48–66, n = 10)**
Cirrus total length	-	**319, n = 1**
Cirrus maximum width	-	**86, n =1**
Distal bulb length	-	**175, n = 1**
Distal bulb width	-	**148, n = 1**
Ovary length	636 (531–755, n = 14)	**754, n = 1**
Egg length	155 (147–165, n = 17)	**Not measured**
Egg width	81 (76–85, n = 17)	**Not measured**
Complex 1 sclerite		
Circumferential length	-	**Not measured**
Total length	381 (238–450, n = 17)	**Not measured**
Shaft length	39 (26–46, n = 13)	**Not measured**
Hook-side curve length	-	**Not measured**
Shaft-side curve length	-	**Not measured**
Total diameter	-	**Not measured**
Inner diameter	-	**Not measured**
Aperture angle	-	**Not measured**
Aperture	-	**Not measured**
Width	-	**Not measured**
Hook length	44 (31–59, n = 13)	**Not measured**

Table 4.4 cont

Hook curve length	-	**Not measured**
Hook aperture angle	-	**Not measured**
Hook aperture	-	**Not measured**
Hook base width	-	**Not measured**
Complex 2 sclerite		
Circumferential length	-	**Not measured**
Total length	488 (350–549, n = 8)	**Not measured**
Shaft length	98 (81–117, n = 7)	**Not measured**
Hook-side curve length	-	**Not measured**
Shaft-side curve length	-	**Not measured**
Total diameter	-	**Not measured**
Inner diameter	-	**Not measured**
Aperture angle	-	**Not measured**
Aperture	-	**Not measured**
Width	-	**Not measured**
Hook length	133 (105–162, n = 7)	**Not measured**
Hook curve length	-	**Not measured**
Hook aperture angle	-	**Not measured**
Hook aperture	-	**Not measured**
Hook base width	-	**Not measured**
Complex 3 sclerite		
Circumferential length	-	**1048, n = 1**
Total length	435 (356–488, n = 11)	**450 ± 6.5(445–454, n = 2)**
Shaft length	88 (56–122, n = 10)*	**452 ± 7.6(446–457, n = 2)**
Hook-side curve length	-	**90 ± 8.4(84–96, n = 2)**
Shaft-side curve length	-	**95 ± 3.4(92–97, n = 2)**
Total diameter	-	**303 ± 7.4(298–308, n = 2)**
Inner diameter	-	**234 ± 7.7(228–239, n = 2)**
Aperture angle	-	**56° ± 6.7(51°–61°, n = 2)**
Aperture	-	**81 ± 10.8(74–89, n = 2)**
Width	-	**73 ± 0.2(72–73, n = 2)**
Hook length	87 (63–99, n = 8)*	**114 ± 10.4(106–121, n = 2)**
Hook curve length	-	**19 ± 0.8(18–19, n = 2)**
Hook aperture angle	-	**117° ± 16.4(106°–129°, n = 2)**
Hook aperture	-	**81 ± 10.8(74–89, n = 2)**
Hook base width	-	**34 ± 1.9(32–35, n = 2)**
Hamulus total length	58 (52–66, n = 13)	**67, n = 1**
Hook point length	-	**13, n = 1**
Hook shank length	-	**30, n = 1**
Total width	-	**37, n = 1**
Distal hook point width	-	**3, n = 1**
Outer aperture angle	-	**30°, n = 1**
Inner aperture angle	-	**58°, n = 1**
Aperture	-	**73, n = 1**

Table 4.4 cont

Hook shank base width	-	**7, n = 1**
Outer root-shaft length	-	**40, n = 1**
Inner root-shaft length	-	**42, n = 1**
Root base width	30(23–38, n = 12)	**33, n = 1**
Root base angle	-	**100°, n = 1**

[1]**Bold script =** all measurements of the present study: vouchers USNPC 080982.00 Paratypes MT25-15A and B.
*Note that sucker sclerite shaft and hook lengths are measured differently in the present study to those of Beverley-Burton and Chisholm (1990) who followed the measurement protocol of Boeger *et al.* (1989). The shaft measurement reflects ambiguity in the terminology of the structure between Wiskin (1970) and Boeger *et al.* (1989). Sclerite structures *sensu* Wiskin (1970) are followed in the present study and as a result are reflected in the measurements.

4.2.6 Callorhynchocotyle sagamiensis Kitamura, Ogawa, Taniuchi and Hirose, 2006

Type host: Chimaera phantasma Jordan and Snyder, 1900 (Chimaeridae, Holocephali).

Type locality: Off Odawara, Sagami Bay, Kanagawa Pref. (35°15'N, 139°15'E), Japan.

Additional localities: Off Enoshima, Sagami Bay, Kanagawa Pref. (35°15'N, 139°30'E), Japan; Tokyo Bay, Japan.

Site on host: Gills.

Material examined: Vouchers SAMCTA 29468 (1 whole mount and 1 haptor digest)

Redescription (Figs. 4.11 and 4.12, Table 4.5)

Total body length (Fig. 4.11A) 4533, n = 1, maximum body width 1111, n = 1. Oral sucker internally papillate, diameter 417, n = 1. Pharynx 78, n = 1 long, 67, n = 1 wide. Branched intestinal caeca unite after testes and extend into the haptor. Asymmetrical haptor 2300, n = 1 long, 1143, n = 1 wide with 3 paired sucker sclerite complexes. Haptoral suckers papillate.

Sclerites of sucker complex 1 (Fig. 4.12A) similar in size to complex 2 and 3 with circumferential length 1217 ± 31.64(1195–1239, n = 2); total length 476 ± 27.02(457–495, n = 2); total diameter 365 ± 44.84(333–396, n = 2); width 83 ± 10.82(75–90, n = 2); shaft length 478 ± 27.17(458–497, n = 2); inner diameter 285 ± 35.34(260–310, n = 2); aperture angle 45°, n = 2; aperture 241 ± 24.64(223–258, n = 2); hook-side curve length 101 ± 15.08(90–111, n = 2) and shaft-side curve length 101 ± 1.36(100–102, n = 2).

Complex 1 sucker sclerite hook length 152 ± 3.74(149–155, n = 2); hook curve length 14 ± 3.51(11–16, n = 2); aperture angle 127° ± 9.89(120°–134°, n = 2); aperture 124 ± 2.23(122–126, n = 2) and base-width 43, n = 2.

Sclerites of sucker complex 2 (Fig. 4.12B): circumferential length 1174 ± 61.37(1130–1217, n = 2); total length 505 ± 19.00(491–518, n = 2); total diameter 359 ± 29.99(338–381, n = 2); width 85 ± 12.12(77–94, n = 2); shaft length 502 ± 18.07(490–515, n = 2); inner diameter 276 ± 19.89(262–290, n = 2); aperture angle 47° ± 6.66(43°–52°, n = 2); aperture 260 ± 51.37(223–296, n = 2); hook-side curve length 99 ± 12.01(90–107, n = 2) and shaft-side curve length 120 ± 21.98(104–135, n = 2). Complex 2 sucker sclerite hook length 150 ± 12.37(142–159, n = 2); hook curve length 19 ± 3.18(17–21, n = 2); aperture angle 124° ± 9.42(117°–131°, n = 2); aperture 117 ± 12.71(108–126, n = 2) and base width 44 ± 1.65(43–45, n = 2).

Sclerites of sucker complex 3 (Fig. 4.12C): circumferential length 1234 ± 27.59(1215–1254, n = 2); total length 495 ± 31.48(473–517, n = 2); total diameter 362 ± 38.31(335–389, n = 2); width 90 ± 4.42(87–93, n = 2); shaft length 494 ± 33.62(470–518, n = 2); inner diameter 274 ± 36.82(248–300, n = 2); aperture angle 48° ± 7.97(43°–54°, n = 2); aperture 257 ± 71.27(207–307, n = 2); hook-side curve length 97 ± 7.45(92–103, n = 2) and shaft-side curve length 120 ± 25.39(102–138, n = 2). Complex 3 sucker sclerite hook length 151 ± 13.59(141–161, n = 2); hook curve length 17 ± 3.54(15–20, n = 2); aperture angle 124° ± 6.99(119°–129°, n = 2); aperture 120 ± 10.72(112–127, n = 2) and base width 46 ± 4.16(43–49, n = 2).

Dorsal haptoral appendix 942, n = 1 long, 368, n = 1 wide. Terminal suckers of appendix 260 ± 8.34(254–266, n = 2) long, 139 ± 0.45(149–150, n = 2) wide. Single pair of hamuli present before terminal suckers.

Hamulus (Fig. 4.12D) total length 65 ± 2.00(64–66, n = 2); hook point length 22 ± 3.89(20–25, n = 2); hook shank length 20, n = 2; total width 30 ± 2.59(28–32, n = 2); distal hook point width 3, n = 2; outer aperture angle 20° ± 0.74(20°–21°, n = 2); inner aperture angle 67° ± 0.87(67°–68°, n = 2); aperture 79 ± 2.01(78–81, n = 2); hook shank base width 8 ± 2.64(6–10, n = 2); inner root-shaft length 44 ± 8.29(38–50, n = 2); outer root-shaft length 47 ± 7.97(41–53, n = 2); root base angle 96° ± 7.47(91°–101°, n = 2), and root base width 23 ± 2.62(22–25, n = 2).

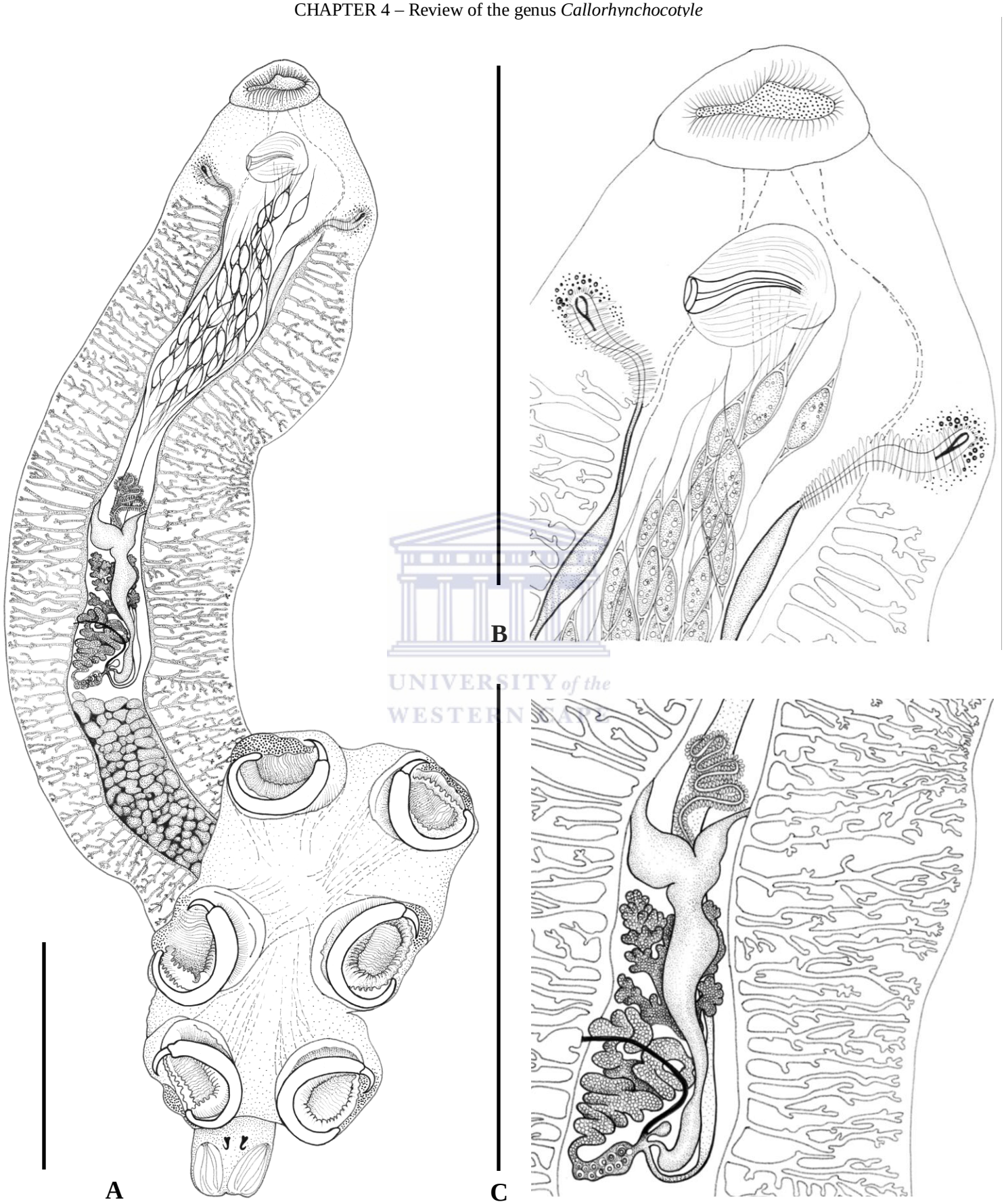

Fig. 4.11 *Callorhynchocotyle sagamiensis.* **A.** Whole mount; **B.** Enlarged anterior section of whole mount; **C.** Enlarged mid-section of whole mount. Scale bars = 1000µm.

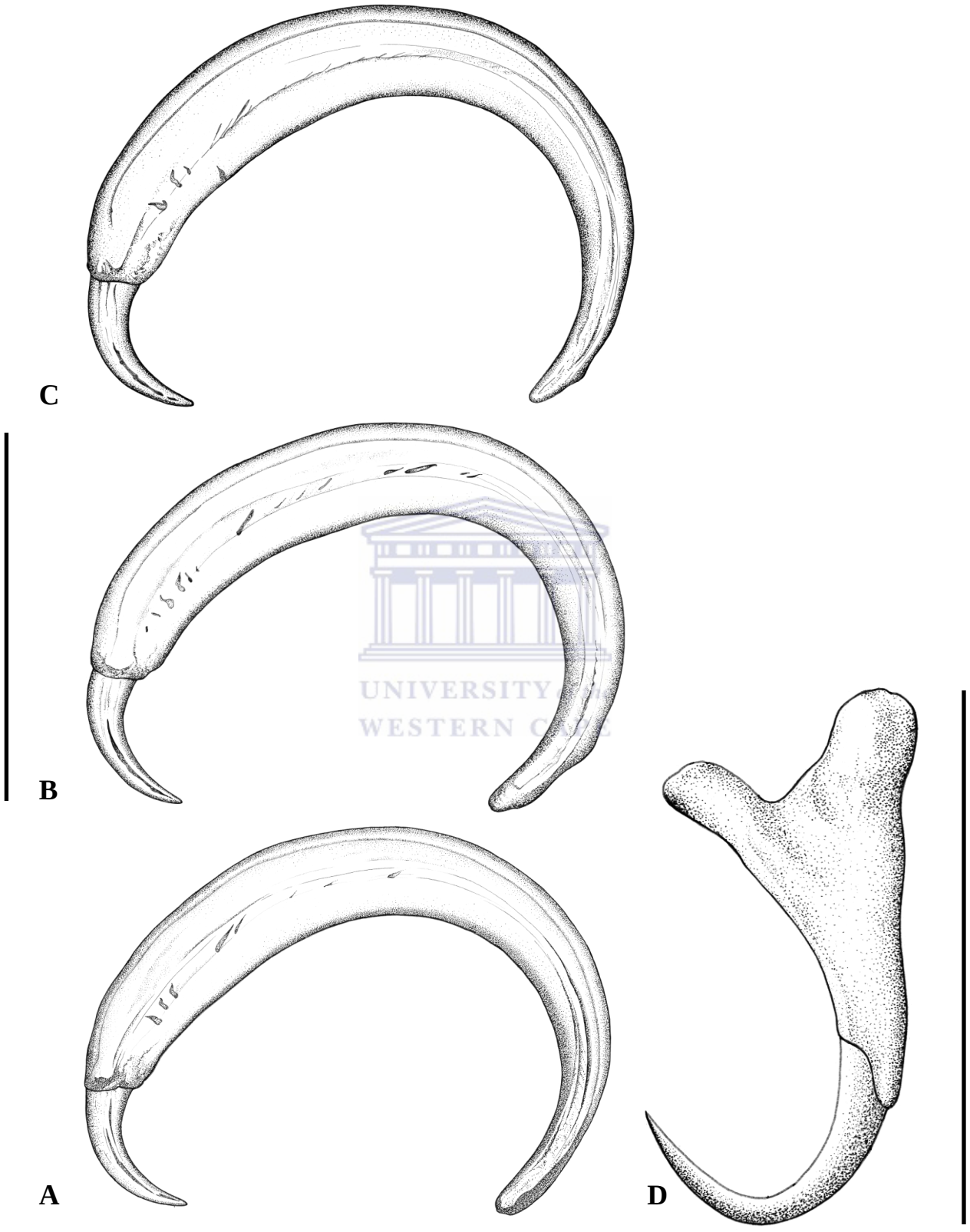

Fig. 4.12 *Callorhynchocotyle sagamiensis* sclerites of sucker complexes 1 (**A**), 2 (**B**) and 3(**C**), and hamulus (**D**). Scale bars = 360µm and 60µm respectively.

Testes irregular in shape, 54, n = 1 in number; 62 ± 5.90(52–70, n = 10) wide. Vas deferens sinuous, surrounded by small gland cells along the majority of its length (Fig. 4.11B). Vas deferens loop proximal to entrance into cirrus, absent. Unarmed muscular cirrus total length 232, n = 1; maximum width 228, n =1.

Ovary (dextral = 1) 678, n = 1 long, anteriorly lobed, coiled posteriorly, ascending to oviduct, branching to sack-like, reduced seminal receptacle (Fig. 4.11C). Oötype smooth, leading to uterus, dorsal to ovary, ventral to vas deferens. Ovate eggs chain-linked by tendrils at each pole. Eggs (*in utero*) 146 ± 5.60(133–149, n = 10) long, 66 ± 2.73(62–69, n = 10) wide. Parallel vaginal ducts with heavily glandulo-muscular distal portion and thin-walled proximal portion. Ventral vaginal pores muscular, lateral to median portion of cirrus. Follicular vitellarium originates posterior to vaginal pores. Excretory pores not observed.

Remarks

Comparative measurements for *C. sagamiensis* are represented in Table 4.5. The voucher whole mount examined in this study (SAMCTA 29468) is comparatively shorter in most soft-body structures which is the result of post flat-fixation after preservation in absolute alcohol prior to shipment to South Africa from Japan. In addition, the voucher is fixed in such a way that the haptor appears symmetrical. This artefact is primarily the result of post flat-fixation and should not be regarded as the exception (Fig. 4.11A). All *Callorhynchocotyle* species possess asymmetrical haptors. Both vouchers agree with the original description in hard characters. However, both outer and inner hamulus root structures have been measured differently, although Kitamura *et al*. (2006) did not indicate how these structures were measured.

Callorhynchocotyle sagamiensis is the most recent addition to the genus and the second *Callorhynchocotyle* species reported from a member of the holocephalan family Chimaeridae. Similar to *C. hydrolagi*, *C. sagamiensis* also possesses haptoral sclerites of similar size throughout all 3 sucker sclerite complexes.

The complex 1 sucker sclerites of *C. sagamiensis* are longer in total, shaft and hook-side curve lengths and wider in total and inner diameter and sclerite widths than those of *C. marplatensis*, *C. callorhynchi* and *C. amatoi*. The complex 1 sucker sclerite

hook length is longer, and the base width wider than those of *C. marplatensis*, *C. callorhynchi* and *C. amatoi*.

The total length of *C. sagamiensis* complex 2 sucker sclerites is shorter than the total length of those of *C. marplatensis*, but the sclerite width is wider than that of both *C. marplatensis* and *C. callorhynchi*. The shaft length is longer than that of *C. amatoi*.

The hook length of complex 2 sucker sclerites is longer than that of *C. amatoi*. The base width is wider than the complex 2 sucker sclerite base widths of *C. marplatensis*, *C. callorhynchi* and *C. amatoi*.

Complex 3 sucker sclerites of *C. sagamiensis* are longer in total length and wider in total width and inner sclerite diameter, than those of *C. hydrolagi*. The shaft length is greater than both that of *C. amatoi* and *C. hydrolagi*, and the hook-side curve length is shorter than that of *C. callorhynchi* and *C. marplatensis*, but longer than that of *C. amatoi* and *C. hydrolagi*.

The complex 3 sucker sclerite hook length is shorter than that of *C. callorhynchi* and *C. marplatensis*, but longer and wider than that of *C. hydrolagi*. The aperture angle is more obtuse than that of both *C. marplatensis* and *C. callorhynchi*.

The hamulus total and hook shank lengths are longer than that of *C. amatoi* and *C. marplatensis*, but less than that of *C. hydrolagi*. Total width is narrower than that of *C. hydrolagi*, and the distal hook point width is wider than that of *C. amatoi*. The outer root-shaft length is longer than that of *C. hydrolagi*.

Callorhynchocotyle sagamiensis can be differentiated from *C. marplatensis* by the presence of papillae in the oral as well as haptoral sclerite suckers, and from *C. callorhynchi* and *C. amatoi* by the similarity in size of all 3 sucker complex sclerites, where those of the former (and *C. marplatensis*) include smaller complex 1 sucker sclerites. *Callorhynchocotyle sagamiensis* is most similar to *C. hydrolagi*, but differs in complex 3 sucker sclerite, hamulus, and cirrus morphology.

The cirrus in *C. sagamiensis* is currently unique amongst the *Callorhynchocotyle* species and serves as a discriminating character as identified by Kitamuro *et al.* (2006). It consists of a single muscular tube not differentiated into the proximal and ovate (bulbous) distal portions of the generic diagnosis of Boeger *et al.* (1989). Additionally, *C. sagamiensis* differs from all of the other *Callorhynchocotyle* species in possessing the widest sclerites of the third sucker complex.

Table 4.5 Comparative measurements for *Callorhynchocotyle sagamiensis* Kitamura, Ogawa, Taniuchi and Hirose, 2006

	Kitamura *et al*. (2006)	**Present study[1] (additional vouchers)**
Total body length	10190 (7200–12100, n = 18)	**4533, n = 1**
Maximum body width	1700 (1400–2000, n = 18)	**1111, n = 1**
Oral sucker diameter	259 (160–340, n = 17)	**417, n = 1**
Pharynx length	96 (70–110, n = 15)	**78, n = 1**
Pharynx width	95 (70–110, n = 15)	**67, n = 1**
Haptor length	4290 (2500–6300, n = 18)	**2300, n = 1**
Haptor width	2320 (1900–2800, n = 18)	**1143, n = 1**
Appendix length	1340 (850–2000, n = 18)	**942, n = 1**
Appendix width	369 (280–450, n = 18)	**368, n = 1**
Terminal appendix sucker length	238 (150–320, n = 16)	**260 ± 8.3(254–266, n = 2)**
Terminal appendix sucker width	246 (170–350, n = 16)	**139 ± 0.5(149–150, n = 2)**
No. of testes	58.1 (52–66, n = 17)	**54, n = 1**
Testes width	-	**62 ± 5.9(52–70, n = 10)**
Cirrus total length	1140 (920–1280, n = 16)	**232, n = 1**
Cirrus maximum width	274 (200–350, n = 17)	**228, n =1**
Ovary length	-	**678, n = 1**
Egg length	147 (110–160, n = 17)	**146 ± 5.6(133–149, n = 10)**
Egg width	65 (50–80, n = 17)	**66 ± 2.7(62–69, n = 10)**
Complex 1 sclerite		
Circumferential length	-	**1217 ± 31.6(1195–1239, n = 2)**
Total length	471 (440–530, n = 32)	**476 ± 27.0(457–495, n = 2)**
Shaft length	-	**478 ± 27.1(458–497, n = 2)**
Hook-side curve length	-	**101 ± 15.0(90–111, n = 2)**
Shaft-side curve length	-	**101 ± 1.3(100–102, n = 2)**
Total diameter	-	**365 ± 44.8(333–396, n = 2)**
Inner diameter	-	**285 ± 35.3(260–310, n = 2)**
Aperture angle	-	**45°, n = 2**
Aperture	-	**241 ± 24.6(223–258, n = 2)**
Width	-	**83 ± 10.8(75–90, n = 2)**
Hook length	-	**152 ± 3.7(149–155, n = 2)**
Hook curve length	-	**14 ± 3.5(11–16, n = 2)**
Hook aperture angle	-	**127° ± 9.8(120°–134°, n = 2)**
Hook aperture	-	**124 ± 2.2(122–126, n = 2)**
Hook base width	-	**43, n = 2**
Complex 2 sclerite		
Circumferential length	-	**1174 ± 61.3(1130–1217, n = 2)**

Table 4.5 cont

Total length	499 (460–560, n = 32)	**505 ± 19.0(491–518, n = 2)**
Shaft length	-	**502 ± 18.0(490–515, n = 2)**
Hook-side curve length	-	**99 ± 12.0(90–107, n = 2)**
Shaft-side curve length	-	**120 ± 21.9(104–135, n = 2)**
Total diameter	-	**359 ± 29.9(338–381, n = 2)**
Inner diameter	-	**276 ± 19.8(262–290, n = 2)**
Aperture angle	-	**47° ± 6.6(43°–52°, n = 2)**
Aperture	-	**260 ± 51.3(223–296, n = 2)**
Width	-	**85 ± 12.1(77–94, n = 2)**
Hook length	-	**150 ± 12.3(142–159, n = 2)**
Hook curve length	-	**19 ± 3.1(17–21, n = 2)**
Hook aperture angle	-	**124° ± 9.4(117°–131°, n = 2)**
Hook aperture	-	**117 ± 12.7(108–126, n = 2)**
Hook base width	-	**44 ± 1.6(43–45, n = 2)**
Complex 3 sclerite		
Circumferential length	-	**1234 ± 27.5(1215–1254, n = 2)**
Total length	494 (440–550, n = 32)	**495 ± 31.4(473–517, n = 2)**
Shaft length	-	**494 ± 33.6(470–518, n = 2)**
Hook-side curve length	-	**97 ± 7.4(92–103, n = 2)**
Shaft-side curve length	-	**120 ± 25.3(102–138, n = 2)**
Total diameter	-	**362 ± 38.3(335–389, n = 2)**
Inner diameter	-	**274 ± 36.8(248–300, n = 2)**
Aperture angle	-	**48° ± 7.9(43°–54°, n = 2)**
Aperture	-	**257 ± 71.2(207–307, n = 2)**
Width	-	**90 ± 4.4(87–93, n = 2)**
Hook length	-	**151 ± 13.5(141–161, n = 2)**
Hook curve length	-	**17 ± 3.5(15–20, n = 2)**
Hook aperture angle	-	**124° ± 6.9(119°–129°, n = 2)**
Hook aperture	-	**120 ± 10.7(112–127, n = 2)**
Hook base width	-	**46 ± 4.1(43–49, n = 2)**
Hamulus total length	62.4 (56–65, n = 16)	**65 ± 2.0(64–66, n = 2)**
Hook point length	16.8 (13–20, n = 16)	**22 ± 3.8(20–25, n = 2)**
Hook shank length	-	**20, n = 2**
Total width	-	**30 ± 2.5(28–32, n = 2)**
Distal hook point width	-	**3, n = 2**
Outer aperture angle	-	**20° ± 0.7(20°–21°, n = 2)**
Inner aperture angle	-	**67° ± 0.8(67°–68°, n = 2)**
Aperture	-	**79 ± 2.0(78–81, n = 2)**
Hook shank base width	-	**8 ± 2.6(6–10, n = 2)**
Outer root-shaft length	11.8 (8–16, n = 16)	**47 ± 7.9(41–53, n = 2)**
Inner root-shaft length	9.8 (6–15, n = 16)	**44 ± 8.2(38–50, n = 2)**
Root base width	-	**23 ± 2.6(22–25, n = 2)**
Root base angle	-	**96° ± 7.4(91°–101°, n = 2)**

[1]**Bold script =** all measurements of the present study: vouchers 08112001 (haptor digest) and 08112002 (whole mount).

4.3 Discussion

The measurements of all *Callorhynchocotyle* species for the present study are compared in Table 4. 6. Sucker complex sclerite and hamulus image overlays are presented in Figs. 4.13–4.21.

Manter (1955) described *S. callorhynchi* Manter, 1955 (junior synonym for *C. callorhynchi*) from a donation of specimens from the University of Cape Town collected from the holocephalan host *Callorhinchus capensis* from South Africa and included this material together with that collected from *C. milii* off New Zealand. The generic designation of this species was based on both Sproston (1946) and Brinkmann (1952). These latter authors had previously disagreed with the synonymy of *Squalonchocotyle* Cerfontaine, 1899 with *Erpocotyle* Beneden and Hesse, 1863 by Price (1942) on the basis of "circumstantial evidence." However, Manter (1955) did indicate that the generic name *Squalonchocotyle* was disputable since *Erpocotyle* pre-dated it by 36 years and that it was likely that *Erpocotyle* might eventually be upheld as valid.

Squalonchocotyle callorhynchi was subsequently redescribed by Dillon and Hargis (1968) as *E. callorhynchi* from new material collected off the South Island of New Zealand from *Callorhinchus milii*, and was also reported by Lebedev and Parukhin (1969) from *Callorhinchus capensis* off Walvis Bay, South West Africa (Namibia). Five years after Lebedev and Parukhin (1969), Euzet and Maillard (1974) separated the synonymised genera *Erpocotyle* and *Squalonchocotyle* based on the presence of large parallel rows of cells lining the oötype in the latter genus.

Suriano and Incorvaia (1982) erected the new genus *Callorhynchocotyle* for *C. marplatensis* from the gills of *Callorhinchus callorhynchus* after *S. callorhynchi* was reported from the same host off Pategonia by Kuznetsova (1970). Although Suriano and Incorvaia (1982) did not refer to the above report or to that of Dillon and Hargis (1868) they disagreed with the generic designation of *S. callorhynchi* of Manter (1955).

Suriano and Incorvaia (1982) differentiated *Callorhynchocotyle* from *Squalonchocotyle* on the basis of the former's smooth oötype. They subsequently differentiated the genus from 3 genera of closest taxonomic similarity *sensu* Euzet and Maillard (1974). *Callorhynchocotyle* was separated from *Heteronchocotyle* Brooks, 1934; *Epicotyle* Euzet and Maillard, 1974 and *Neonchocotyle* Kitary and Maillard, 1972. It was separated by the morphology of the seminal receptacle, dissimilarity between the

first complex pair and the second and third complex pairs of sucker sclerites and morphology of the cirrus complex, position of vaginal pores and presence of oncomiracidial anterior rostrum respectively (Suriano and Incorvaia 1982). The position of the vaginal pores were noted as located "ventro-laterally on the body walls" and this difference was also used as a genus-specific feature, although it has subsequently been indicated by Boeger *et al*. (1989) as incorrect and is confirmed as incorrect in the present study.

Boeger *et al.* (1989) in their revision of *Callorhynchocotyle* and shortly thereafter in the revision of Hexabothriidae (Boeger and Kritsky 1989), differentiated *Callorhynchocotyle* from all other hexabothriid genera by the combined presence of the extensive glandular region of the vas deferens, bulbous distal cirrus, dorsal haptoral appendix, and glandulo-muscular distal portion of the vaginae.

Boeger *et al*. (1989) redescribed *S. callorhynci* and transferred it to *Callorhynchocotyle*. In addition they separated a new species *C. amatoi* Boeger *et al*., 1989 from the South African material of *C. callorhynchi* based on the shorter shafts and hooks of all 3 sucker complex sclerites of all the material from *C. milii* from New Zealand.

Suriano and Incorvaia (1982) measured the total length of *C. marplatensis* excluding the haptor which was measured separately. Boeger *et al.* (1989) included the haptor into the total length of *C. marplatensis, C. callorhynchi* and *C. amatoi*. Beverley-Burton and Chisholm (1990) and Kitamura *et al.* (2006) excluded the haptor from the total body length measurements. Beverley-Burton and Chisholm (1990) cautioned against its inclusion since its orientation influenced the total length measurement. In the present study, total body length is measured exclusive of the haptor *sensu* Beverley-Burton and Chisholm (1990) and it should be noted that the measurement of the haptor along its true longitudinal axis *sensu* Boeger and Kritsky (1989) cannot be made if the haptor is included in the total length measurement in *Callorhynchocotyle*. The haptor of *Callorhynchocotyle* species is asymmetrical and the longitudinal haptoral axis described in Boeger and Kritsky (1989) falls at an angle to the longitudinal axis of the body proper.

Beverley-Burton and Chisholm (1990) indicated that the hamulus measurements of Boeger *et al.* (1989) for *C. marplatensis, C. callorhynchi* and *C. amatoi* were incorrect. These errors are confirmed in the present study as presented comparatively in Tables 4.1,

4.2 and 4.3. However, the appendix width measurement for *C. amatoi* in the original description of Boeger *et al.* (1989) is also incorrect (see table 4.3), as is the cirrus length measurement for *C. callorhynchi* in Beverley-Burton and Chisholm (1990) (*pers. comm*. Leslie A. Chisholm). The measurements of total length of all three sucker complex sclerites for *C. marplatensis* of Suriano and Incorvaia (1982) as well as the total length of the hamulus do not appear to be accurate.

Sclerite shaft measurements for all 3 sucker complex sclerites of *C. marplatensis*, *C. callorhynchi* and *C. amatoi* as measured in this study and that of the existing literature reflects the ambiguity in descriptive nomenclature. Wiskin (1970) was the first to identify and separate hexabothriid sclerites into hook and shaft. Subsequently, the present study follows this example. As a result, the interpretation of the sclerite shaft of Boeger *et al*. (1989), subsequently adopted by Beverley-Burton and Chisholm (1990), is disputed and the respective measurements of the hook and shaft re-measured in this study follow the priority given to the structures identified by Wiskin (1970) (see Chapters 1 and 2).

The generic diagnosis of *Callorhynchocotyle* is amended to re-include the unciliated, blind oncomiracidium with 10 marginal hooklets (Suriano and Incorvaia 1982) which was previously excluded from the revision of the genus by Boeger *et al.* (1989). *Callorhynchocotyle* oncomiracidia were not included by Glennon *et al.* (2005) in discussing all prior hexabothriid larval descriptions in comparison to the larval description of *Branchotenthes octohamatus* Glennon, Chisholm and Whittington, 2005.

Table 4.6 Comparative measurements for all *Callorhynchocotyle* species measured in the present study

	C. marplatensis	***C. callorhynchi****	***C. amatoi****	***C. hydrolagi***	***C. sagamiensis****
Total body length	**9750 ± 1021.4(8300–10600, n = 4)**	**6885 ± 1214.6(5500–11100, n = 24)**	**6892 ± 359.1(6625–7300, n = 3)**	**4450 ± 212.1(4300–4600, n = 2)**	**4533, n = 1**
Maximum body width	**917 ± 171.6(683–1095, n = 4)**	**1700 ± 161.7(1425–2100, n = 24)**	**1349 ± 114.5(1254–1476, n = 3)**	**897 ± 78.6(841–952, n = 2)**	**1111, n = 1**
Oral sucker diameter	**335 ± 36.6(290 – 365, n = 4)**	**360 ± 34.3(269–411, n = 24)**	**366 ± 35.6(338–406, n = 3)**	**922 ± 397.7(340–903, n = 2)**	**417, n = 1**
Pharynx length	**75 ± 5.8(67–81, n = 4)**	**91 ± 9.7(68–113, n = 24)**	**109 ± 9.6(103–120, n = 3)**	**85 ± 1.4(84–85, n = 2)**	**78, n = 1**
Pharynx width	**79 ± 5.3(72–84, n = 4)**	**89 ± 7.1(75–100, n = 24)**	**91 ± 4.0(88–96, n = 3)**	**63 ± 10.4(55–70, n = 2)**	**67, n = 1**

Table 4.6 cont	***C. marplatensis***	***C. callorhynchi****	***C. amatoi****	***C. hydrolagi***	***C. sagamiensis****
Haptor length	**2930 ± 995.8(1933–3920, n = 4)**	**2494 ± 362.9(2025–3640, n = 24)**	**2750 ± 534.1(2200–3267, n = 3)**	**1990 ± 70.7(1940–2040, n = 2)**	**2300, n = 1**
Haptor width	**1225 ± 646.5(277–1640, n = 4)**	**1595 ± 169.8(1275–1960, n = 24)**	**1138 ± 282.3(937–1460, n = 3)**	**1076 ± 175.1(952–1200, n = 2)**	**1143, n = 1**
Appendix length	**1655 ± 124.7(1514–1773, n = 4)**	**1223 ± 183.7(918–1668, n = 20)**	**1016 ± 223.9(810–1254, n = 3)**	**739, n = 1**	**942, n = 1**
Appendix width	**286 ± 48.4(223 – 337, n = 4)**	**423 ± 62.5(248–509, n = 23)**	**434 ± 18.3(413 – 444, n = 3)**	**320, n = 1**	**368, n = 1**
Terminal appendix sucker length	**232 ± 28.5(193–264, n = 6)**	**293 ± 30.9(243–364, n = 44)**	**287 ± 28.1(257–330, n = 6)**	**Not measured**	**260 ± 8.3(254–266, n = 2)**
Terminal appendix sucker width	**142 ± 20.1(120–167, n = 6)**	**146 ± 15.7(118–188, n = 44)**	**142 ± 6.5(135–154, n = 3)**	**Not measured**	**139 ± 0.5(149–150, n = 2)**
No. of testes	**83 ± 18.7(65–107, n = 4)**	**88 ± 12.5(57–111, n = 22)**	**103 ± 7.8(94–109, n = 8)**	**65, n = 1**	**54, n = 1**
Testes width	**95 ± 9.2(83–109, n = 10)**	**82 ± 13.0(62–109, n = 18)**	**86 ± 9.9(74–103, n = 17)**	**57 ± 5.6(48–66, n = 10)**	**62 ± 5.9(52–70, n = 10)**
Cirrus total length	**410 ± 29.3(374–447, n = 4)**	**472 ± 67.2(356–623, n = 20)**	**429, n = 1**	**319, n = 1**	**232, n = 1**
Cirrus maximum width	**38 ± 4.1(34–44, n = 4)**	**74 ± 7.9(49–85, n = 20)**	**70, n = 1**	**86, n =1**	**228, n =1**
Distal bulb length	**67 ± 2.7(65–70, n = 4)**	**125 ± 21.8(76–175, n = 20)**	**85, n = 1**	**175, n = 1**	**NA**
Distal bulb width	**62 ± 3.1(58–66, n = 4)**	**113 ± 7.9(97–131, n = 20)**	**85, n = 1**	**148, n = 1**	**NA**
Ovary length	**1422 ± 141.9(1265–1530, n = 4)**	**1110 ± 176.2(845–1527, n = 19)**	**763 ± 59.2(694–799, n = 3)**	**754, n = 1**	**678, n = 1**
Egg length	**154 ± 5.3(146–165, n = 13)**	**173 ± 9.9(158–189, n = 9)**	**163 ± 16.1(151–186, n = 4)**	**Not measured**	**146 ± 5.6(133–149, n = 10)**
Egg width	**67 ± 7.6(57–83, n = 13)**	**72 ± 4.1(65–77, n = 9)**	**82 ± 12.6(70–100, n = 4)**	**Not measured**	**66 ± 2.7(62–69, n = 10)**
Complex 1 sclerite					
Circumferential length	**922 ± 30.7(896–966, n = 4)**	**858 ± 44.4(758–953, n = 30)**	**863 ± 27.8(837–892, n = 3)**	**Not measured**	**1217 ± 31.6(1195–1239, n = 2)**
Total length	**418 ± 11.6(401–423, n = 4)**	**381 ± 19.5(347–428, n = 30)**	**401 ± 69.9(323–458, n = 3)**	**Not measured**	**476 ± 27.0(457–495, n = 2)**
Shaft length	**420 ± 9.2(408–427, n = 4)**	**384 ± 19.6(347–432, n = 30)**	**402 ± 66.2(328–456, n = 3)**	**Not measured**	**478 ± 27.1(458–497, n = 2)**
Hook-side curve length	**68 ± 4.0(64–72, n = 4)**	**61 ± 5.7(50–71, n = 30)**	**61 ± 10.3(49–69, n = 3)**	**Not measured**	**101 ± 15.0(90–111, n = 2)**
Shaft-side curve length	**101 ± 14.0(88–114, n = 4)**	**89 ± 11.6(74–111, n = 30)**	**86 ± 20.9(62–99, n = 3)**	**Not measured**	**101 ± 1.3(100–102, n = 2)**
Total diameter	**262 ± 20.1(233–280, n = 4)**	**246 ± 15.0(219–271, n = 30)**	**257 ± 17.0(237–267, n = 3)**	**Not measured**	**365 ± 44.8(333–396, n = 2)**
Inner diameter	**222 ± 15.3(199–232, n = 4)**	**209 ± 14.1(183–235, n = 30)**	**217 ± 21.6(192–232, n = 3)**	**Not measured**	**285 ± 35.3(260–310, n = 2)**

Table 4.6 cont	***C. marplatensis***	***C. callorhynchi****	***C. amatoi****	***C. hydrolagi***	***C. sagamiensis****
Aperture angle	**59° ± 5.3(53°–65°, n = 4)**	**60° ± 5.7(49°–70°, n = 30)**	**64° ± 28.6(34°–92°, n = 3)**	**Not measured**	**45°, n = 2**
Aperture	**270 ± 18.7(256–297, n = 4)**	**253 ± 25.6(199–306, n = 30)**	**47 ± 0.7(46–48, n = 3)**	**Not measured**	**241 ± 24.6(223–258, n = 2)**
Width	**42 ± 5.3(35–47, n = 4)**	**40 ± 4.9(29–49, n = 30)**	**43 ± 4.0(38–46, n = 3)**	**Not measured**	**83 ± 10.8(75–90, n = 2)**
Hook length	**59 ± 4.4(54–64, n = 4)**	**56 ± 4.8(44–65, n = 30)**	**56 ± 0.5(56–57, n = 3)**	**Not measured**	**152 ± 3.7(149–155, n = 2)**
Hook curve length	**15 ± 1.1(13–16, n = 4)**	**12 ± 1.8(7–16, n = 30)**	**10 ± 0.7(10–11, n = 3)**	**Not measured**	**14 ± 3.5(11–16, n = 2)**
Hook aperture angle	**100° ± 5.1(93°–104°, n = 4)**	**105° ± 7.7(91°–124°, n = 30)**	**112° ± 2.6(109°–114°, n = 3)**	**Not measured**	**127° ± 9.8(120°–134°, n = 2)**
Hook aperture	**47 ± 5.3(40–53, n = 4)**	**45 ± 4.9(32–54, n = 30)**	**47 ± 0.7(46–48, n = 3)**	**Not measured**	**124 ± 2.2(122–126, n = 2)**
Hook base width	**16 ± 0.8(15–17, n = 4)**	**14 ± 1.2(11–18, n = 30)**	**15 ± 0.6(15–16, n = 3)**	**Not measured**	**43, n = 2**
Complex 2 sclerite					
Circumferential length	**1244 ± 43.9(1206–1306, n = 4)**	**1191 ± 59.8(1076–1292, n = 30)**	**1111 ± 98.3(998–1172, n = 3)**	**Not measured**	**1174 ± 61.3(1130–1217, n = 2)**
Total length	**541 ± 22.7(511–566, n = 4)**	**525 ± 19.4(484–558, n = 30)**	**451 ± 37.1(410–481, n = 3)**	**Not measured**	**505 ± 19.0(491–518, n = 2)**
Shaft length	**523 ± 26.0(498–559, n = 4)**	**482 ± 19.5(441–520, n = 30)**	**451 ± 35.8(411–479, n = 3)**	**Not measured**	**502 ± 18.0(490–515, n = 2)**
Hook-side curve length	**105 ± 9.5(99–119, n = 4)**	**110 ± 12.4(81–127, n = 30)**	**80 ± 3.6(78–84, n = 3)**	**Not measured**	**99 ± 12.0(90–107, n = 2)**
Shaft-side curve length	**140 ± 9.5(99–119, n = 4)**	**123 ± 13.4(89–145, n = 30)**	**116 ± 20.6(99–139, n = 3)**	**Not measured**	**120 ± 21.9(104–135, n = 2)**
Total diameter	**344 ± 22.8(320–374, n = 4)**	**351 ± 15.7(323–381, n = 30)**	**336 ± 32.4(299–357, n = 3)**	**Not measured**	**359 ± 29.9(338–381, n = 2)**
Inner diameter	**280 ± 14.0(265–299, n = 4)**	**285 ± 13.5(255–310, n = 30)**	**264 ± 28.2(232–282, n = 3)**	**Not measured**	**276 ± 19.8(262–290, n = 2)**
Aperture angle	**54° ± 2.5(51°–57°, n = 4)**	**55° ± 4.4(45°–67°, n = 30)**	**48° ± 15.1(31°–59°, n = 3)**	**Not measured**	**47° ± 6.6(43°–52°, n = 2)**
Aperture	**303 ± 19.0(278–318, n = 4)**	**305 ± 23.7(249 – 348, n = 30)**	**248 ± 97.1(137–311, n = 3)**	**Not measured**	**260 ± 51.3(223–296, n = 2)**
Width	**66 ± 8.2(59–78, n = 4)**	**66 ± 7.6(54–83, n = 30)**	**73 ± 3.7(69–76, n = 3)**	**Not measured**	**85 ± 12.1(77–94, n = 2)**
Hook length	**153 ± 12.0(145–171, n = 4)**	**162 ± 19.2(118–191, n = 30)**	**126 ± 7.7(120–135, n = 3)**	**Not measured**	**150 ± 12.3(142–159, n = 2)**
Hook curve length	**30 ± 3.3(28–35, n = 4)**	**35 ± 4.2(28–41, n = 30)**	**18 ± 0.3(17–18, n = 3)**	**Not measured**	**19 ± 3.1(17–21, n = 2)**
Hook aperture angle	**109° ± 5.4(102°–115°, n = 4)**	**107° ± 6.8(89°–118°, n = 30)**	**120° ± 5.5(113°–123°, n = 3)**	**Not measured**	**124° ± 9.4(117°–131°, n = 2)**
Hook aperture	**121 ± 9.9(110–134, n = 4)**	**132 ± 17.3(93–157, n = 30)**	**102 ± 7.1(96–110, n = 3)**	**Not measured**	**117 ± 12.7(108–126, n = 2)**
Hook base width	**38 ± 3.5(35–43, n = 4)**	**40 ± 3.0(32–46, n = 30)**	**37 ± 3.2(35–41, n = 3)**	**Not measured**	**44 ± 1.6(43–45, n = 2)**
Complex 3 sclerite					
Circumferential length	**1197 ± 42.9(1149–1249, n = 4)**	**1179 ± 57.8(1070–1320, n = 30)**	**1092 ± 96.4(989–1181, n = 3)**	**1048, n = 1**	**1234 ± 27.5(1215–1254, n = 2)**
Total length	**535 ± 27.2(503–562, n = 4)**	**530 ± 20.4(488–585, n = 30)**	**428 ± 12.0(415–436, n = 3)**	**450 ± 6.5(445–454, n = 2)**	**495 ± 31.4(473–517, n = 2)**

Table 4.6 cont	***C. marplatensis***	***C. callorhynchi****	***C. amatoi****	***C. hydrolagi***	***C. sagamiensis****
Shaft length	**506 ± 21.6(480–528, n = 4)**	**476 ± 20.9(431–532, n = 30)**	**421 ± 13.3(407–433, n = 3)**	**452 ± 7.6(446–457, n = 2)**	**494 ± 33.6(470–518, n = 2)**
Hook-side curve length	**116 ± 5.3(113–120, n = 4)**	**113 ± 9.1(96–132, n = 30)**	**77 ± 1.8(75–79, n = 3)**	**90 ± 8.4(84–96, n = 2)**	**97 ± 7.4(92–103, n = 2)**
Shaft-side curve length	**134 ± 6.3(126–141, n = 4)**	**119 ± 12.0(90–140, n = 30)**	**130 ± 10.7(118–138, n = 3)**	**95 ± 3.4(92–97, n = 2)**	**120 ± 25.3(102–138, n = 2)**
Total diameter	**336 ± 17.0(314–355, n = 4)**	**345 ± 16.5(310–374, n = 30)**	**319 ± 15.9(304–336, n = 3)**	**303 ± 7.4(298–308, n = 2)**	**362 ± 38.3(335–389, n = 2)**
Inner diameter	**269 ± 11.1(255–280, n = 4)**	**277 ± 14.9(236–310, n = 30)**	**249 ± 14.2(237–265, n = 3)**	**234 ± 7.7(228–239, n = 2)**	**274 ± 36.8(248–300, n = 2)**
Aperture angle	**56° ± 4.5(50°–61°, n = 4)**	**57° ± 4.33(48°–68°, n = 30)**	**40° ± 5.9(35°–46°, n = 3)**	**56° ± 6.7(51°–61°, n = 2)**	**48° ± 7.9(43°–54°, n = 2)**
Aperture	**302 ± 25.8(278–332, n = 4)**	**311 ± 24.70(257–357, n = 30)**	**191 ± 31.5(158–221, n = 3)**	**81 ± 10.8(74–89, n = 2)**	**257 ± 71.2(207–307, n = 2)**
Width	**69 ± 6.5(60–76, n = 4)**	**70 ± 5.7(61–81, n = 30)**	**71 ± 2.8(68–74, n = 3)**	**73 ± 0.2(72–73, n = 2)**	**90 ± 4.4(87–93, n = 2)**
Hook length	**172 ± 5.3(168–180, n = 4)**	**168 ± 16.2(138–205, n = 30)**	**138 ± 5.2(134–144, n = 3)**	**114 ± 10.4(106–121, n = 2)**	**151 ± 13.5(141–161, n = 2)**
Hook curve length	**30 ± 7.7(23–41, n = 4)**	**37 ± 4.2(31–48, n = 30)**	**18 ± 2.8(15–21, n = 3)**	**19 ± 0.8(18–19, n = 2)**	**17 ± 3.5(15–20, n = 2)**
Hook aperture angle	**110° ± 9.5(96°–118°, n = 4)**	**104° ± 6.4(89°–119°, n = 30)**	**121° ± 4.7(117°–127°, n = 3)**	**117° ± 16.4(106°–129°, n = 2)**	**124° ± 6.9(119°–129°, n = 2)**
Hook aperture	**138 ± 3.7(134–142, n = 4)**	**135 ± 15.5(106–178, n = 30)**	**109 ± 4.4(106–114, n = 3)**	**81 ± 10.8(74–89, n = 2)**	**120 ± 10.7(112–127, n = 2)**
Hook base width	**46 ± 5.0(41–53, n = 4)**	**43 ± 3.7(35–50, n = 30)**	**40 ± 0.4(39–40, n = 3)**	**34 ± 1.9(32–35, n = 2)**	**46 ± 4.1(43–49, n = 2)**
Hamulus total length	**58 ± 3.3(56–61, n = 2)**	**63 ± 2.5(56–67, n = 25)**	**54 ± 1.6(53–56, n = 3)**	**67, n = 1**	**65 ± 2.0(64–66, n = 2)**
Hook point length	**14 ± 0.3, n = 2**	**15 ± 0.8(13–16, n = 25)**	**15 ± 0.3(15–16, n = 3)**	**13, n = 1**	**22 ± 3.8(20–25, n = 2)**
Hook shank length	**17 ± 0.1, n = 2**	**20 ± 1.4(16–22, n = 25)**	**16 ± 0.8(15–17, n = 3)**	**30, n = 1**	**20, n = 2**
Total width	**26 ± 2.1(25–28, n = 2)**	**31 ± 1.3(28–33, n = 25)**	**29 ± 0.8(28–30, n = 3)**	**37, n = 1**	**30 ± 2.5(28–32, n = 2)**
Distal hook point width	**4 ± 0.3, n = 2**	**3 ± 0.3(3–4, n = 25)**	**3 ± 0.2, n = 3**	**3, n = 1**	**3, n = 2**
Outer aperture angle	**17° (n = 2)**	**20° ± 1.3(16°–22°, n = 25)**	**17° ± 0.3(16°–17°, n = 3)**	**30°, n = 1**	**20° ± 0.7(20°–21°, n = 2)**
Inner aperture angle	**67° ± 18.2(54°–80°, n = 2)**	**78° ± 3.7(67°–85°, n = 25)**	**86° ± 3.8(83°–90°, n = 3)**	**58°, n = 1**	**67° ± 0.8(67°–68°, n = 2)**
Aperture	**88 ± 19.6(74–102, n = 2)**	**94 ± 5.6(82–105, n = 25)**	**102 ± 4.5(98–107, n = 3)**	**73, n = 1**	**79 ± 2.0(78–81, n = 2)**
Hook shank base width	**6 ± 0.7, n = 2**	**7 ± 1.0(5–9, n = 25)**	**6 ± 0.6(5–7, n = 3)**	**7, n = 1**	**8 ± 2.6(6–10, n = 2)**
Outer root-shaft length	**41 ± 3.9(38–44, n = 2)**	**47 ± 1.9(43–50, n = 25)**	**44 ± 2.9(41–49, n = 3)**	**40, n = 1**	**47 ± 7.9(41–53, n = 2)**
Inner root-shaft length	**45 ± 1.8(44–46, n = 2)**	**37 ± 2.2(33–43, n = 25)**	**42 ± 1.3(40–44, n = 3)**	**42, n = 1**	**44 ± 8.2(38–50, n = 2)**
Root base width	**23 ± 1.0(23–24, n = 2)**	**26 ± 1.9(22–30, n = 25)**	**23 ± 1.4(20–24, n = 3)**	**33, n = 1**	**23 ± 2.6(22–25, n = 2)**
Root base angle	**105° ± 4.5(102°–108°, n = 2)**	**115° ± 14.1(86°–139°, n = 25)**	**105° ± 10.7(93°–118°, n = 3)**	**100°, n = 1**	**96° ± 7.4(91°–101°, n = 2)**

*Measurements of new vouchers material contributed by the present study.
NA – Not applicable (i.e. no distal bulb in *C. sagamiensis*).

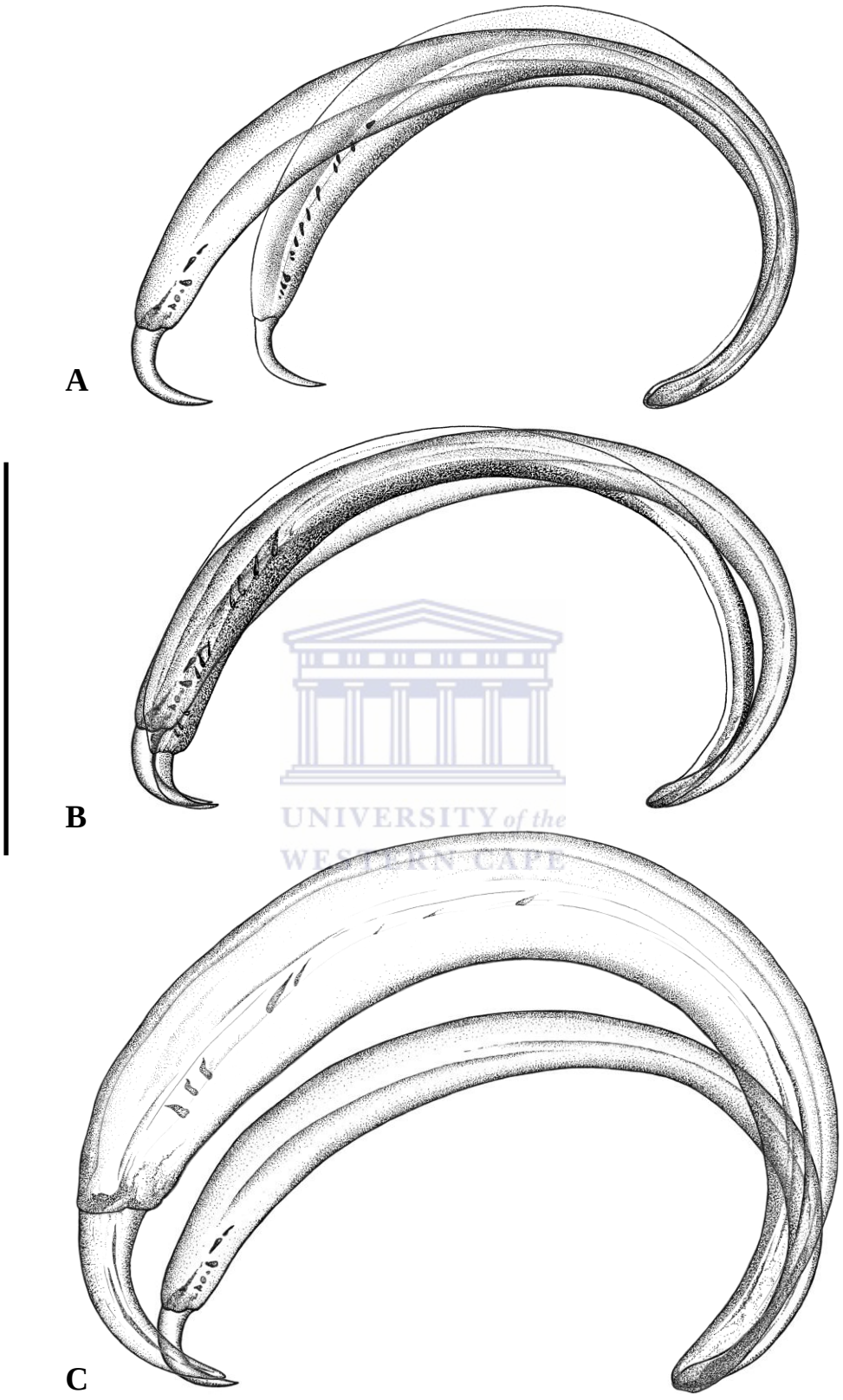

Fig. 4.13 Complex 1 sucker sclerite overlays. **A.** *Callorhynchocotyle marplatensis* and *C. callorhynchi*, **B.** *C. marplatensis* and *C. amatoi*, **C.** *C. marplatensis* and *C. sagamiensis*. Scale bar = 260µm.

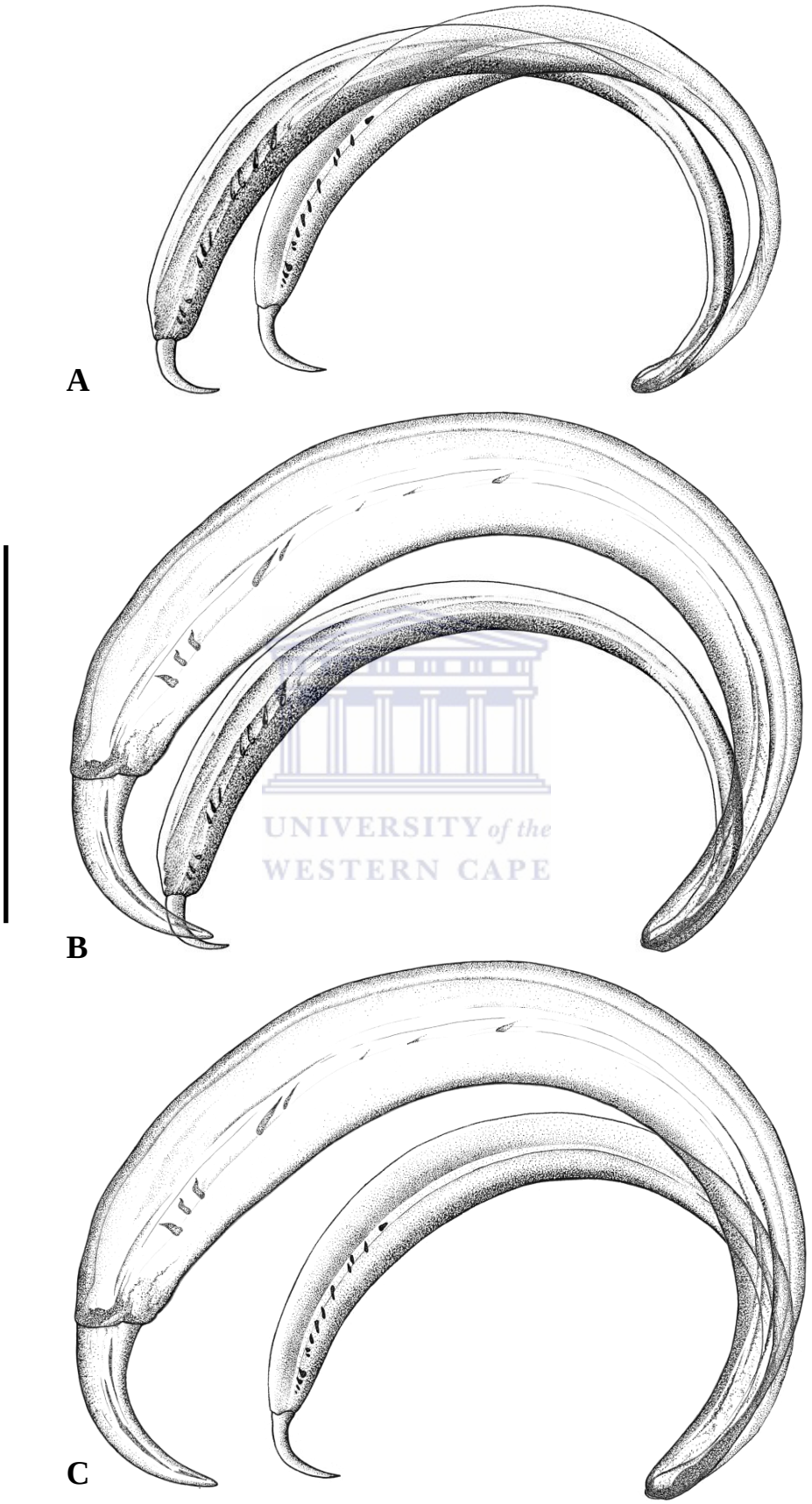

Fig. 4.14 Complex 1 sucker sclerite overlays. **A.** *Callorhynchocotyle callorhynchi* and *C. amatoi*, **B.** *C. callorhynchi* and *C. sagamiensis*, **C.** *C. amatoi* and *C. sagamiensis.* Scale bar = 260µm.

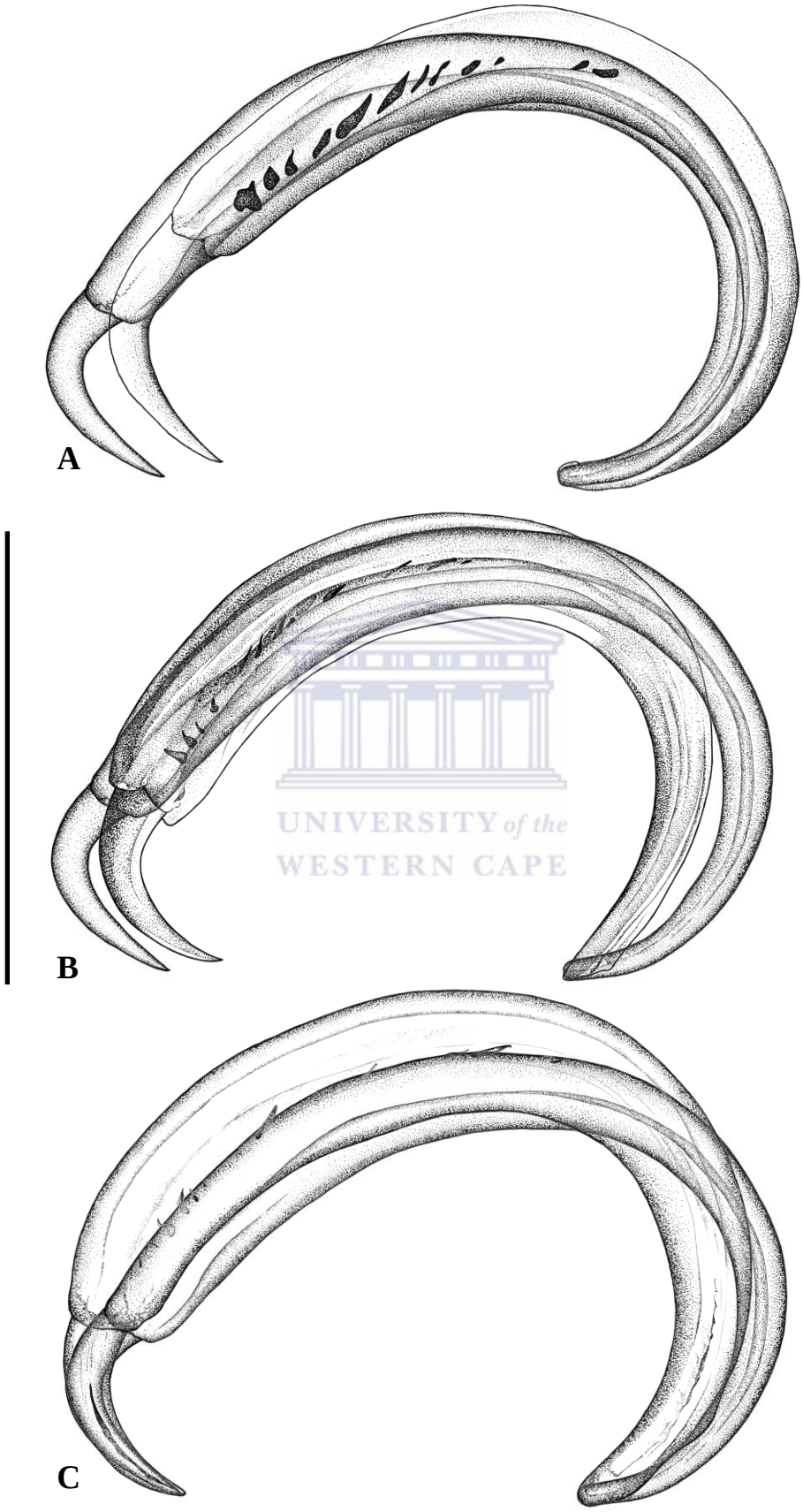

Fig. 4.15 Complex 2 sucker sclerite overlays. **A.** *Callorhynchocotyle marplatensis* and *C. callorhynchi*, **B.** *C. marplatensis* and *C. amatoi*, **C.** *C. marplatensis* and *C. sagamiensis*. Scale bar = 340µm.

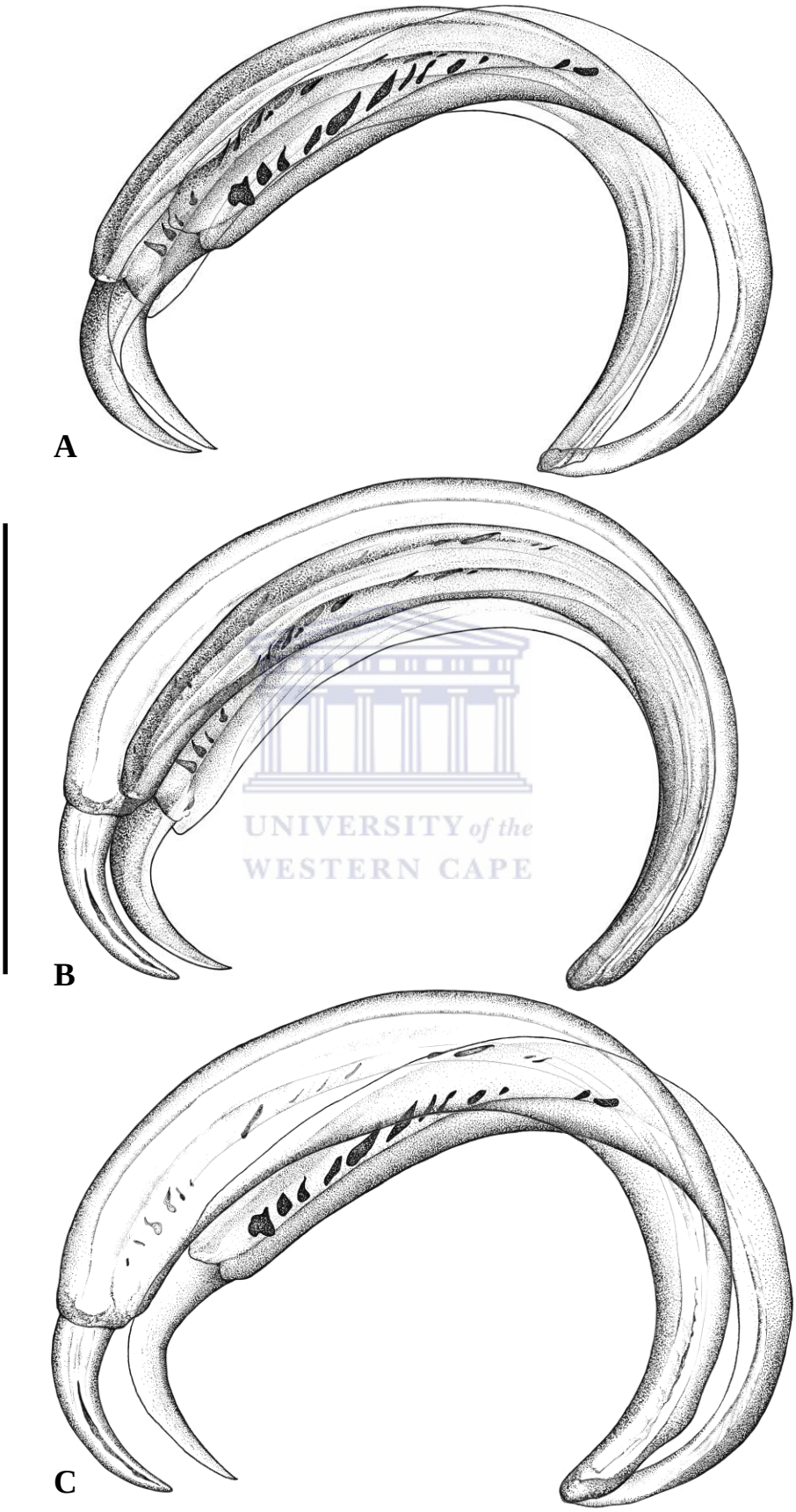

Fig. 4.16 Complex 2 sucker sclerite overlays. **A.** *Callorhynchocotyle callorhynchi* and *C. amatoi*, **B.** *C. amatoi* and *C. sagamiensis*, **C.** *C. callorhynchi* and *C. sagamiensis.* Scale bar = 340µm.

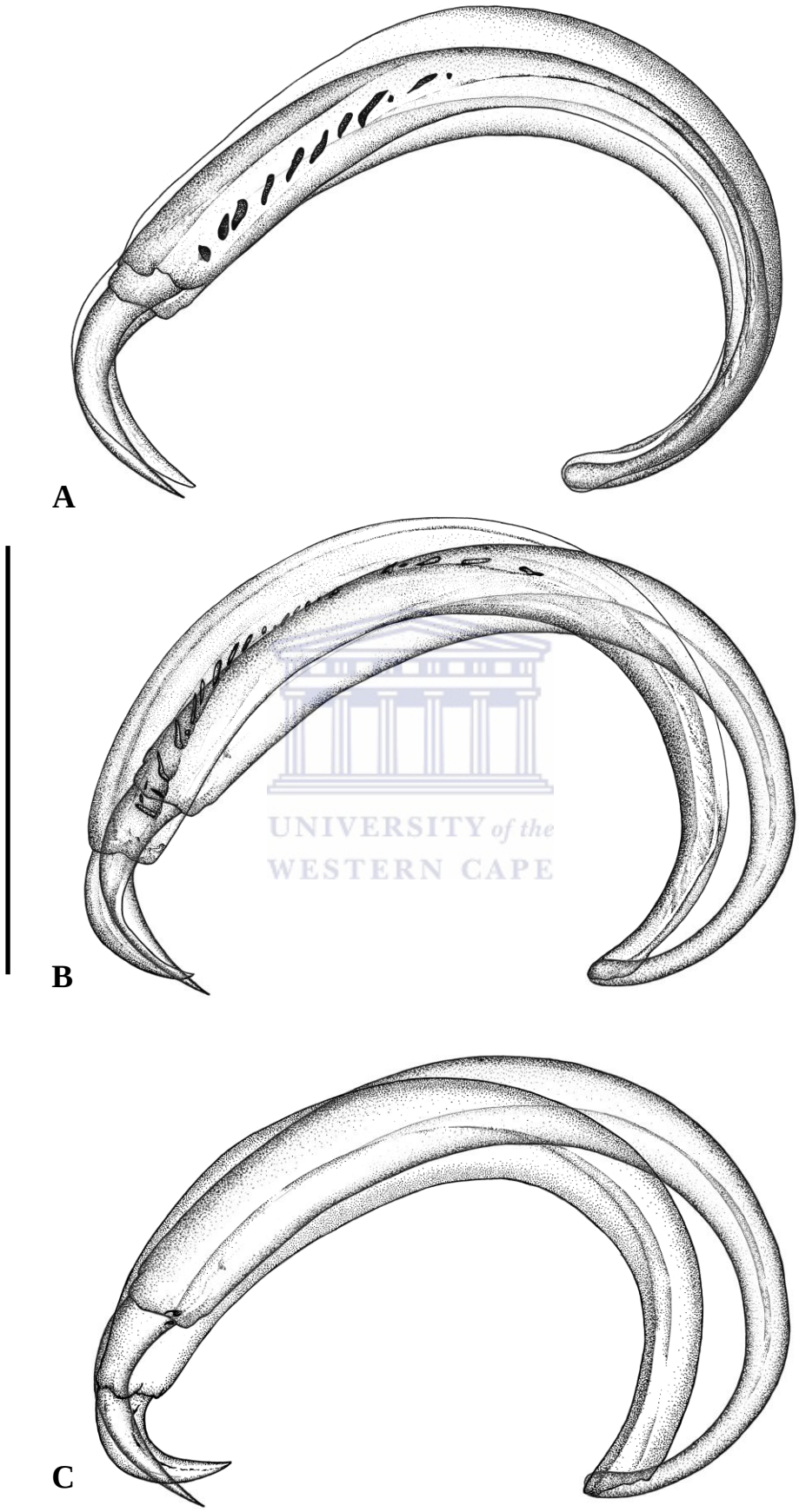

Fig. 4.17 Complex 3 sucker sclerite overlays. **A.** *Callorhynchocotyle marplatensis* and *C. callorhynchi*, **B.** *C. marplatensis* and *C. amatoi*, **C.** *C. marplatensis* and *C. hydrolagi*. Scale bar = 340µm.

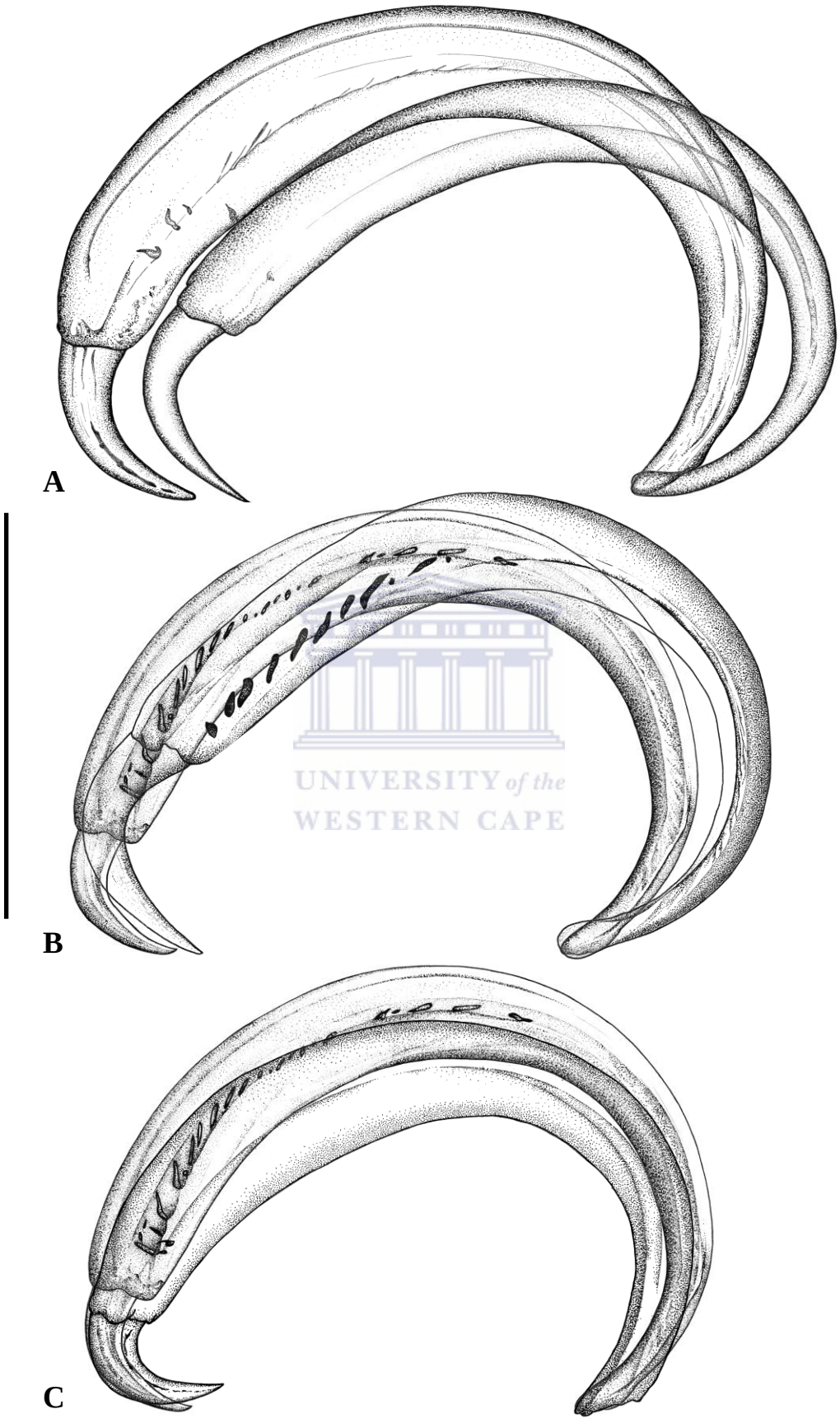

Fig. 4.18 Complex 3 sucker sclerite overlays. **A.** *Callorhynchocotyle marplatensis* and *C. sagamiensis*, **B.** *C. callorhynchi* and *C. amatoi*, **C.** *C. amatoi* and *C. hydrolagi*. Scale bar = 340µm.

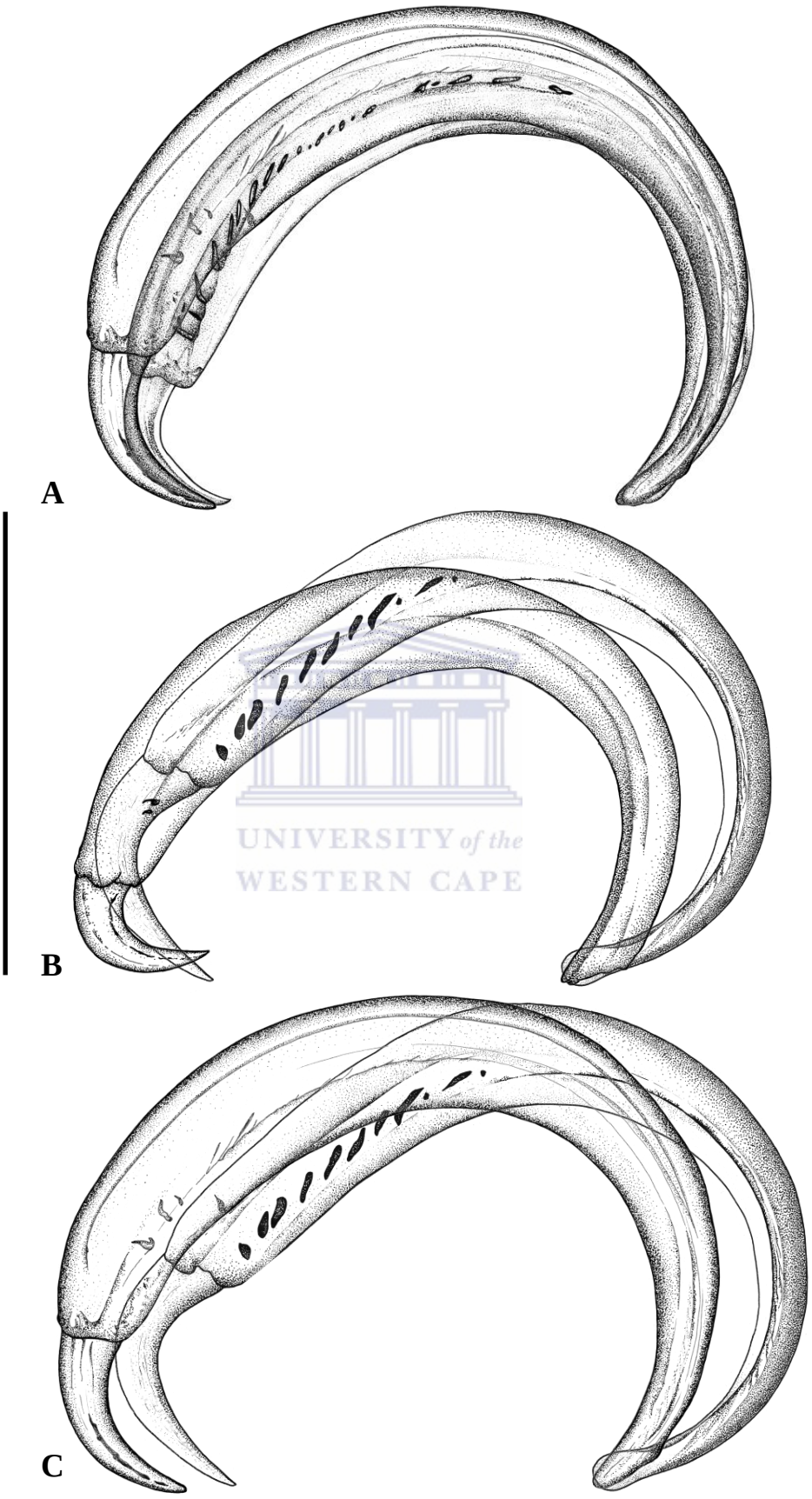

Fig. 4.19 Complex 3 sucker sclerite overlays. **A.** *Callorhynchocotyle amatoi* and *C. sagamiensis*, **B.** *C. callorhynchi* and *C. hydrolagi*, **C.** *C. callorhynchi* and *C. sagamiensis.* Scale bar = 340µm.

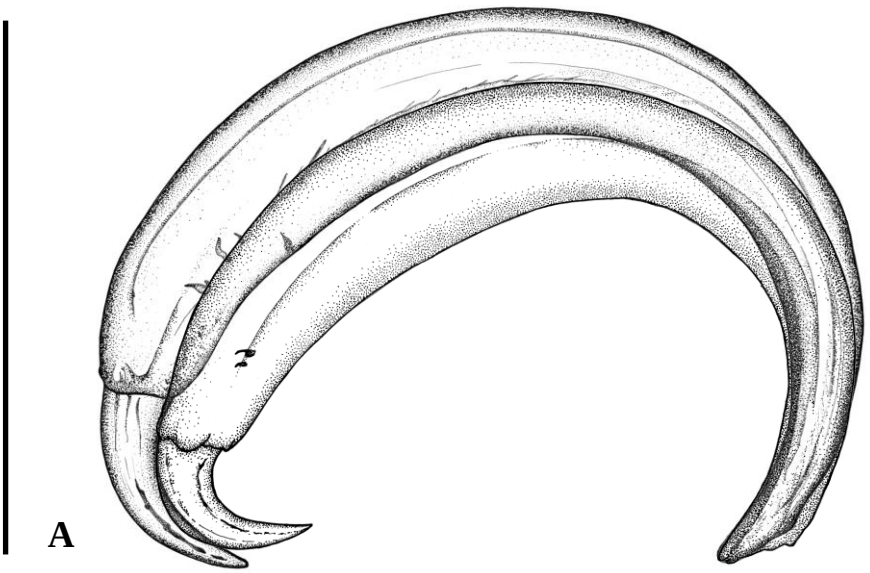

Fig. 4.20 Complex 3 sucker sclerite overlay. **A.** *Callorhynchocotyle sagamiensis* and *C. hydrolagi* Scale bar = 340µm.

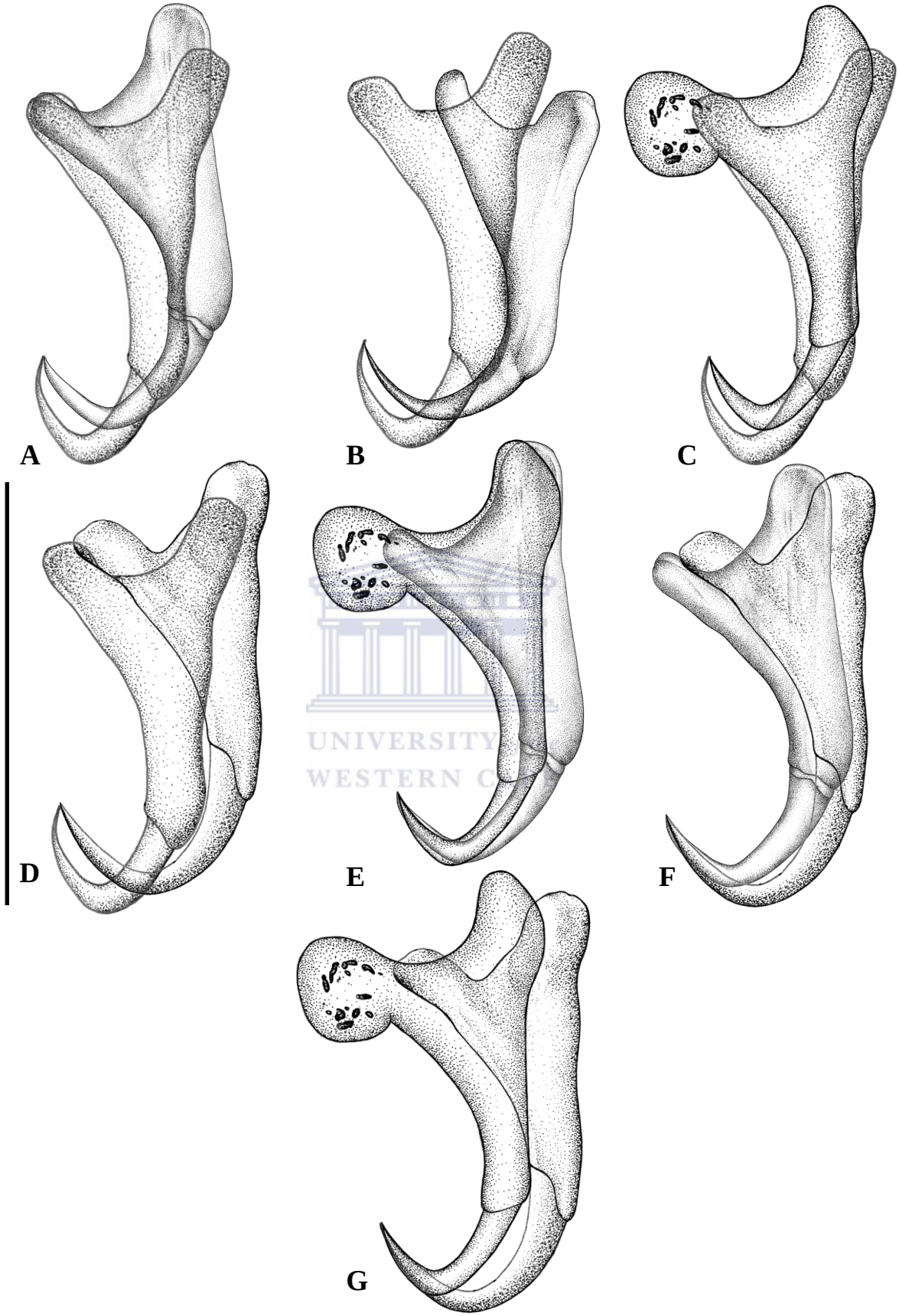

Fig. 4.21 Hamulus overlays. **A.** *Callorhynchocotyle marplatensis* and *C. callorhynchi*, **B.** *C. marplatensis* and *C. amatoi*, **C.** *C. marplatensis* and *C. hydrolagi*, **D.** *C. marplatensis* and *C. sagamiensis*, **E.** *C. callorhynchi* and *C. hydrolagi*, **F.** *C. callorhynchi* and *C. sagamiensis*, **G.** *C. hydrolagi* and *C. sagamiensis.* Scale bar = 60µm.

CHAPTER 5

Review of *Branchotenthes* Bullard and Dippenaar, 2003

5.1 Introduction

Branchotenthes is the most recent genus described in Hexabothriidae Price, 1942. Two species have been described to date; the type species *B. robinoverstreeti* from the East coast of South Africa, and *B. octohamatus* from Kingston point near Adelaide, Australia.

Both *Branchotenthes* species are recorded from rhinobatoid hosts. Rhinobatoids were previously recorded as hosts only to the hexabothriid genus *Rhinobatonchocotyle* (Doran 1953, Boeger and Kritsky 1989, Oliva and Luque 1995).

Branchotenthes was differentiated from all of the other hexabothriid genera because members species possess 2 distally dilated glandular sperm ducts (*sensu* Glennon *et al*. 2005) which join to form the vas deferens (Bullard and Dippenaar 2003). Additionally, Bullard and Dippenaar (2003) separated *Branchotenthes* from *Erpocotyle* and *Squalonchocotyle* on the morphology of the lobed proximal ovary, the elongate distal portion of the cirrus (ovate in *Erpocotyle*) and the thick-walled, muscular distal vaginae, opposed to those of *Squalonchocotyle*.

5.2 Branchotenthes Bullard and Dippenaar, 2003

5.2.1 Amended diagnosis

Symmetrical haptor with three sucker sclerite complex pairs (*sensu* Boeger and Kritsky 1989) of similar size. Haptoral longitudinal axis continuous with that of the body proper. Appendix terminal with pair of hamuli between pair of terminal appendix suckers. Two distally dilated sperm ducts (*sensu* Glennon *et al*. 2005) with glandular walls join medially to form vas deferens. Cirrus unarmed, thick-walled with tapering, elongate distal and oblong proximal parts (*sensu* Glennon *et al*. 2005). Parallel vaginae with thin or thick-walled distal glandulo-muscular distal part and highly sinuous, thin-walled, glandular proximal part. Ovary anteriorly lobate, with sinuous descending and straight ascending ovarian branches. Thin-walled seminal receptacle between descending and ascending ovarian branches. Oötype smooth. Ovate eggs either chain-linked by elongate

tendril at each end, or singular, unattached by tendrils. Branch of intestinal caecum splits into three in the haptoral appendix. Gill parasites of rhinobatoid hosts.

5.2.2 Branchotenthes robinoverstreeti Bullard and Dippenaar, 2003

Type host: Rhina ancylostoma Bloch and Schneider, 1801.
Type locality: Coast of Trafalgar (30.57°S, 30.17°E), KwaZulu Natal, South Africa.
Site on host: Gills.
Material examined: SAMCTA A29445 (1 paratype), USNPC 092533.00: paratype MT31: 23 O.
Redescription (Figs. 5.1 and 5.2, Table 5.1)
Body elongate. Total body length (excluding haptor) 4775 ± 35.35(4750–4800, n = 2). Maximum body width 1858 ± 24.74(1840–1875, n = 2) (Fig. 5.1A). Oral sucker non-papillate 629 ± 23.56(613–646, n = 2) in diameter, covered by conspicuous, tightly arranged cells, with one pair of anterior-marginal glands opening anteriorly. Pharynx 124 ± 27.12(105–143, n = 2) long, 96 ± 6.4(91–100, n = 2) wide. Branched intestinal caeca unite posterior to testes, extending into haptor. Haptor symmetrical, 2583 ± 683.53(2100–3067, n = 2) long, 1367 ± 895.67(733–2000, n = 2) wide with 3 paired sucker sclerite complexes *sensu* Boeger *et al.* (1989). Haptoral suckers non-papillate. Sucker complex sclerites all of similar size.

Sclerite of sucker complex 1 (Fig. 5.2A) circumferential length 1337 ± 78.06(1282–1392, n = 2); total length 548 ± 32.48(515–581, n = 2); total diameter 372 ± 38.04(335–413, n = 2); width 97 ± 16.65(78–112, n = 2); shaft length 546 ± 36.5(508–586, n = 2); inner diameter 276 ± 22.50(250–303, n = 2); aperture angle 62° ± 6.85(54°–69°, n = 2); aperture 333 ± 30.10(305–373, n = 2); hook-side curve length 99 ± 9.15(90–112, n = 2) and shaft-side curve length 103 ± 18.68(81–124, n = 2). Complex 1 sucker sclerite hook length 146 ± 5.94(140–151, n = 2); hook curve length 10 ± 1.18(9–11, n = 2); aperture angle 124° ± 3.8(119°–128°, n = 2); aperture 134 ± 6.07(127–141, n = 2) and base width 59 ± 3.75(56–63, n = 2).

Sclerite of sucker complex 2 (Fig. 5.2B) circumferential length 1405, n = 1; total length 599 ± 10.84(592–612, n = 2); total diameter 378 ± 27.01(352–406, n = 2); width 104 ± 14.21(94–121, n = 2); shaft length 592 ± 15.16(580–609, n = 2); inner diameter

273 ± 17.16(254–286, n = 2); aperture angle 61° ± 4.89(56°–66°, n = 2); aperture 323 ± 14.50(315–343, n = 2); hook-side curve length 112 ± 4.97(109–117, n = 2) and shaft-side curve length 143 ± 3.70(139–145, n = 2). Complex 2 sucker sclerite hook length 166 ± 0.60(166–167, n = 2); hook curve length 11 ± 2.96(7–12, n = 2); aperture angle 130° ± 4.55(125°–134°, n = 2); aperture 154 ± 5.87(147–158, n = 2) and base width 63 ± 8.32(54–69, n = 2).

Sclerite of sucker complex 3 (Fig. 5.2C) circumferential length 1388 ± 16.57(1377–1400, n = 2); total length 618 ± 25.75(594–649, n = 2); total diameter 400 ± 23.69(373–422, n = 2); width 104 ± 15.39(91–121, n = 2); shaft length 613 ± 28.24(586–644, n = 2); inner diameter 297 ± 10.24(283–308, n = 2); aperture angle 65° ± 3.87(62°–71°, n = 2); aperture 380 ± 37.26(353–435, n = 2); hook-side curve length 119 ± 3.89(113–122, n = 2) and shaft-side curve length 126 ± 9.91(113–137, n = 2). Complex 3 sucker sclerite hook length 167 ± 2.34(164–169, n = 2); hook curve length 10 ± 2.58(7–12, n = 2); aperture angle 130° ± 3.72(128°–135°, n = 2); aperture 159 ± 3.00(157–162, n = 2) and base width 67 ± 1.12(65–68, n = 2).

Terminal haptoral appendix 1437 ± 135.19(1341–1532, n = 2) long, 596 ± 172.91(473–718, n = 2) wide. Terminal suckers of appendix 385 ± 50.98(287–396, n = 2) long, 181 ± 29.67(144–213, n = 2) wide. Pair of hamuli present between terminal suckers of appendix. Hamulus (Fig. 5.2D) total length 84, n = 1; hook point length 24, n = 1; hook shank length 14, n = 1; total width 42, n = 1; distal hook point width 5, n = 1; outer aperture angle 33°, n = 1; inner aperture angle 40°, n = 1; aperture 13, n = 1; hook shank base width 13, n = 1; inner root-shaft length 63, n = 1; outer root-shaft length 64, n = 1; root base angle 105°, n = 1, and root base width 37, n = 1.

Testes irregular in shape, numerous and tightly packed. Vas deferens extends ventro-laterally as thin walled tube into base of proximal portion of cirrus. Cirrus unarmed, thick-walled with tapering, elongate distal and oblong proximal parts. Cirrus total length 1499 ± 100.00(1429–1570, n = 2); maximum width 269 ± 28.40(249–289, n = 2).

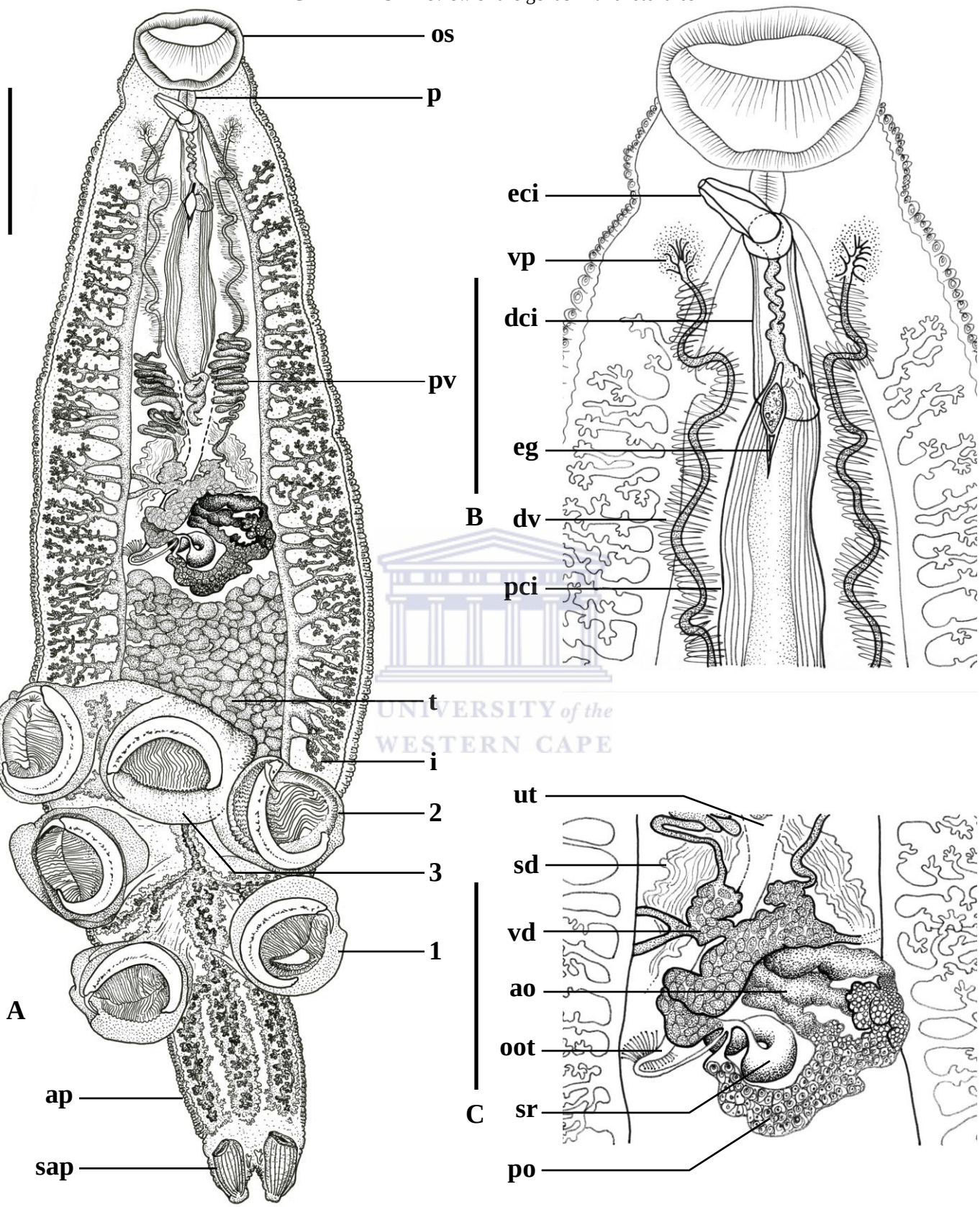

Fig. 5.1 *Branchotenthes robinoverstreeti* **A.** whole mount; **B.** Enlarged anterior section of whole mount; **C.** enlarged mid section of whole mount. **Abbreviations:** ao – anterior section of ovary; ap – appendix; dci – distal section of cirrus; dv – distal section of vagina; eci – everted portion of cirrus; eg – egg; i – intestinal caecum; oot – oötype with Mehlis' glands; os – oral sucker; p – pharynx; pci – proximal section of cirrus; po – posterior section of ovary; pv – proximal section of vagina; sap – sucker of appendix; sd – sperm duct; sr – seminal receptacle; t – testes; ut – uterus; vd – vitteline duct; vp – vaginal pore; 1–3 – sucker-sclerite complexes 1–3. Scale bars = 1000µm and 700µm respectively.

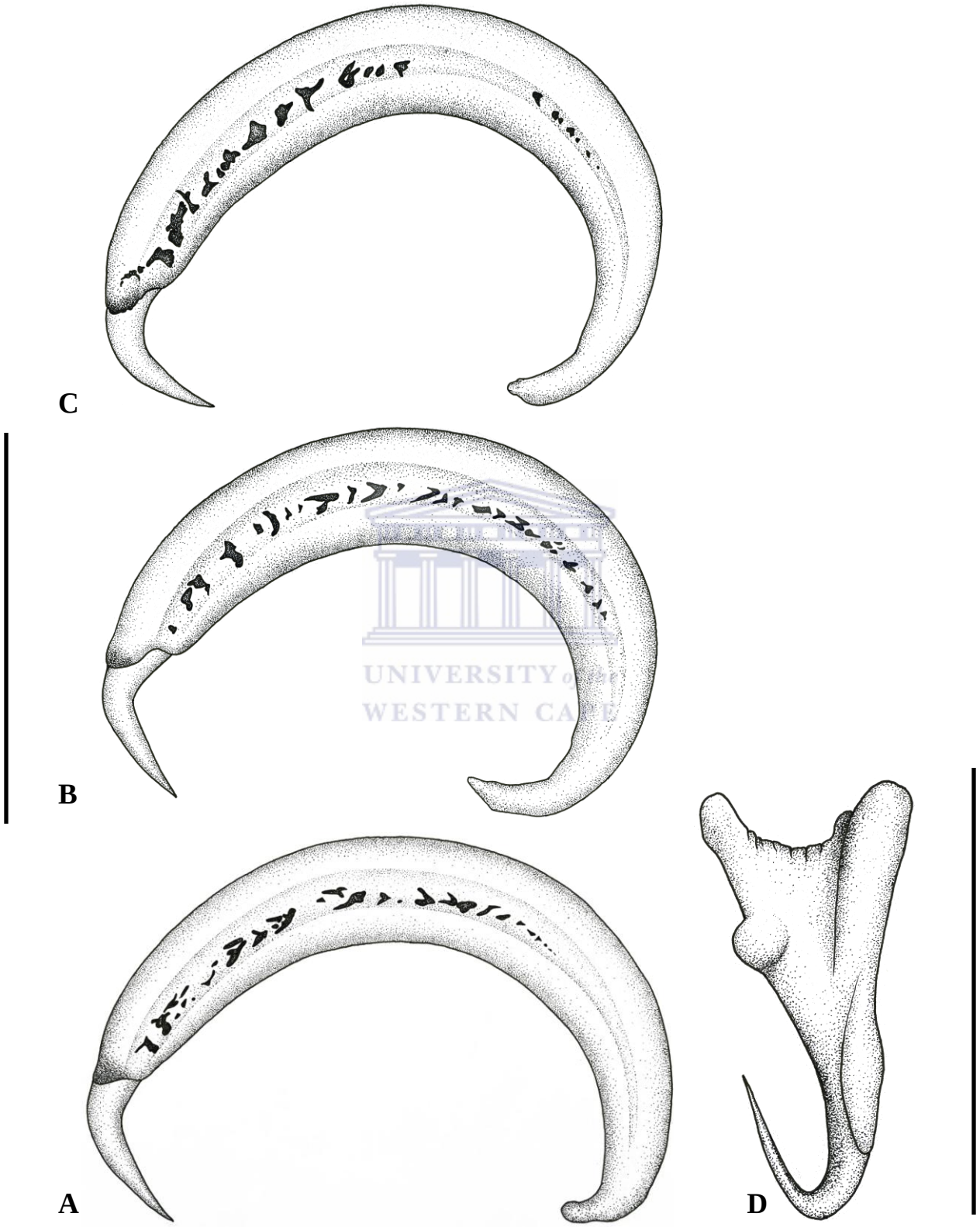

Fig. 5.2 *Branchotenthes robinoverstreeti* sclerites of sucker complexes 1 (**A**), 2 (**B**) and 3(**C**), and hamulus (**D**). Scale bars = 370µm and 84µm respectively.

Ovary (dextral = 1, sinistral = 1) 666 ± 55.83(626–705, n = 2) in total length, anteriorly lobate with sinuous descending and straight ascending ovarian branches (Fig. 5.1B). Thin-walled seminal receptacle between descending and ascending ovarian branches.

Oötype smooth, leading to uterus, dorsal to ovary, ventral to vas deferens. Ovate eggs not linked to each other by tendrils at each pole. Eggs (*in utero*) 163 ± 11.46(155–172, n = 2) long, 63 ± 4.47(58–67, n = 3) wide. Parallel vaginae with thick-walled distal glandulo-muscular distal part and highly sinuous, thin-walled, glandular proximal part. Ventral vaginal pores muscular, lateral to distal portion of cirrus (Fig. 5.1C).

Remarks

The paratype SAMCTA A29445 was cracked along the coverslip on arrival from the South African Iziko Museum and was subsequently re-constituted in xylene and re-mounted in Canada balsam with permission from the Museum. Bullard and Dippenaar (2003) did not indicate the presence of the pair of anterior glands found on the anterior margin of the oral sucker. These glands are not indicated in the whole mount of Fig. 5.1, and are inconspicuous in the paratype SAMCTA A29445.

The presence of 2 distally dilated glandular sperm ducts, although indicated by Bullard and Dippenaar (2003) to be a unique character to *Branchotenthes*, is shared with 6 species representing 4 genera within Hexabothriidae (Glennon *et al.* 2005). However, the combination of characters adequately differentiates the genus from the other 14 valid genera within the family.

Comparative measurements for *B. robinoverstreeti* are given in Table 5.1. A discrepancy in the measurements of the terminal appendix suckers exists for those taken by Bullard and Dippenaar (2003) and those measured from the paratypes in the present study. Although Bullard and Dippenaar (2003) reported the measurement of both the length and width of the terminal appendix suckers as a range between 99–204 µm and 99–152 µm respectively, their scale bar for these structures in their Fig. 18 does not agree. However, the scale bar is in agreement with the measurements of these structures taken in the present study using the 2 paratypes. Therefore, it is likely that these structures were measured incorrectly in the original description.

The total length measurement of *B. robinoverstreeti* eggs is measured by Bullard and Dippenaar (2003) inclusive of both tendrils attached to the poles of the egg capsule. It must be cautioned that measuring hexabothriid eggs in this manor cannot be consistent. Not all hexabothriids possess eggs that are separated from each other. Many hexabothriids produce eggs attached to each other in long chains, joined by these polar tendrils. Eggs of *B. octohamatus* conform to the latter and can therefore not be compared accurately to those of *B. robinoverstreeti*. As such, all eggs are measured in the present study from the total length of the egg capsule which remains constant.

Table 5.1 Comparative measurements for *Branchotenthes robinoverstreeti* Bullard and Dippenaar, 2003

	Bullard and Dippenaar (2003)	**Present study**
Total body length	4890–5297, n = 6	**4775 ± 35.4(4750–4800, n = 2)**
Maximum body width	1467–2119, n = 6	**1858 ± 24.7(1840–1875, n = 2)**
Oral sucker diameter	514–692, n = 5	**629 ± 23.6(613–646, n = 2)**
Pharynx length	-	**124 ± 27.1(105–143, n = 2)**
Pharynx width	109–129, n = 5	**96 ± 6.4(91–100, n = 2)**
Haptor length	1350–1768, n = 6	**2583 ± 683.5(2100–3067, n = 2)**
Haptor width	1105–1350, n = 6	**1367 ± 895.7(733–2000, n = 2)**
Appendix length	1150–1470, n = 5	**1437 ± 135.2(1341–1532, n = 2)**
Appendix width	-	**596 ± 172.9(473–718, n = 2)**
Terminal appendix sucker length	99–204, n = 5	**385 ± 50.9(287–396, n = 4)**
Terminal appendix sucker width	99–152, n = 5	**181 ± 29.7(144–213, n = 4)**
Cirrus total length	1689–2167, n = 5	**1499 ± 100.0(1429–1570, n = 2)**
Cirrus maximum width	247–307, n = 6	**269 ± 28.4(249–289, n = 2)**
Ovary total length	-	**666 ± 55.8(626–705, n = 2)**
Egg length	423–530, n = 3[1]	**163 ± 11.5(155–172, n = 2)**
Egg width	59–60, n = 3	**63 ± 4.5(58–67, n = 3)**
Complex 1 sclerite		
Circumferential length	-	**1337 ± 78.0(1282–1392, n = 2)**
Total length	554–662, n = 4*****	**548 ± 32.5(515–581, n = 2)**
Shaft length	-	**546 ± 36.5(508–586, n = 2)**
Hook-side curve length	-	**99 ± 9.2(90–112, n = 2)**
Shaft-side curve length	-	**103 ± 18.6(81–124, n = 2)**
Total diameter	-	**372 ± 38.0(335–413, n = 2)**
Inner diameter	-	**276 ± 22.5(250–303, n = 2)**
Aperture angle	-	**62° ± 6.8(54°–69°, n = 2)**
Aperture	-	**333 ± 30.1(305–373, n = 2)**
Width	79–99, n = 4	**97 ± 16.7(78–112, n = 2)**
Hook length	-	**146 ± 5.9(140–151, n = 2)**
Hook curve length	-	**10 ± 1.1(9–11, n = 2)**

Table 5.1 cont

Hook aperture angle	-	**124° ± 3.8(119°–128°, n = 2)**
Hook aperture	-	**134 ± 6.0(127–141, n = 2)**
Hook base width	-	**59 ± 3.8(56–63, n = 2)**
Complex 2 sclerite		
Circumferential length	-	**1405, n = 1**
Total length	-	**599 ± 10.8(592–612, n = 2)**
Shaft length	-	**592 ± 15.2(580–609, n = 2)**
Hook-side curve length	-	**112 ± 4.9(109–117, n = 2)**
Shaft-side curve length	-	**143 ± 3.7(139–145, n = 2)**
Total diameter	-	**378 ± 27.0(352–406, n = 2)**
Inner diameter	-	**273 ± 17.2(254–286, n = 2)**
Aperture angle	-	**61° ± 4.8(56°–66°, n = 2)**
Aperture	-	**323 ± 14.5(315–343, n = 2)**
Width	-	**104 ± 14.2(94–121, n = 2)**
Hook length	-	**166 ± 0.6(166–167, n = 2)**
Hook curve length	-	**11 ± 2.9(7–12, n = 2)**
Hook aperture angle	-	**130° ± 4.6(125°–134°, n = 2)**
Hook aperture	-	**154 ± 5.8(147–158, n = 2)**
Hook base width	-	**63 ± 8.3(54–69, n = 2)**
Complex 3 sclerite		
Circumferential length	-	**1388 ± 16.5(1377–1400, n = 2)**
Total length	-	**618 ± 25.8(594–649, n = 2)**
Shaft length	-	**613 ± 28.2(586–644, n = 2)**
Hook-side curve length	-	**119 ± 3.9(113–122, n = 2)**
Shaft-side curve length	-	**126 ± 9.9(113–137, n = 2)**
Total diameter	-	**400 ± 23.7(373–422, n = 2)**
Inner diameter	-	**297 ± 10.2(283–308, n = 2)**
Aperture angle	-	**65° ± 3.8(62°–71°, n = 2)**
Aperture	-	**159 ± 3.0(157–162, n = 2)**
Width	-	**104 ± 15.4(91–121, n = 2)**
Hook length	-	**167 ± 2.3(164–169, n = 2)**
Hook curve length	-	**10 ± 2.5(7–12, n = 2)**
Hook aperture angle	-	**130° ± 3.8(128°–135°, n = 2)**
Hook aperture	-	**159 ± 3.0(157–162, n = 2)**
Hook base width	-	**67 ± 1.1(65–68, n = 2)**
Hamulus total length	70–80, n = 5	**84, n = 1**
Hook point length	-	**24, n = 1**
Hook shank length	-	**14, n = 1**
Total width	-	**42, n = 1**
Distal hook point width	-	**5, n = 1**
Outer aperture angle	-	**33°, n = 1**
Inner aperture angle	-	**40°, n = 1**
Aperture	-	**13, n = 1**
Hook shank base width	-	**13, n = 1**
Outer root-shaft length	-	**64, n = 1**
Inner root-shaft length	-	**63, n = 1**

Table 5.1 cont

Root base width	30–45, n = 5	**37, n = 1**
Root base angle	-	**105°, n = 1**

Bold script = all measurements of the present study.
[1]Egg length of Bullard and Dippenaar (2003) is measured inclusive of the tendrils attached to each pole. In the present study for consistency, eggs are measured for the egg capsule, excluding the tendrils. The measurement of egg tendrils is unnecessary and promotes a high variability in total length measurements. In addition this measurement of total egg length cannot be repeated for many hexabothriid species as their eggs are joined to each other by these tendrils in long consecutive chains.
*Note that Bullard and Dippenaar did not specify which sucker complex sclerites were being measured. However, since they are all of similar size, they are included into the above Table 5.1 under complex 1.

5.2.3 Branchotenthes octohamatus Glennon, Chisholm and Whittington, 2005

Type host: Trygonorrhina fasciata Müller and Henle, 1841.

Additional hosts: Aptychotrema vincentiana, A. rostrata, Trygonorrhina sp. A (Glennon *et al.* 2008).

Type locality: Kingston point, Seacliff, Adelaide, South Australia.

Additional localities: Fremantle, Western Australia; Adelaide, South Australia; Newcastle, New South Wales, and Stradbroke Island, Queensland, Australia.

Site on host: Gills.

Material examined: Queensland Museum paratypes QM AHC 28768, 28769 and 28771, USNPC 095759.00 paratype MT 33-4 No.5.

Redescription (Figs. 5.3 and 5.4, Table 5.2)

Body elongate. Total body length (excluding haptor) 5267 ± 2140.95(3200–8200, n = 4). Maximum body width 1837 ± 568.52(1300–2400, n = 4) (Fig. 5.3A). Oral sucker non-papillate 377 ± 93.04(306–510, n = 4) in diameter, covered by conspicuous, tightly arranged cells, with one pair of anterior-marginal gland duct openings. Pharynx 113 ± 14.87(101–133, n = 4) long, 109 ± 10.07(97–121, n = 4) wide. Branched intestinal caeca unite posterior to testes, extending into haptor. Haptor symmetrical, 3798 ± 693.60(2920–4600, n = 4) long, 2455 ± 580.89(1800–3200, n = 2) wide with 3 paired sucker sclerite complexes *sensu* Boeger *et al.* (1989) (Fig. 5.3A). Haptoral suckers non-papillate. Sucker sclerites all of similar size.

Sclerite of sucker complex 1 (Fig. 5.4A) circumferential length 1126 ± 31.80(1103–1148, n = 2); total length 589 ± 64.47(518–689, n = 4); total diameter 398 ±

67.88(345–509, n = 4); width 84 ± 19.55(69–117, n = 4); shaft length 586 ± 68.81(513–692, n = 4); inner diameter 317 ± 49.53(274–400, n = 4); aperture angle 60° ± 4.94(52°–68°, n = 4); aperture 367 ± 37.73(323–416, n = 4); hook-side curve length 106 ± 15.62(85–128, n = 4) and shaft-side curve length 118 ± 9.67(103–132, n = 4). Complex 1 sucker sclerite hook length 135 ± 18.49(107–157, n = 4); hook curve length 13 ± 2.24(9–16, n = 4); aperture angle 117° ± 5.68(112°–127°, n = 4); aperture 117 ± 16.10(92–132, n = 4) and base width 55 ± 9.33(41–68, n = 4).

Sclerite of sucker complex 2 (Fig. 5.4B) circumferential length 1135 ± 7.67(1130–1141, n = 4); total length 614 ± 73.32(531–729, n = 8); total diameter 402 ± 63.32(349–507, n = 4); width 85 ± 19.25(71–116, n = 4); shaft length 609 ± 76.88(524–729, n = 4); inner diameter 319 ± 45.24(276–396, n = 4); aperture angle 62° ± 3.22(57°–68°, n = 4); aperture 392 ± 40.07(351–447, n = 4); hook-side curve length 102 ± 16.04(84–127, n = 4) and shaft-side curve length 122 ± 15.79(101–140, n = 4). Complex 2 sucker sclerite hook length 137 ± 16.53(115–160, n = 4); hook curve length 13 ± 1.43(11–15, n = 4); aperture angle 118° ± 5.44(110°–125°, n = 4); aperture 117 ± 15.06(98–135, n = 4) and base width 55 ± 6.72(45–62, n = 4).

Sclerite of sucker complex 3 (Fig. 5.4C) circumferential length 1097 ± 2.53(1096–1099, n = 4); total length 613 ± 72.80(513–699, n = 4); total diameter 404 ± 65.68(323–483, n = 4); width 84 ± 15.87(68–104, n = 4); shaft length 606 ± 78.60(506–705, n = 4); inner diameter 324 ± 53.21(255–392, n = 4); aperture angle 62° ± 3.98(58°–67°, n = 4); aperture 398 ± 49.74(311–350, n = 4); hook-side curve length 100 ± 18.54(84–130, n = 4) and shaft-side curve length 120 ± 21.98(95–155, n = 4). Complex 3 sucker sclerite hook length 134 ± 13.49(116–150, n = 4); hook curve length 12 ± 1.22(11–15, n = 4); aperture angle 117° ± 3.75(112°–123°, n = 4); aperture 123 ± 13.80(99–135, n = 4) and base width 57 ± 6.22(46–65, n = 4).

Terminal haptoral appendix 1635 ± 344.13(1197–1920, n = 4) long, 874 ± 180.27(627–1049, n = 4) wide. Terminal suckers of appendix 434 ± 83.90(339–559, n = 8) long, 209 ± 32.64(160–250, n = 8) wide. Pair of hamuli present between terminal suckers of appendix. Hamulus (Fig. 5.4D) total length 84 ± 2.28(83–87, n = 3); hook point length 19 ± 5.46(13–24, n = 3); hook shank length 14 ± 2.39(12–16, n = 3); total width 35 ± 5.23(29–38, n= 3); distal hook point width 5 ± 1.33(3–6, n = 3); outer aperture angle 53° ± 12.88(43°–68°, n = 3); inner aperture angle 71° ± 13.59(58°–85°, n = 3);

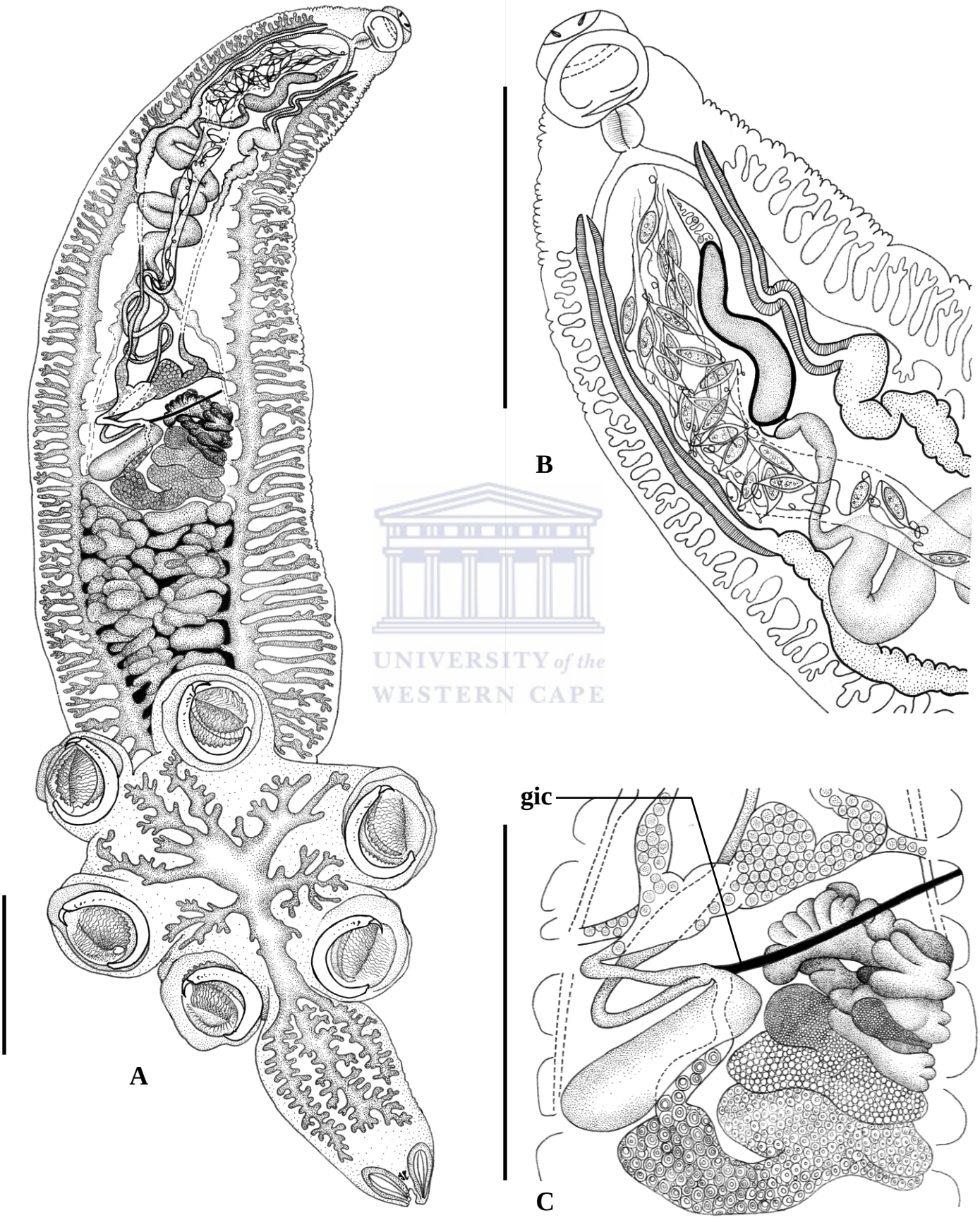

Fig. 5.3 *Branchotenthes octohamatus.* **A.** whole mount; **B.** enlarged anterior section of whole mount; **C.** enlarged mid section of whole mount. **Abbreviation:** gic – genito-intestinal canal. Scale bars = 1000µm.

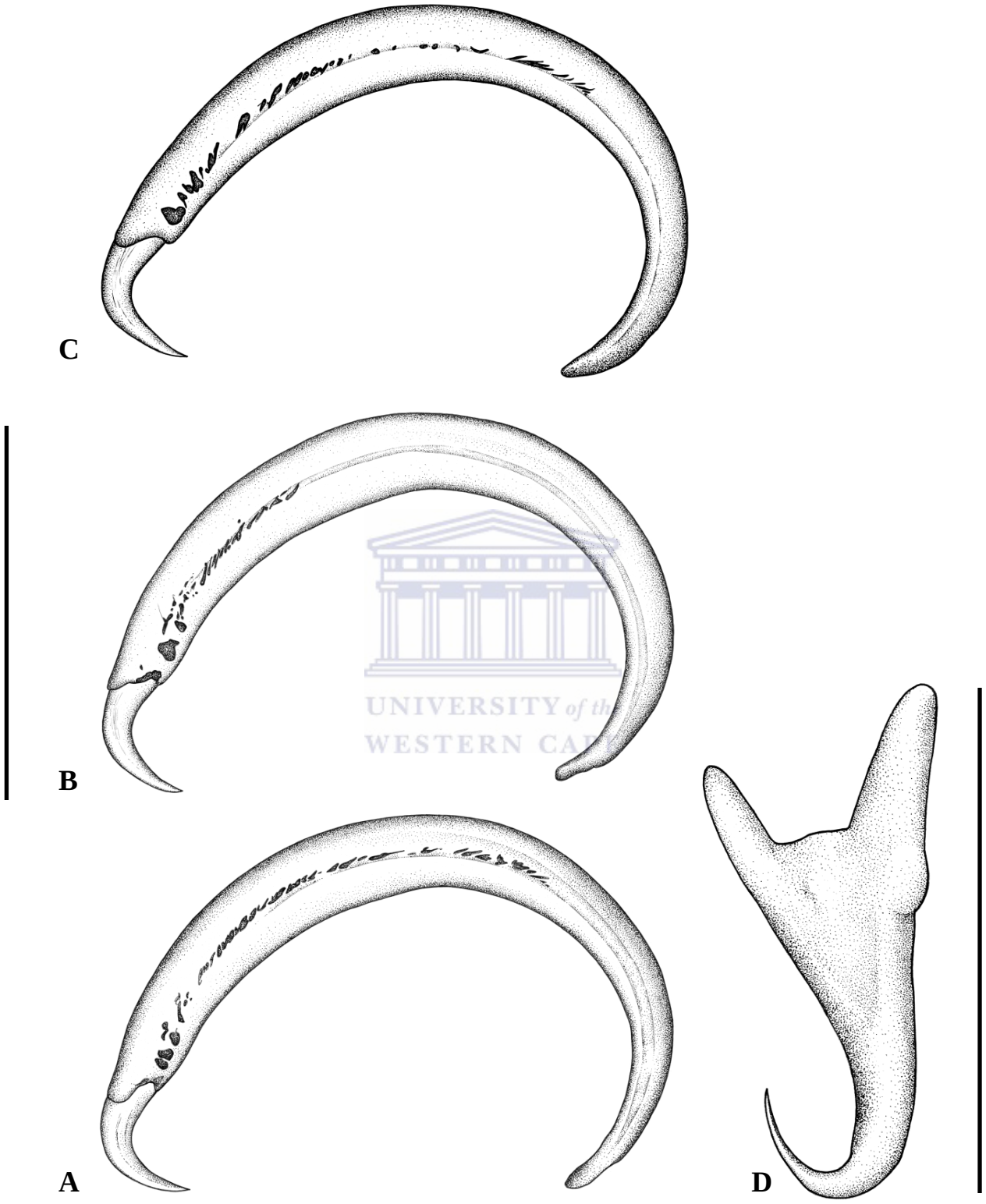

Fig. 5.4 *Branchotenthes octohamatus* sclerites of sucker complexes 1 (**A**), 2 (**B**) and 3(**C**), and hamulus (**D**). Scale bars = 390µm and 80µm respectively.

aperture 17 ± 2.12(14–18, n = 3); hook shank base width 8 ± 1.92(6–10, n = 3); inner root-shaft length 61 ± 12.64(47–72, n = 3); outer root-shaft length 52 ± 13.69(37–64, n = 3); root base angle 94° ± 8.80(89°–104°, n = 3), and root base width 35 ± 4.43(30–38, n = 3).

Testes irregular in shape, numerous and tightly packed. Vas deferens extends ventro-laterally as thin walled tube into base of proximal portion of cirrus. Cirrus unarmed, with tapering, elongate distal and oblong proximal parts (Fig. 5.3B). Cirrus total length 937, n = 1; maximum width 122, n = 1.

Ovary (sinistral = 4) 791 ± 261.92(571–1171, n = 4) in total length, anteriorly lobate with sinuous descending and straight ascending ovarian branches (Fig. 5.3C). Thin-walled seminal receptacle between descending and ascending ovarian branches. Oötype smooth leading to uterus, dorsal to ovary, ventral to vas deferens. Ovate eggs not linked to each other by tendrils at each pole. Eggs (*in utero*) 121 ± 8.90(108–137, n = 14) long, 60 ± 7.25(50–72, n = 14) wide. Parallel vaginae with thick-walled distal glandulo-muscular distal part and sinuous, thin-walled, glandular proximal part. Ventral vaginal pores muscular, lateral to distal portion of cirrus (Fig. 5.3B).

Remarks

Comparative measurements for *B. octohamatus* are given in Table 5.2. Most measurements of the same structures measured in the present study fall within the range given by Glennon *et al*. (2005). It must however be remembered that Glennon *et al.* (2005) measured a range of different size classes including juveniles. Only type material representing adult specimens were measured in the present study. Some small differences in macro measurements are present as well as egg measurements. The differences in macro measurements may be the result of measurement interpretation (e.g. maximum haptor width) while egg measurements are expected to differ depending on which eggs (*in utero*) were measured, as some would be in different stages of development. Sucker complex sclerite and hamulus image overlays for both species are presented in Fig. 5.5 and 5.6 respectively. Comparative measurements of *B. octohamatus* and *B. robinoverstreeti* are given in table 5.3. *Branchotenthes octohamatus* differs from *B. robinoverstreeti* in possessing a shorter complex 2 sucker sclerite hook length and a more acute hook aperture angle than those of *B. robinoverstreeti*. The hook-side curve length

of *B. octohamatus* complex 3 sucker sclerites is shorter than that of *B. robinoverstreeti*. The complex 3 sucker sclerite hook length of *B. octohamatus* is shorter, base width narrower, and aperture angle more acute than that of *B. robinoverstreeti*.

Branchotenthes octohamatus was differentiated from *B. robinoverstreeti* partly on the morphology of the cirrus for which *Glennon et al*. (2005) could not discern a true proximal and distal portion, rather a separation of the 2 regions by a constriction. However, distal and proximal portions of the cirrus of both species were easily distinguishable in the respective type series of each species. The muscular cirrus wall of *B. octohamatus* is comparatively thinner. Additionally, *Glennon et al*. (2005) separated *B. octohamatus* from *B. robinoverstreeti* by the presence of the comparatively thinner muscular region surrounding the distal vaginae and the proportionately longer vas deferens. Although Glennon *et al.* (2005) identified the hamulus as a differentiating character based on the pronounced third process of the hamulus of *B. robinoverstreeti*, the hamuli of *B. octohamatus* also possess a much-reduced third process. However, it is likely that hamulus morphometric measurements could separate the species but not enough resolution could be obtained from the type material examined in the present study to confirm this.

Table 5.2 Comparative measurements for *Branchotenthes octohamatus* Glennon, Chisholm and Whittington, 2005

	Glennon *et al*. (2005)	**Present study**
Total body length	6627 (2548–10528, n = 9)	**5267 ± 2140.9(3200–8200, n = 4)**
Maximum body width	1384 (670–2364, n = 9)	**1837 ± 568.6(1300–2400, n = 4)**
Oral sucker diameter	-	**377 ± 93.0(306–510, n = 4)**
Pharynx length	-	**113 ± 14.9(101–133, n = 4)**
Pharynx width	104 (49–143, n = 9)	**109 ± 10.1(97–121, n = 4)**
Haptor length	-	**3798 ± 693.6(2920–4600, n = 4)**
Haptor width	1949 (961–2971, n = 9)	**2455 ± 580.9(1800–3200, n = 2)**
Appendix length	1294 (490–2051, n = 10)	**1635 ± 344.1(1197–1920, n = 4)**
Appendix width	819 (297–1206, n = 10)	**874 ± 180.3(627–1049, n = 4)**
Terminal appendix sucker length	386 (150–530, n = 11)	**434 ± 83.9(339–559, n = 8)**
Terminal appendix sucker width	180 (66–297, n = 11)	**209 ± 32.6(160–250, n = 8)**
Cirrus total length	-	**937, n = 1**
Cirrus maximum width	-	**122, n = 1**
Ovary total length	-	**791 ± 261.9(571–1171, n = 4)**

Table 5.2 cont

Egg length	139 (135–141, n = 10)	**121 ± 8.9(108–137, n = 14)**
Egg width	68 (64–73, n = 10)	**60 ± 7.3(50–72, n = 14)**
Complex 1 sclerite		
Circumferential length	-	**1126 ± 31.8(1103–1148, n = 2)**
Total length	-	**589 ± 64.5(518–689, n = 4)**
Shaft length	1039 (533–1461, n = 31)	**586 ± 68.8(513–692, n = 4)**
Hook-side curve length	-	**106 ± 15.6(85–128, n = 4)**
Shaft-side curve length	-	**118 ± 9.6(103–132, n = 4)**
Total diameter	-	**398 ± 67.9(345–509, n = 4)**
Inner diameter	-	**317 ± 49.5(274–400, n = 4)**
Aperture angle	-	**60° ± 4.9(52°–68°, n = 4)**
Aperture	-	**367 ± 37.7(323–416, n = 4)**
Width	102 (84–144, n = 8)	**84 ± 19.6(69–117, n = 4)**
Hook length	185 (150–229, n = 10)	**135 ± 18.5(107–157, n = 4)**
Hook curve length	-	**13 ± 2.2(9–16, n = 4)**
Hook aperture angle	-	**117° ± 5.7(112°–127°, n = 4)**
Hook aperture	-	**117 ± 16.1(92–132, n = 4)**
Hook base width	-	**55 ± 9.3(41–68, n = 4)**
Complex 2 sclerite		
Circumferential length	-	**1135 ± 7.6(1130–1141, n = 4)**
Total length	-	**614 ± 73.3(531–729, n = 4)**
Shaft length	-	**609 ± 76.9(524–729, n = 4)**
Hook-side curve length	-	**102 ± 16.0(84–127, n = 4)**
Shaft-side curve length	-	**122 ± 15.7(101–140, n = 4)**
Total diameter	-	**402 ± 63.3(349–507, n = 4)**
Inner diameter	-	**319 ± 45.2(276–396, n = 4)**
Aperture angle	-	**62° ± 3.2(57°–68°, n = 4)**
Aperture	-	**392 ± 40.0(351–447, n = 4)**
Width	-	**85 ± 19.3(71–116, n = 4)**
Hook length	-	**137 ± 16.5(115–160, n = 4)**
Hook curve length	-	**13 ± 1.43(11–15, n = 4)**
Hook aperture angle	-	**118° ± 5.4(110°–125°, n = 4)**
Hook aperture	-	**117 ± 15.0(98–135, n = 4)**
Hook base width	-	**55 ± 6.7(45–62, n = 4)**
Complex 3 sclerite		
Circumferential length	-	**1097 ± 2.5(1096–1099, n = 4)**
Total length	-	**613 ± 72.8(513–699, n = 4)**
Shaft length	-	**404 ± 65.7(323–483, n = 4)**
Hook-side curve length	-	**100 ± 18.5(84–130, n = 4)**
Shaft-side curve length	-	**120 ± 21.9(95–155, n = 4)**
Total diameter	-	**404 ± 65.7(323–483, n = 4)**
Inner diameter	-	**324 ± 53.2(255–392, n = 4)**
Aperture angle	-	**62° ± 3.9(58°–67°, n = 4)**
Aperture	-	**398 ± 49.7(311–350, n = 4)**
Width	-	**84 ± 15.9(68–104, n = 4)**
Hook length	-	**134 ± 13.5(116–150, n = 4)**

Table 5.2 cont

Hook curve length	-	**12 ± 1.2(11–15, n = 4)**
Hook aperture angle	-	**117° ± 3.8(112°–123°, n = 4)**
Hook aperture	-	**123 ± 13.8(99–135, n = 4)**
Hook base width	-	**57 ± 6.2(46–65, n = 4)**
Hamulus total length	86 (75–98,n = 22)	**84 ± 2.3(83–87, n = 3)**
Hook point length	-	**19 ± 5.5(13–24, n = 3)**
Hook shank length	-	**14 ± 2.4(12–16, n = 3)**
Total width	-	**35 ± 5.2(29–38, n= 3)**
Distal hook point width	-	**5 ± 1.3(3–6, n = 3)**
Outer aperture angle	-	**53° ± 12.8(43°–68°, n = 3)**
Inner aperture angle	-	**71° ± 13.5(58°–85°, n = 3)**
Aperture	-	**17 ± 2.1(14–18, n = 3)**
Hook shank base width	-	**8 ± 1.9(6–10, n = 3)**
Outer root-shaft length	-	**52 ± 13.7(37–64, n = 3)**
Inner root-shaft length	-	**61 ± 12.6(47–72, n = 3)**
Root base width	-	**35 ± 4.4(30–38, n = 3)**
Root base angle	-	**94° ± 8.8(89°–104°, n = 3)**

Bold script = all measurements of the present study
*Note that Glennon *et al.* (2005) did not specify which sucker complex sclerites were being measured. However, since they are all of similar size, they are included into the above Table 5.2 under complex 1.

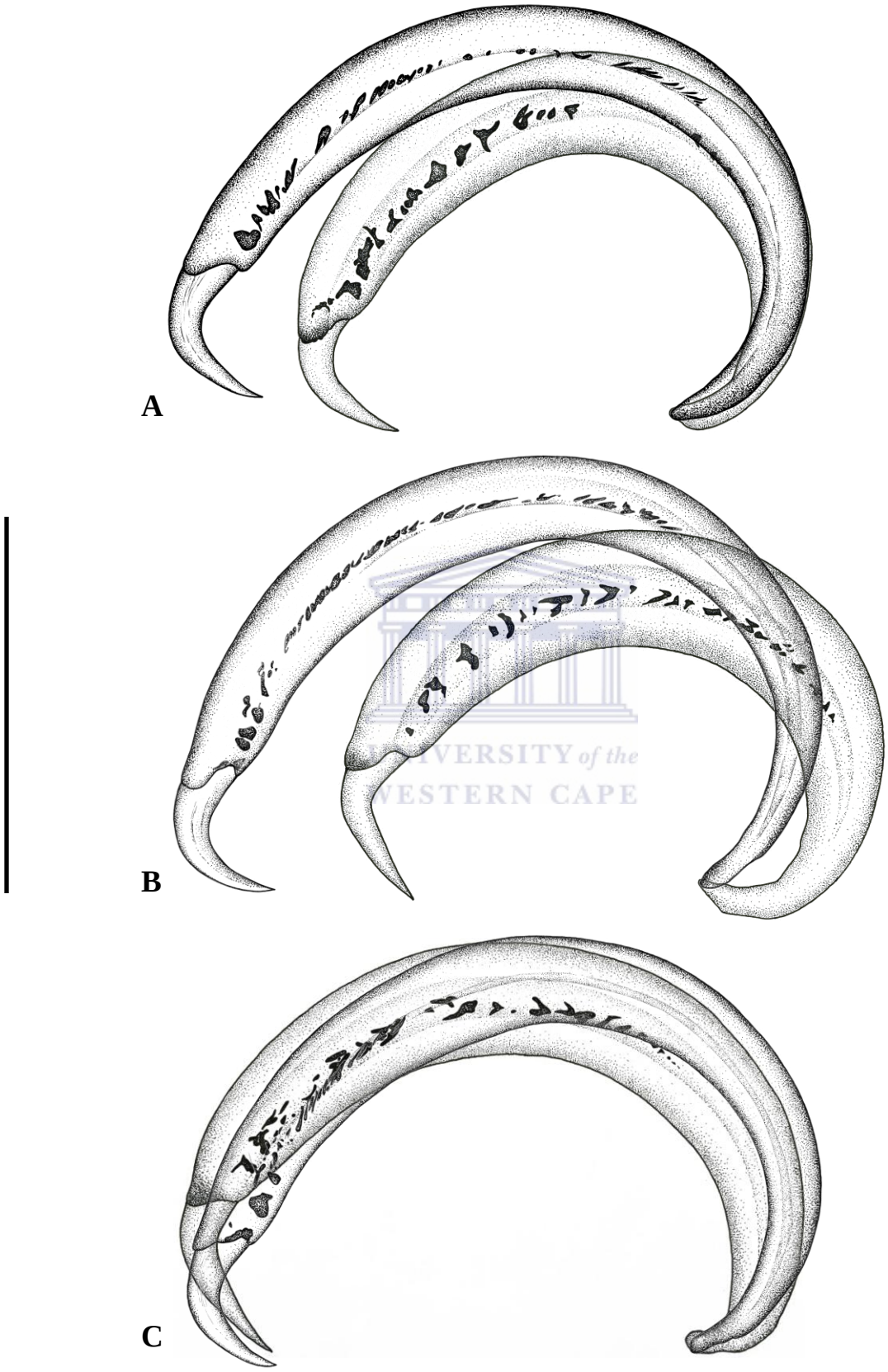

Fig. 5.5 *Branchotenthes octohamatus* and *B. rovinoverstreeti* sclerite image overlays. **A.** complex 1, **B.** complex 2, **C.** complex 3. Scale bar = 390µm.

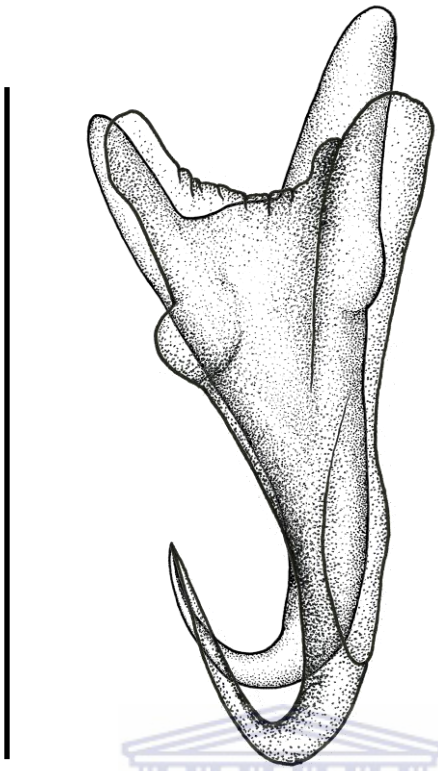

Fig. 5.6 *Branchotenthes octohamatus* and *B. rovinoverstreeti* hamulus image overlays. Scale bar = 80µm.

Table 5.3 Comparative measurements for both *Branchotenthes* species measured in the present study

	B. robinovertstreeti	***B. octohamatus***
Total body length	**4775 ± 35.4(4750–4800, n = 2)**	**5267 ± 2140.9(3200–8200, n = 4)**
Maximum body width	**1858 ± 24.7(1840–1875, n = 2)**	**1837 ± 568.6(1300–2400, n = 4)**
Oral sucker diameter	**629 ± 23.6(613–646, n = 2)**	**377 ± 93.0(306–510, n = 4)**
Pharynx length	**124 ± 27.1(105–143, n = 2)**	**113 ± 14.9(101–133, n = 4)**
Pharynx width	**96 ± 6.4(91–100, n = 2)**	**109 ± 10.1(97–121, n = 4)**
Haptor length	**2583 ± 683.5(2100–3067, n = 2)**	**3798 ± 693.6(2920–4600, n = 4)**
Haptor width	**1367 ± 895.7(733–2000, n = 2)**	**2455 ± 580.9(1800–3200, n = 2)**
Appendix length	**1437 ± 135.2(1341–1532, n = 2)**	**1635 ± 344.1(1197–1920, n = 4)**
Appendix width	**596 ± 172.9(473–718, n = 2)**	**874 ± 180.3(627–1049, n = 4)**
Terminal appendix sucker length	**385 ± 50.9(287–396, n = 4)**	**434 ± 83.9(339–559, n = 8)**
Terminal appendix sucker width	**181 ± 29.7(144–213, n = 4)**	**209 ± 32.6(160–250, n = 8)**
Cirrus total length	**1499 ± 100.0(1429–1570, n = 2)**	**937, n = 1**
Cirrus maximum width	**269 ± 28.4(249–289, n = 2)**	**122, n = 1**
Ovary total length	**666 ± 55.8(626–705, n = 2)**	**791 ± 261.9(571–1171, n = 4)**

Table 5.3 cont	*B. robinovertstreeti*	***B. octohamatus***
Egg length	**163 ± 11.5(155–172, n = 2)**	**121 ± 8.9(108–137, n = 14)**
Egg width	**63 ± 4.5(58–67, n = 3)**	**60 ± 7.3(50–72, n = 14)**
Complex 1 sclerite		
Circumferential length	**1337 ± 78.0(1282–1392, n = 2)**	**1126 ± 31.8(1103–1148, n = 2)**
Total length	**548 ± 32.5(515–581, n = 2)**	**589 ± 64.5(518–689, n = 4)**
Shaft length	**546 ± 36.5(508–586, n = 2)**	**586 ± 68.8(513–692, n = 4)**
Hook-side curve length	**99 ± 9.2(90–112, n = 2)**	**106 ± 15.6(85–128, n = 4)**
Shaft-side curve length	**103 ± 18.6(81–124, n = 2)**	**118 ± 9.6(103–132, n = 4)**
Total diameter	**372 ± 38.0(335–413, n = 2)**	**398 ± 67.9(345–509, n = 4)**
Inner diameter	**276 ± 22.5(250–303, n = 2)**	**317 ± 49.5(274–400, n = 4)**
Aperture angle	**62° ± 6.8(54°–69°, n = 2)**	**60° ± 4.9(52°–68°, n = 4)**
Aperture	**333 ± 30.1(305–373, n = 2)**	**367 ± 37.7(323–416, n = 4)**
Width	**97 ± 16.7(78–112, n = 2)**	**84 ± 19.6(69–117, n = 4)**
Hook length	**146 ± 5.9(140–151, n = 2)**	**135 ± 18.5(107–157, n = 4)**
Hook curve length	**10 ± 1.1(9–11, n = 2)**	**13 ± 2.2(9–16, n = 4)**
Hook aperture angle	**124° ± 3.8(119°–128°, n = 2)**	**117° ± 5.7(112°–127°, n = 4)**
Hook aperture	**134 ± 6.0(127–141, n = 2)**	**117 ± 16.1(92–132, n = 4)**
Hook base width	**59 ± 3.8(56–63, n = 2)**	**55 ± 9.3(41–68, n = 4)**
Complex 2 sclerite		
Circumferential length	**1405, n = 1**	**1135 ± 7.6(1130–1141, n = 4)**
Total length	**599 ± 10.8(592–612, n = 2)**	**614 ± 73.3(531–729, n = 4)**
Shaft length	**592 ± 15.2(580–609, n = 2)**	**609 ± 76.9(524–729, n = 4)**
Hook-side curve length	**112 ± 4.9(109–117, n = 2)**	**102 ± 16.0(84–127, n = 4)**
Shaft-side curve length	**143 ± 3.7(139–145, n = 2)**	**122 ± 15.7(101–140, n = 4)**
Total diameter	**378 ± 27.0(352–406, n = 2)**	**402 ± 63.3(349–507, n = 4)**
Inner diameter	**273 ± 17.2(254–286, n = 2)**	**319 ± 45.2(276–396, n = 4)**
Aperture angle	**61° ± 4.8(56°–66°, n = 2)**	**62° ± 3.2(57°–68°, n = 4)**
Aperture	**323 ± 14.5(315–343, n = 2)**	**392 ± 40.0(351–447, n = 4)**
Width	**104 ± 14.2(94–121, n = 2)**	**85 ± 19.3(71–116, n = 4)**
Hook length	**166 ± 0.6(166–167, n = 2)**	**137 ± 16.5(115–160, n = 4)**
Hook curve length	**11 ± 2.9(7–12, n = 2)**	**13 ± 1.43(11–15, n = 4)**
Hook aperture angle	**130° ± 4.6(125°–134°, n = 2)**	**118° ± 5.4(110°–125°, n = 4)**
Hook aperture	**154 ± 5.8(147–158, n = 2)**	**117 ± 15.0(98–135, n = 4)**
Hook base width	**63 ± 8.3(54–69, n = 2)**	**55 ± 6.7(45–62, n = 4)**
Complex 3 sclerite		
Circumferential length	**1388 ± 16.5(1377–1400, n = 2)**	**1097 ± 2.5(1096–1099, n = 4)**
Total length	**618 ± 25.8(594–649, n = 2)**	**613 ± 72.8(513–699, n = 4)**
Shaft length	**613 ± 28.2(586–644, n = 2)**	**404 ± 65.7(323–483, n = 4)**
Hook-side curve length	**119 ± 3.9(113–122, n = 2)**	**100 ± 18.5(84–130, n = 4)**
Shaft-side curve length	**126 ± 9.9(113–137, n = 2)**	**120 ± 21.9(95–155, n = 4)**
Total diameter	**400 ± 23.7(373–422, n = 2)**	**404 ± 65.7(323–483, n = 4)**
Inner diameter	**297 ± 10.2(283–308, n = 2)**	**324 ± 53.2(255–392, n = 4)**
Aperture angle	**65° ± 3.8(62°–71°, n = 2)**	**62° ± 3.9(58°–67°, n = 4)**
Aperture	**159 ± 3.0(157–162, n = 2)**	**398 ± 49.7(311–350, n = 4)**
Width	**104 ± 15.4(91–121, n = 2)**	**84 ± 15.9(68–104, n = 4)**

Table 5.3 cont	*B. robinovertstreeti*	***B. octohamatus***
Hook length	**167 ± 2.3(164–169, n = 2)**	**134 ± 13.5(116–150, n = 4)**
Hook curve length	**10 ± 2.5(7–12, n = 2)**	**12 ± 1.2(11–15, n = 4)**
Hook aperture angle	**130° ± 3.8(128°–135°, n = 2)**	**117° ± 3.8(112°–123°, n = 4)**
Hook aperture	**159 ± 3.0(157–162, n = 2)**	**123 ± 13.8(99–135, n = 4)**
Hook base width	**67 ± 1.1(65–68, n = 2)**	**57 ± 6.2(46–65, n = 4)**
Hamulus total length	**84, n = 1**	**84 ± 2.3(83–87, n = 3)**
Hook point length	**24, n = 1**	**19 ± 5.5(13–24, n = 3)**
Hook shank length	**14, n = 1**	**14 ± 2.4(12–16, n = 3)**
Total width	**42, n = 1**	**35 ± 5.2(29–38, n= 3)**
Distal hook point width	**5, n = 1**	**5 ± 1.3(3–6, n = 3)**
Outer aperture angle	**33°, n = 1**	**53° ± 12.8(43°–68°, n = 3)**
Inner aperture angle	**40°, n = 1**	**71° ± 13.5(58°–85°, n = 3)**
Apertutre	**13, n = 1**	**17 ± 2.1(14–18, n = 3)**
Hook shank base width	**13, n = 1**	**8 ± 1.9(6–10, n = 3)**
Outer root-shaft length	**64, n = 1**	**52 ± 13.7(37–64, n = 3)**
Inner root-shaft length	**63, n = 1**	**61 ± 12.6(47–72, n = 3)**
Root base width	**37, n = 1**	**35 ± 4.4(30–38, n = 3)**
Root base angle	**105°, n = 1**	**94° ± 8.8(89°–104°, n = 3)**

Bold script = all measurements of the present study

5.3 Discussion

Bullard and Dippenaar (2003) in their generic diagnosis indicated *Branchotenthes* as "gill parasites of elasmobranchs." This statement is non-specific since all but one hexabothriid genus (*Callorhynchocotyle*) are gill parasites of elasmobranchs. Glennon *et al*. (2005) made reference to members of *Branchotenthes* in their amended generic diagnosis as "gill parasites of rhynchobatids (sharkfin guitarfish) and rhinobatids (shovelnose rays)." *Branchotenthes robinoverstreeti* and *B. octohamatus* are gill parasites of *Rhina ancylostoma*, and *Trigonorrina spp.* and *Aptychotrema* spp. respectively. Host genera belong to Rhinobatidae. None of the host genera are rhynchobatid (genus *Rhynchobatus* Forsskål, 1775) and no *Branchotenthes* species have to date been collected from any rhynchobatids.

Glennon *et al.* (2005) reported that the oncomiracidium of *B. octohamatus* possesses only 8 marginal hooklets. Although they excluded the larval description of Suriano and Incorvaia (1982) when mentioning that all previous hexabothriid larva

descriptions included 10 marginal hooklets, *B. octohamatus* in currently the only hexabothriid species to possess 8.

Seven *T. fasciata* were collected by Glennon *et al.* (2005) and maintained in a 1000ℓ aquarium for a period of time to allow parasite numbers to increase prior to the study. Two individual *T. fasciata* were euthanased and adult *B. octohamatus* removed for identification (Glennon *et al*. 2005). Remaining hosts were individually removed to separate bins to allow parasites to produce eggs which were subsequently filtered from the water for incubation and larval description (Glennon *et al*. 2005). The above experiment assumes that all host fishes were parasitised by a single hexabothriid taxon, where it is known that representatives of both *Rhinobatonchocotyle* and *Branchotenthes* are gill parasites of rhinobatoid hosts. There is no mention by Glennon *et al*. (2005) that the remaining *T. fasciata* were euthanased to check whether indeed they were dealing with a hexabothriid monoculture. Therefore, future experimentation for obtaining eggs for larval descriptions should either be done *in vitro* or host fishes should be euthanased post-experimentation to confirm the monoculture.

The generic diagnosis of *Branchotenthes* is amended to include the splitting of the intestinal caecum branch in the haptoral appendix into 3.

CHAPTER 6

Discussion

Supporting the on-going development of proactive quarantine procedures for captive chondrichthyan fishes in public aquaria, through the dissemination of information on host-parasite associations, is crucial. Chondrichthyan fishes including elasmobranchs and holocephalans are in general, highly vulnerable to human-induced pressures such as over-fishing (and by-catch), pollution and habitat destruction. As such, emphasis in public aquaria internationally is placed on their conservation, providing an educational platform from which to launch the plight of these fishes to the general public. Smith *et al*. (2004) highlighted the importance of ever-improving captive health care and management of captive chondrichthyan fishes emphasising that public aquaria have a role to play in providing crucial information on various unknown aspects of their biology, ecology and health.

Callorhinchus capensis and *Rhina ancylostoma* are sought after aquarium chondrichthyan fishes both with a history of being kept with varying degrees of success in captivity although not without parasite problems. The Two Oceans Aquarium maintained an exhibit of *Callorhinchus capensis* for up to a year on 2 separate occasions from 1999-2003 and experienced chronic mortalities as a result of monogenean infestations of the gills (*Callorhynchocotyle callorhynchi* and *Callorhynchicola multitesticulatus* Manter, 1955). These monogeneans were quantified post-mortem and reported on at the 6th International Symposium on Fish Parasites held in Bloemfontein in 2003 (Christison *et al.* 2003). The aquarium-held *C. capensis* monogenean intensities were compared to those of wild-caught *C. capensis* collected off the West coast of South Africa. Intensities of *Callorhynchocotyle callorhynchi* in the aquarium-held fish were significantly higher. Recently, mortalities of captive *R. ancylostoma* in Dubai were linked to monogenean infestations. However, these were not retained for identification (*pers. comm*. Paul Lötter, Director of Large exhibits, Atlantis, Dubai). This raises an important problem. It appears that aquatic animal health workers in public aquaria remain ignorant about the importance of monogeneans in captivity often until it is too late. This could possibly be partly due to the lack of specific training in the field of aquatic animal health

specifically in developing countries like South Africa, but also the lack of information available to them on the specific health problems associated with aquarium-held fishes.

Monogeneans, in general are of particularly high importance to public aquarium fish collections because of their ability to reproduce so successfully under captive conditions often resulting in host mortalities in a short space of time. A large void exists however, between the advancement of aquatic parasitology in the global aquaculture and public aquarium industries where the former industry has historically been subjected to massive economic losses as a result of monogeneans. This has resulted in a reciprocal investment in the active and proactive management of problematic species (see examples Bakke *et al*. 2007; Bondad-Reantasso *et al*. 2005; Ernst *et al*. 2005; Hayward *et al*. 2007; Lackenby *et al*. 2007; Mooney *et al*. 2006; Williams *et al*. 2007). Monogeneans are generally host-specific with a few exceptions (e.g. Deveney *et al*. 2001; Vaughan and Chisholm 2009). Monocultures of fishes are therefore particularly vulnerable to the affects of their associated monogeneans with the potential to infect entire culture populations. Public aquarium exhibits on the other hand are usually made up of mixed species kept together and therefore the impacts of their associated monogeneans are often only seen at the species-level. As such, monogeneans in public aquaria are often not considered as large a threat to an entire mixed exhibit as those in aquaculture monocultures. This is however misleading since the cumulative affects of monogenean-related mortalities of all those species affected in an aquarium are not taken into account together but are rather considered as isolated incidents.

Hexabothriids are important monogeneans of captive chondrichthyan fishes (see Bullard *et al*. 2001, Wiskin 1970). However, the lack of consensus in the morphometric discrimination of hexabothriid species, including conflicting measurement protocols for sucker complex sclerites of *Callorhynchocotyle* species provides much confusion and ambiguity throughout the historical literature including obvious errors (see Chapter 1). Linear measurements should be considered as dimensions between homologous points or between points of constant topography (Brower and Veinus 1978). However, in the historic and current hexabothriid literature many linear measurements of sucker complex sclerites are not made relative to a consistent set of reference axes (see Chapter 1). In the present study all characters were orientated within a rectangular reference grid after the

example of Browner and Veinus (1978) to consistently return points of measurement and to reduce variance associated with measurement interpretation (ambiguous measurements). The accurate identification of hexabothriid species is of utmost importance since an understanding of species is essential in understanding their specific biology and host associations. This ultimately facilitates the correct treatments employed to control and manage them in captivity based on host-parasite profiling. For this reason, the new sets of variables for the characters of the haptoral armature were tested, to introduce a new protocol that could be used to support robust species discriminations without being subjected to the inconsistencies of different methods.

Monogenean taxonomy is qualitative. Demands on taxonomists to support their conclusions and hypotheses quantitatively using statistical techniques (see Shinn *et al*. 1994, 1996, 2001; Du Preez and Maritz 2006), are increasing. And in some cases with the added support of molecular evidence (see Bakke *et al*. 2007). This quantitative approach was preliminarily applied to test 15 sucker complex sclerite and 13 hamulus character variables proposed for hexabothriids using the species examples representing *Callorhynchocotyle* and the control hexabothriid taxon *Rajonchocotyle alba*. All sucker complex sclerites are allometric, affected in size by age given their ontogeny (see Wiskin 1970), therefore having an effect on the variability of certain measures. As a result, the surrogate variable for age (circumferential length) was selected to be used to ratio-transform the data of age-dependant variables determined from correlations of variables to reduce age-associated variance prior to multivariate analysis. Character variables with consistently high coefficient of variance after the elimination of age-associated variance and/or log and Cos-transformations were disqualified for use in the multivariate analysis. High variances in these variables was likely due to their small size and therefore due to measurement error.

The sensitivity of univariate analyses was compromised due to the small samples sizes of specimens measured for each species. Obvious differences between the complex 1 sucker-complex sclerites of *C. sagamiensis* and the other species were not considered significant although it is suspected that they would be, given larger samples sizes. No single character variable could separate all the *Callorhynchocotyle* species from each other. However, the variables C1A, C2AA, C2A, C3AA, C3A and HHPL could

significantly separate all the *Callorhynchocotyle* species from the control taxon *R. alba*. The characters combined were tested for their robustness in discriminating all the *Callorhynchocotyle* species using multivariate analysis. Here, the control taxon and *C. sagamiensis* were clearly separated from each other and the other *Callorhynchocotyle* species, but these could not be separated from each other. *Callorhynchocotyle sagamiensis* is one of 2 species found on hosts belonging to Chimaeridae. The second species belonging to a chimaerid host, *C. hydrolagi*, was excluded from all analyses due to a lack of data resulting from the poor condition of specimens representing the 2 type specimens examined. The other species are all found on hosts in Callorhynchidae. Multivariate analyses require large data sets. Specimens with incomplete data for characters and variables cannot be used. It is unfortunate that additional specimens of *C. hydrolagi* could not be accessed to provide some clarity on the relationship of this species' set of characters with those of the other species.

Various combinations of the sucker-complex sclerites and hamulus could not be performed because of the small samples sizes for the specimens measured, however the results of the analysis of combined characters showed merit. The overall limitations experienced by the small sample sizes of species used in the present study provided some support to accept the first hypothesis that the haptoral armature consists of robust characters for separating hexabothriid taxa. The second hypothesis that the new morphometric method will separate the *Callorhynchocotyle* species representing the host families Callorhynchidae and Chimaeridae cannot be conclusively supported at this time due to the lack of availability of specimens.

The combination of the 3 sucker-complex sclerite characters was used to separate *C. amatoi* from *C. callorhynchi* and *C. marplatensis* by Boeger *et al*. (1989). These authors indicated that *C. amatoi* differed from *C. callorhynchi* and *C. marplatensis* by having shorter shafts and points of all 3 sucker complex sclerites, and an indistinct angle between the "point and shaft of sclerite 3." With the addition of new material and the inclusion of a larger sample size for *C. callorhynchi*, these differences may only represent differences in size or age between the specimens examined historically and may therefore not represent true differences. Further investigation of these characters but in various combinations supported by larger sample sizes, is therefore warranted.

Traditionally, little emphasis has been given to the importance of hexabothriid hamuli in species descriptions with the exception of Beverley-Burton and Chisholm (1990) for *C. hydrolagi*, and Glennon *et al*. (2005) for *B. octohamatus*. Kitamura *et al*. (2006) provided some basic differences in the hamulus of *C. sagamiensis* and *C. hydrolagi*. The hamulus character, however, is used extensively in the identification of other monogenean taxa including *Gyrodactylus* (see Shinn *et al*. 1996, 1001, 2004; Bakke *et al*. 2007). In the present study, factor loading of the various retained character variables identified 8 of 12 hamulus variables significantly influencing the analysis. The hamulus character has the potential to provide species-level information previously not considered in detail in hexabothriid literature. The use of this character for future hexabothriid species identifications is therefore suggested.

All *Callorhynchocotyle* species possess hamuli with acute root base angles while the control taxon possesses hamuli with an obtuse angle. It should be noted that traditional hamulus measurements for monogeneans with a hamulus bearing an inner and an outer root performed poorly with higher variable variances compared to the variables of the new protocol using initial character quadrangular orientation. This may have significance when critically reviewing members of various monogenean taxa.

Callorhynchocotyle species from hosts of Callorhynchidae are all very similar to each other. *Callorhynchocotyle callorhynchi* is the most similar species to *C. marplatensis*, based on the results of the new morphometric method. Dillon and Hargis (1968) did not consider the possibility that the *Callorhynchocotyle* species (now *C. amatoi*) they collected from New Zealand, was a different species when compared to the type material of *C. callorhynchi* even though they indicated considerable differences in the lengths of the sucker complex sclerite hooks between the 2 species. *Callorhynchocotyle amatoi* is more similar to *C. marplatensis* when considering the structure of the hamulus, however, the complex 2 and 3 sucker complex sclerites are similarly to those of the species representing the host family Chimaeridae (see similarity in sclerite overlays of Chapter 4).

Callorhynchocotyle marplatensis remains the only species without papillae lining the inner wall of the sclerite suckers of the haptor, as well as the oral sucker. All literature subsequent to Suriano and Incorvaia (1982), however, failed to question why, in the

original description, Suriano and Incorvaia (1982) specifically indicated the presence of many small cuticular tubercles[4] (papillae) on the inner surface of the oral sucker. The voucher specimens examined in the present study are representatives of the species collected by Boeger *et al*. (1989) off Uruguay from the same host species *Callorhinchus callorhynchus*. No papillae were present in the oral or haptoral suckers of these vouchers, scrutinised under high magnification and phase-contrast microscopy, supporting the redescription of Boeger *et al.* (1989). Of the original type material, Boeger *et al.* (1989) only examined the Holotype (MP 12 – Museo de La Plata), which they admitted was mounted upside down, excessively flattened, contracted and damaged. Therefore, emphasis is placed on the recommendation that paratype material should be used in future comparison to the vouchers of Boeger *et al*. (1989) to confirm the absence of papillae in original material. Or, if possible, additional vouchers from the type locality should be collected and scrutinised in comparison to these vouchers. It could be possible that Suriano and Incorvaia (1982) were mistaken in their indication of oral sucker papillae, made difficult to confirm or deny by the condition of the Holotype (Boeger *et al.* 1989). Alternatively, these differences could indicate the possibility of separate populations represented by slightly different phenotypes.

Shinn *et al*. (1996, 2001) provided resolution between previously morphologically indistinguishable *Gyrodactylus* species using a similar, yet more advanced set of exploratory multivariate analyses of the sclerotised haptoral armature. Given the similar approach to finding resolution between hexabothriid species examples in the present study it may be possible to provide evidence of population structures with the inclusion of more advanced analyses concentrating on hexabothriid species over large geographical distributions. Future investigations of other hexabothriid taxa from South Africa will include a separation of specimens from each trawl location along the coastline prior to comparative analysis.

Several published errors have been addressed in the present study. Total body length measurements of hexabothriids inclusive of the haptor in those which possess asymmetrical haptors (e.g. *Callorhynchocotyle* spp.) cannot be accurately measured because the true longitudinal axes of the body proper and the haptor do not support a continuous measurement. Therefore all hexabothriids measured in the present study

[4]From the translation of the original manuscript into English.

included separate body proper and haptor measurements *sensu* Beverley-Burton and Chisholm (1990). Erroneous hamulus measurements of *Callorhynchocotyle* species of Boeger *et al*. (1989) (Beverley-Burton and Chisholm 1990), and erroneous appendix measurements of *C. amatoi* of Boeger *et al.* (1989) were re-measured using some of the original type series specimens and additional vouchers. The erroneous cirrus length measurement for *C. callorhynchi* in Beverley-Burton and Chisholm (1990) is also corrected using existing and additional voucher material. In addition to measurements of characters, the inconsistencies in character nomenclature in historic literature is addressed using the example of Wiskin (1970) who was the first author to separate the sucker complex sclerites and hamulus into 2 defined working areas, namely the hook and shaft. There is a lack of nomenclature discriminating the shaft from the hook of the sucker complex sclerites and hamulus of hexabothriids prior to her investigation of *R. emarginata*. After Wiskin (1970) however, many authors made reference to the same separation of the sucker complex sclerites into these 2 basic components, without consistency in their nomenclature. This may have led to the confusion in nomenclature for the sucker complex sclerite hook as the shaft in Boeger *et al*. (1989), subsequently followed by Beverley-Burton and Chisholm (1990) for *C. hydrolagi*.

Currently, only 2 *Branchotenthes* species have been recorded, the first from South African waters and the second, *B. octohamatus* from Australia. *Branchotenthes octohamatus* can be separated from *B. robinoverstreeti* using the morphometrics of the new method tested on *Callorhynchocotyle* in addition to the characters of Bullard and Dippenaar (2003) and Glennon *et al*. (2005). *B. octohamatus* possesses larger overall sucker complex sclerites although *B. robinoverstreeti* sucker complex hook lengths of complex 2 and 3 sucker sclerites are longer. Convention, until recently, supported the idea that hexabothriids were host-specific. Glennon *et al.* (2008), however, provided evidence that *B. octohamatus* was not strictly host-specific. *Branchotenthes octohamatus* was collected from 4 different rhinobatoid hosts with overlapping distributions. If evidence of this species' lack of host-specificity is a reflection of the potential of hexabothriids in general, given the overlapping distributions of many chondrichthyan fishes, it remains possible that hexabothriids in captivity may present a previously overlooked problem to mixed species exhibits. This is certainly true for other

monogeneans of chondrichthyan fishes in captivity, given the evidence provided by Vaughan *et al*. (2008) and Vaughan and Chisholm (2009) for the potential of *Dendromonocotyle* species (Monogenea: Monocotylidae) infecting stingrays to host-switch under captive conditions. Of the 21 chondrichthyan fish taxa for which unconfirmed hexabothriid species have been collected from South Africa, 9 are earmarked for future collection and public exhibition. It is anticipated that future work will address the taxonomy of hexabothriids associated with these host fishes, using an approach similar to that of the present study, with the aim of providing much needed resolution to Hexabothriidae while supporting the captive husbandry of chondrichthyan fishes.

CHAPTER 7

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Levene test of homogeneity of variance (Statistica 6) for all character variables.

Variables	SS effect	df effect	MS effect	SS error	df error	MS error	F	*p*
C1CL	0.000	3.000	0.000	0.005	35.000	0.000	0.944	0.429
C1TL	**0.005**	**3.000**	**0.001**	**0.008**	**35.000**	**0.000**	**7.811**	**0.000**
C1TD	0.000	3.000	0.001	0.006	35.000	0.000	0.756	0.526
C1SW	0.000	3.000	0.000	0.037	35.000	0.001	0.132	0.940
C1SSL	**0.004**	**3.000**	**0.001**	**0.007**	**35.000**	**0.000**	**7.283**	**0.000**
C1ID	0.000	3.000	0.000	0.008	35.000	0.000	0.811	0.496
C1AA	**0.149**	**3.000**	**0.049**	**0.181**	**35.000**	**0.005**	**9.600**	**0.000**
C1A	**0.054**	**3.000**	**0.018**	**0.044**	**35.000**	**0.001**	**14.539**	**0.000**
C1HSCL	0.002	3.000	0.000	0.014	35.000	0.000	2.070	0.121
C1SSCL	**0.009**	**3.000**	**0.003**	**0.029**	**35.000**	**0.000**	**3.657**	**0.021**
C1HL	0.002	3.000	0.000	0.016	35.000	0.000	2.083	0.120
C1HCL	0.006	3.000	0.002	0.057	35.000	0.001	1.373	0.266
C1HAA	0.019	3.000	0.006	0.145	35.000	0.004	1.581	0.211
C1HA	0.004	3.000	0.001	0.031	35.000	0.000	1.621	0.202
C1HBW	0.002	3.000	0.000	0.021	35.000	0.000	1.194	0.326
C2CL	0.000	3.000	0.000	0.003	35.000	0.000	2.134	0.113
C2TL	0.000	3.000	0.000	0.003	35.000	0.000	1.955	0.138
C2TD	0.000	3.000	0.000	0.004	35.000	0.000	2.532	0.072
C2SW	0.001	3.000	0.000	0.026	35.000	0.000	0.636	0.596
C2SSL	0.000	3.000	0.000	0.004	35.000	0.000	1.291	0.292
C2ID	0.001	3.000	0.000	0.005	35.000	0.000	2.712	0.059
C2AA	**0.026**	**3.000**	**0.008**	**0.073**	**35.000**	**0.002**	**4.270**	**0.011**
C2A	**0.047**	**3.000**	**0.015**	**0.023**	**35.000**	**0.000**	**23.512**	**0.000**
C2HSCL	0.002	3.000	0.000	0.033	35.000	0.000	0.710	0.552
C2SSCL	0.004	3.000	0.001	0.026	35.000	0.000	1.818	0.161
C2HL	0.002	3.000	0.000	0.037	35.000	0.001	0.753	0.527
C2HCL	0.004	3.000	0.001	0.026	35.000	0.000	2.054	0.124
C2HAA	0.004	3.000	0.001	0.138	35.000	0.003	0.360	0.782
C2HA	0.003	3.000	0.001	0.046	35.000	0.001	0.821	0.490
C2HBW	0.000	3.000	0.000	0.013	35.000	0.000	0.506	0.680
C3CL	0.000	4.000	0.000	0.004	36.000	0.000	1.712	0.169
C3TL	0.000	4.000	0.000	0.003	36.000	0.000	1.048	0.396
C3TD	0.000	4.000	0.000	0.005	36.000	0.000	1.190	0.331
C3SW	0.002	4.000	0.000	0.011	36.000	0.000	2.104	0.100
C3SSL	0.000	4.000	0.000	0.004	36.000	0.000	0.682	0.608
C3ID	0.001	4.000	0.000	0.007	36.000	0.000	1.534	0.213
C3AA	0.003	4.000	0.000	0.072	36.000	0.002	0.375	0.824
C3A	**0.007**	**4.000**	**0.001**	**0.016**	**36.000**	**0.000**	**4.301**	**0.006**
C3HSCL	0.002	4.000	0.000	0.009	36.000	0.000	2.289	0.078
C3SSCL	0.004	4.000	0.001	0.022	36.000	0.000	1.890	0.133
C3HL	0.002	4.000	0.000	0.021	36.000	0.000	1.087	0.377

Levene test cont

C3HCL	0.006	4.000	0.001	0.038	36.000	0.001	1.616	0.191
C3HAA	0.024	4.000	0.006	0.149	36.000	0.004	1.459	0.234
C3HA	0.004	4.000	0.001	0.029	36.000	0.000	1.270	0.299
C3HBW	0.002	4.000	0.000	0.017	36.000	0.000	1.036	0.402
HTL	0.000	4.000	0.000	0.004	28.000	0.000	0.388	0.815
HTW	0.000	4.000	0.000	0.003	28.000	0.000	1.956	0.128
HHPL	**0.014**	**4.000**	**0.003**	**0.005**	**28.000**	**0.000**	**18.240**	**0.000**
HDHPW	0.001	4.000	0.000	0.004	28.000	0.000	1.541	0.217
HHSL	0.002	4.000	0.000	0.013	28.000	0.000	1.162	0.384
HOAA	0.000	4.000	0.000	0.000	28.000	0.000	1.089	0.380
HIAA	**0.054**	**4.000**	**0.013**	**0.043**	**28.000**	**0.001**	**8.817**	**0.000**
HA	**0.005**	**4.000**	**0.001**	**0.006**	**28.000**	**0.000**	**6.219**	**0.001**
HHSBW	0.011	4.000	0.002	0.044	28.000	0.001	1.820	0.152
HORL	**0.003**	**4.000**	**0.000**	**0.003**	**28.000**	**0.000**	**7.177**	**0.000**
HIRL	**0.003**	**4.000**	**0.000**	**0.005**	**28.000**	**0.000**	**5.232**	**0.002**
HRBA	0.000	4.000	0.000	0.002	28.000	0.000	0.718	0.586
HBW	0.005	4.000	0.001	0.025	28.000	0.000	1.480	0.234

Character variables representing parametric data are highlighted in red. These variables were subjected to ANOVA using the post-hoc Tukey HSD test for unequal N (Spjotvoll/Stoline). All remaining variables, representing non-parametric data, were subjected to Kriskal-Wallis ANOVA of ranks.

Abbreviations: C1 – complex 1 sucker sclerite; C2 – complex 2 sucker sclerite; C3 – complex 3 sucker sclerite; H – hamulus; CL – circumferential length; TL – total length; TD – total diameter, SW – shaft width; SSL – scletite shaft length; ID – inner diameter; AA – aperture angle; A – aperture; HSCL – hook-side curve length; SSCL – shaft-side curve length; HL – hook length; HPL – hook point length; DHPW – distal hook point width; HSL – hook shank length; OAA – outer aperture angle; IAA – inner aperture angle; HSBW – hook shank base width; ORL – outer root length; IRL – inner root length; RBA – root base angle; BW – base width.

Complex 1 sucker sclerite character variables

	TL	TD	SW	SSL	ID	AA	A	HSCL	SSCL	HL	HCL	HAA	HA	HBW
CL	**0.498**	**0.614**	-0.090	**0.539**	**0.636**	**0.379**	0.021	0.344	**0.430**	0.033	-0.057	-0.004	0.013	0.124
	p= .005	**p= .000**	p= .634	**p= .002**	**p= .000**	**p= .039**	p= .912	p= .062	**p= .017**	p= .861	p= .762	p= .982	p= .942	p= .513

Fig. A. *Callorhynchocotyle callorhynchi* (n = 30): Complex 1 sucker sclerite character variable correlation between the surrogate variable for age (CL) and all remaining variables indicating *R* and *P* values. Significant ($p = \leq 0.05$) correlations are highlighted in red for the age-dependant variables. Abbreviations: CL – circumferential length; TL – total length; TD – total diameter; SW – sclerite width; SSL – sclerite shaft length; ID – inner diameter; AA – aperture angle; A – aperture; HSCL – hook-side curve length; SSCL – shaft-side curve length; HL – hook length; HCL – hook curve length; HAA – hook aperture angle; HA – hook aperture; HBW – hook base width.

Complex 2 sucker sclerite character variables

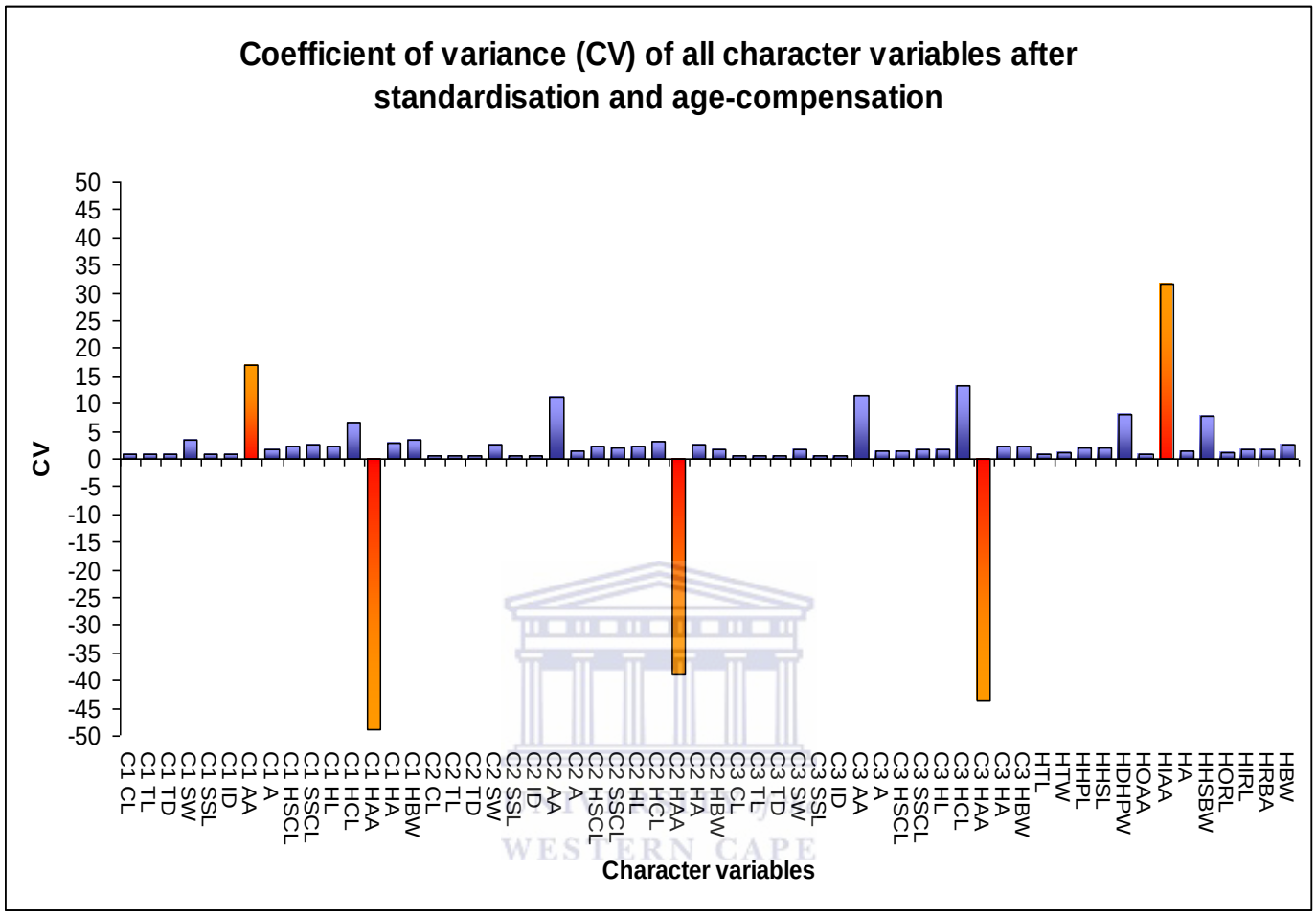
	TL	TD	SW	SSL	ID	AA	A	HSCL	SSCL	HL	HCL	HAA	HA	HBW
CL	**0.692**	**0.677**	0.307	**0.699**	**0.608**	0.333	-0.017	**0.525**	**0.520**	**0.557**	**0.522**	-0.176	**0.457**	**0.551**
	p= .000	**p= .000**	p= .098	**p= .000**	**p= .000**	p= .072	p= .925	**p= .003**	**p= .003**	**p= .001**	**p= .003**	p= .352	**p= .011**	**p= .002**

Fig. B. *Callorhynchocotyle callorhynchi* (n = 30): Complex 2 sucker sclerite character variable correlation between the surrogate variable for age (CL) and all remaining variables indicating *R* and *P* values. Significant ($p = \leq 0.05$) correlations are highlighted in red for the age-dependant variables.

Complex 3 sucker sclerite character variables

	TL	TD	SW	SSL	ID	AA	A	HSCL	SSCL	HL	HCL	HAA	HA	HBW
CL	**0.688**	**0.755**	**0.367**	**0.695**	**0.703**	**0.428**	0.111	**0.480**	**0.638**	**0.395**	**0.397**	-0.029	0.273	0.278
	p= .000	**p= .000**	**p= .046**	**p= .000**	**p= .000**	**p= .018**	p= .954	**p= .007**	**p= .000**	**p= .031**	**p= .030**	p= .879	p= .144	p= .136

Fig. C. *Callorhynchocotyle callorhynchi* (n = 30): Complex 3 sucker sclerite character variable correlation between the surrogate variable for age (CL) and all remaining variables indicating *R* and *P* values. Significant ($p = \leq 0.05$) correlations are highlighted in red for the age-dependant variables.



Callorhynchocotyle callorhynchi (n = 30): Coefficient of variance (CV) of transformed data for all character variables. Character variables disqualified due to high CV values resulting from possible measurement error, are indicated in red. Negative values originate from the Cosine-transformation of obtuse angles.

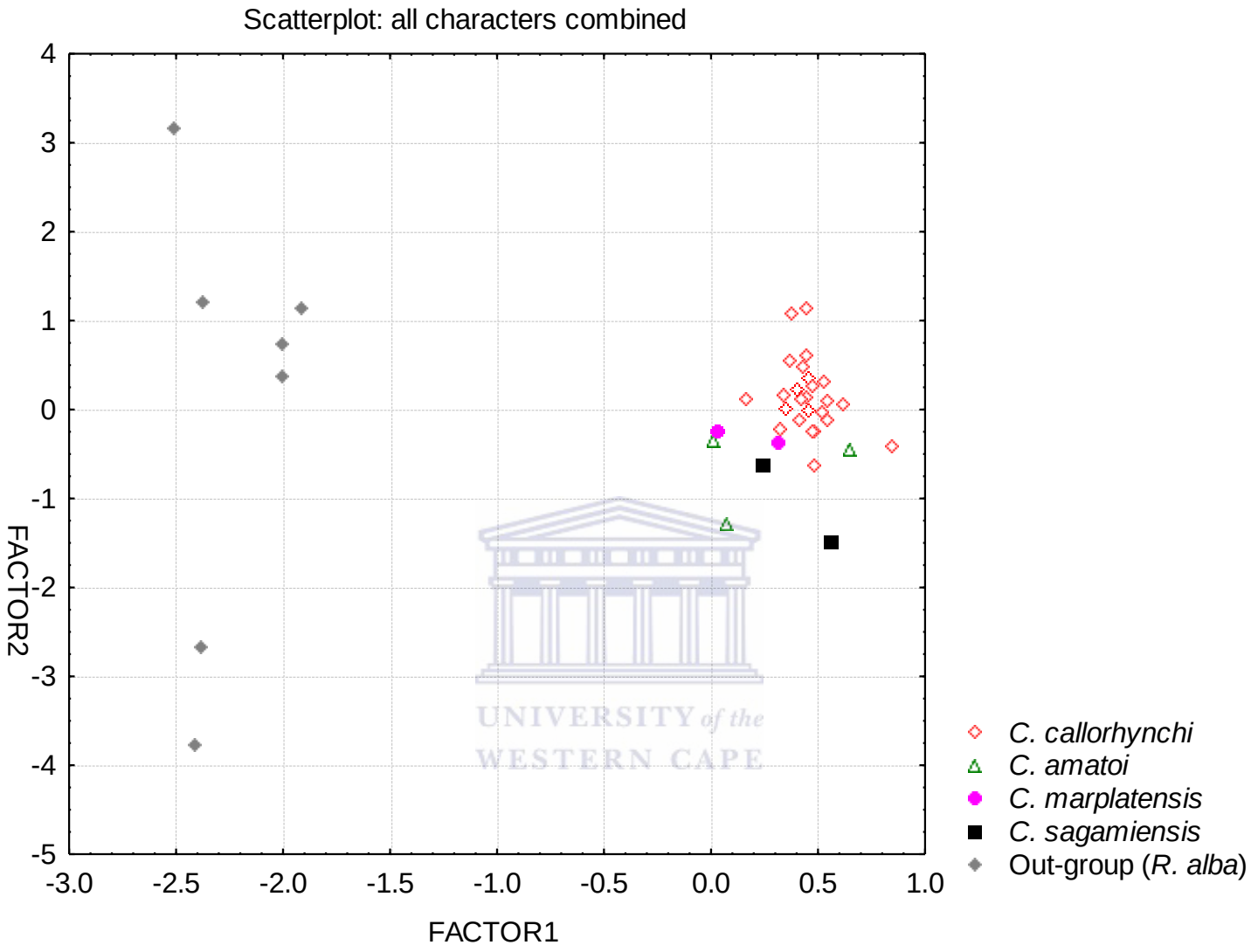
APPENDIX 4 – Factor loadings for multivariate analysis

Character		Factor 1	Factor 2	Factor 3
	Eigenvalue	**24.438**	**8.209**	**5.181**
	Total variance explained %	**46.109**	**15.489**	**9.776**
	Cumulative variance %	**46.109**	**61.599**	**71.376**
C1	Circumferential length	**-0.838**	-0.306	0.295
C1	Total length	-0.688	0.227	-0.243
C1	Total diameter	**-0.723**	0.160	0.406
C1	Shaft width	-0.415	-0.150	**0.737**
C1	Shaft length	-0.698	0.243	-0.255
C1	Inner diameter	**-0.801**	0.159	0.207
C1	Aperture	**-0.916**	-0.064	-0.114
C1	Hook-side curve length	**-0.790**	-0.142	0.388
C1	Shaft-side curve length	-0.258	0.118	0.003
C1	Hook length	0.084	-0.228	**0.841**
C1	Hook curve length	**0.839**	-0.107	0.113
C1	Hook aperture	0.226	-0.206	**0.807**
C1	Hook base width	-0.329	-0.289	**0.815**
C2	Circumferential length	-0.563	-0.369	-0.103
C2	Total length	-0.080	**0.933**	0.098
C2	Total diameter	0.010	**0.849**	0.307
C2	Shaft width	0.655	0.099	0.366
C2	Shaft length	-0.436	0.767	0.306
C2	Inner diameter	-0.339	**0.836**	0.134
C2	Aperture angle	**0.829**	-0.090	0.330
C2	Aperture	**-0.818**	0.225	-0.239
C2	Hook-side curve length	0.583	0.489	-0.020
C2	Shaft-side curve length	0.379	0.468	0.312
C2	Hook length	**0.972**	0.091	-0.007
C2	Hook curve length	**0.943**	0.087	-0.183
C2	Hook aperture	**0.967**	0.087	-0.019
C2	Hook base width	**0.973**	0.065	0.108
C3	Circumferential length	-0.570	-0.394	0.006
C3	Total length	0.070	**0.924**	-0.089
C3	Total diameter	0.066	**0.898**	0.286
C3	Shaft width	**0.816**	0.197	0.382
C3	Shaft length	-0.562	0.663	0.144
C3	Inner diameter	-0.275	**0.857**	0.069
C3	Aperture angle	**0.754**	-0.191	0.324
C3	Aperture	**-0.729**	0.319	-0.253
C3	Hook-side curve length	0.573	0.598	-0.062
C3	Shaft-side curve length	0.333	0.363	0.341
C3	Hook length	**0.982**	0.058	-0.031
C3	Hook curve length	**0.816**	-0.000	-0.263
C3	Hook aperture	**0.975**	0.039	-0.031
C3	Hook base width	**0.965**	-0.028	0.044
Ham	Total length	**0.743**	-0.137	-0.137
Ham	Total width	0.514	-0.029	-0.259

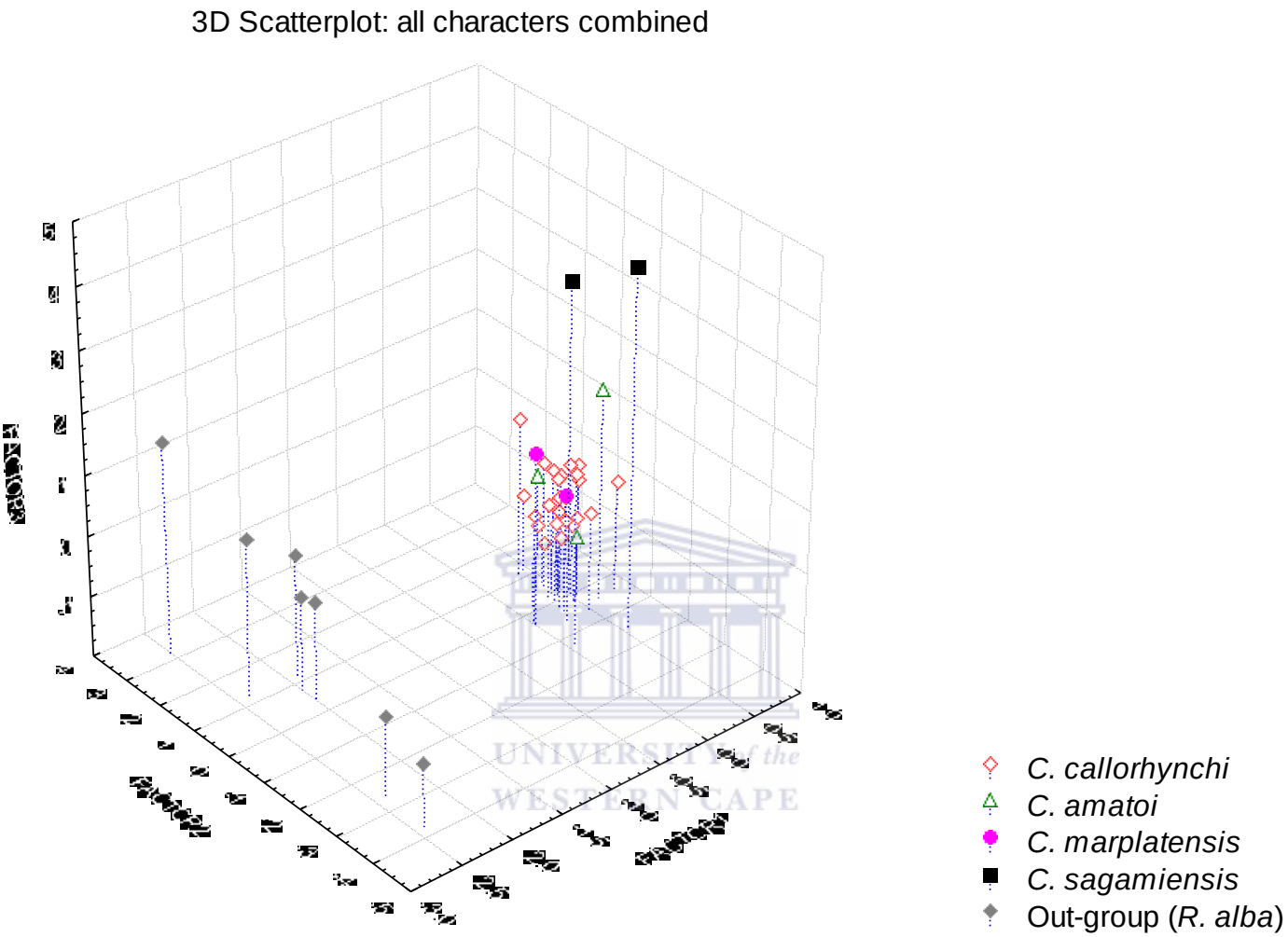
Factor loadings cont

Ham	Hook point length	**0.912**	-0.002	-0.092
Ham	Hook shank length	**0.825**	-0.247	0.322
Ham	Distal hook point width	**0.788**	0.090	-0.019
Ham	Outer aperture angle	**-0.863**	-0.069	0.093
Ham	Aperture	0.195	0.217	-0.352
Ham	Hook shank base width	**0.726**	-0.046	0.046
Ham	Outer root length	**0.876**	-0.066	-0.033
Ham	Inner root length	-0.317	-0.552	0.233
Ham	Root base angle	0.283	-0.001	0.539
Ham	Base width	**0.861**	0.080	-0.192

All characters combined. Factor and variable correlation, eigenvalues and associated total and cumulative variance (%) of factors 1, 2 and 3. Loading scores in red indicate the most significant factor to variable correlations.



Scatterplot of all characters combined from the PCA using factor 1 *versus* factor 2.



Scatterplot of all characters combined from the PCA using factor 1 *versus* factor 2 *versus* factor 3.