A closer look at the taxonomic and genetic diversity of indigenous South African *Marphysa* Quatrefages, 1865 ~~in South Africa.~~

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Abstract

The current study investigates ~~specimens identified as *Marphysa corallina*,~~ the final unresolved cosmopolitan species ~~in this genusof~~ *Marphysa* in South Africa, *Marphysa corallina*, collected from KwaZulu Natal, Eastern and Western Cape provinces, ~~and a *Marphysa*~~together with another species collected from ~~a single location in~~ northern KwaZulu Natal. ~~Extensive m~~Morphological and genetic ~~investigations established~~prove that *M. corallina*, ~~which was~~ originally described from Hawaii, does not occur in South Africa. ~~In these specimens, t~~The curvature of the inner base on maxilla I, ~~and~~ the elevated inner base of maxilla II, and the ventral cirrus as transverse welt with rounded tip allow us to identify~~ied~~ it as a new species of~~being in the genus~~ *Treadwellphysa*, ~~. Unique characteristics of *T. readwellphysa*~~ *izinqa* sp. nov. (common name brown wonderworm). Characteristic traits include the colour of the subacicular hook, ~~the consistent length of falcigers throughout the body,~~ shape of the postchaetal lobe and ~~falcigers of consistent length all along the body and importantly, the presence of~~ tridentate ~~falcigers,~~ which is ~~a character~~ re~~c~~p~~o~~r~~d~~ted for the first time for the genus. ~~This~~ The species~~, here assigned the name brown wonderworm,~~ is ~~co~~harves~~ll~~ec~~t~~ed as bait on the south coast, ~~and can~~although less than the more common blood wonderworm, ~~be distinguished from~~ *Marphysa haemasona*, ~~the more widely harvested blood wonderworm, by~~which shows a ~~its~~ more uniform brown colouration and white-tipped antennae. ~~The~~ A second ~~taxon~~species, *Marphysa mzingazia* sp. nov., is characterized by the ~~presence of~~ red eyes, six branchial filaments ~~that~~ extend~~ing~~ to the posterior body end ~~of the body~~, the colour of aciculae, the shape and colour of subacicular hooks, and the spinigers ~~presence of~~having similar size~~d spinigers throughout the length of~~all along the body. A molecular ~~A~~analysis based on~~of~~ cytochrome oxidase I fragments confirm ~~that~~ both taxa ~~are~~ as different from all previously sequenced species. A key for all South African species of *Marphysa* ~~species in South Africa~~ is included.

Keywords: bait polychaetes, *Marphysa*, *Treadwellphysa*, taxonomic revision, COI, Phylogeny

Introduction

~~The process of i~~Identifying, classifying~~,~~ and naming species allows ~~the~~ discover~~ingy of foundational~~ key biodiversity information, that is subsequently ~~forming the~~ foundational for ~~other research in~~ molecular ecology, biogeography, and conservation research (Pamungkas et al. 2019; Hutchings and Lavesque 2020; Monckton et al. 2020). ~~Consequently, i~~Incorrect taxonomic identifications lead to incomplete understanding of regional biogeography, morpholog~~ical characters~~, and phylogeny, among others (Hutchings and Lavesque 2020; Simon et al. 2022), ~~with~~ which may have drastic environmental management and conservation implications (Bickford et al. 2007; Nygren 2014).

South African polychaetes are ~~A~~a good example, ~~d is the case of the polychaete fauna in South Africa. D~~due to the extensive Day's (1967) ~~polychaete~~ monograph, ~~developed by Day (1967), South Africa's polychaete~~Their diversity was long-time considered reasonably well-

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resolved (Griffiths et al. 2010), despite. Tthe monograph listed more than half the species as having widespread or cosmopolitan distributions leading to 20 – 35% of to estimates of polychaete endemism to be between 20 – 35% (Day 1967; Awad et al. 2002). However, taxonomic investigations that usually include molecular methods have uncovered substantial previously unknown diversity of indigenous polychaetes, that were previously unknown, with restricted distributions for the regionhas been uncovered (e.g., Lewis and Karageorgopoulos 2008; Clarke et al. 2010; Sikorski and Pavlova 2016; Simon et al. 2017, 2019a, 2019b; Sedick and Simon 2019; Kara et al. 2018; Kara et al. 2020a; Kara et al. 2020b), proving that the level of endemism . These findings provided evidence that Day (1967) and Awad et al. (2002)had been significantly underestimated. Accordinglypolychaete endemism, suggesting that our knowledge on the diversity and biogeography of indigenous South African polychaetes fauna revealed to bein South Africa is poor, and incomplete, and thus requirequiring furtherres attention, while numerous (Simon et al. 2022). As such, Simon et al. (2022) revised the species recorded in Day (1967) and provided an extensive list of taxa that should be prioritised for taxonomic revision.

Until the 1900's, As for other ttaxa, polychaete descriptions developed before the 1900'stended to be are often vague and, include poor illustrations and do not include diagnostic characters that are currently understood as important for at generic, nor atnd species, levels designations (Klautau et al. 1999; Hutchings and Kupriyanova 2018; Simon et al. 2022). Tthe number of species described by 1900 wasere low at, and the number of comparable distinctive characters to distinguish between them was correspondingly small. Consequently, there was a bias toward the only known, easy-to-identify using obvious characters as they were the only ones known and were easier to identify. Among Eunicidae Berthold, 1827, this was the case ofFor example, the presence/absence, shape, and distribution of branchiae, which is were considered diagnostic characters for generic delimitation in family Eunicidae Berthold, 1827, but today, these characters are fo undknown to vary within species and, thus, as has been deemed uninformative at generic level (Carrera-Parra and Salazar Vallejo 1998; Zanol et al. 2014). Taxonomic rRevisionsg of species using type and extensive non-type material and combined withthe use of molecular data have become increasingly important as itbecame key in helps to character standardizinge important characters for species delimitation of species (Molina-Acevedo and Idris 2020; Molina-Acevedo and Idris 2021; Zanol et al. 2021), as it. This is evident and has been well-documented for the genus *Marphysa* Quatrefages, 1865 in family Eunicidae (Fauchald 1970; Glasby and Hutchings 2010; Idris et al. 2014; Zanol et al. 2014; Molina-Acevedo and Carrera-Parra 2015; Zanol et al. 2017).

In South Africa, the genus *Marphysa* was among thehas been prioritised taxa in South Africafor taxonomic revision (Simon et al. 2022), but also and this may also apply to those in the East African region (Simon et al. 2021a). Among them, different species Several species within this genus areare used as baits for recreational and subsistence fishing in the region and although different taxa are used, while inconsistent use of common names can complicate identification and hampers management strategies. For example, in South Africa, fisherman use dUp to six common names refer tofor two species of *Marphysa* species, and also applied the most widely usedwhile the name (wonderworm) includes also to species of *Eunice* and *Lysidice* (Simon et al. 2021a, b; 2022). Correctly clarifying species identity and indigenous diversity It is therefore imperative that species are identified correctly, and indigenous diversity clarified, to facilitate their management and to avoid over resource exploitation of resources (Simon et al. 2019c; Simon et al. 2021a; Simon et al. 2021b).

Of the seven southern African species of *Marphysa* ~~species recorded for southern Africa~~ in Day (1967), only three were indigenous~~. species were recorded for the region, while t~~The remaining four ~~species~~ have type localities ~~outside of the region~~abroad and were considered unresolved cosmopolitan ~~species~~ complexes (*sensu* Darling and Carlton, 2020). However, recent studies ~~revisions of *Marphysa* species reported locally (Lewis and Karageogropoulos 2008, Molina-Acevedo 2018, Kara et al. 2020, Molina-Acevedo and Idris 2021) resulted in the~~ reinstatedment of two incorrectly synonymised indigenous species *Marphysa haemasona* Quatrefages, 1866 (=*Marphysa sanguinea, M. elityeni* Lewis and Karageorgopoulos, 2008) and *Marphysa durbanensis* Day, 1934 (=*Marphysa macintoshi* Crossland, 1903), ~~the~~ describedption of one new indigenous species *Marphysa sherlockae* Kara, Molina-Acevedo, Zanol, Simon & Idris, 2020 (=*Marphysa depressa* (Schmarda, 1861)), and ~~the~~ placedment of two indigenous species in two newly erected genera; *Nicidion posterobranchia* Day, 1962 and *Paucibranchia purcellana* (Willey, 1904) (Lewis and Karageogropoulos 2008, Molina-Acevedo 2018, Kara et al. 2020, Molina-Acevedo and Idris 2021). ~~Thus~~Accordingly, there are currently five (instead of seven) southern African ~~number of~~ species of *Marphysa* ~~species for the region changed from seven to five~~, four of which ~~are~~ being valid indigenous species.

The only remaining unresolved cosmopolitan species ~~*Marphysa* species from South Africa~~, *M~~arphisa~~. *corallina* (Kinberg, 1865) ~~has a type locality~~was originally described from ~~in~~ Honolulu~~,~~ (Hawai'i) and has since been recorded from ~~several disjunct localities such as~~ Mozambique, Madagascar, New Zealand, the Red Sea, Kahului, Australia, Marshall Islands, Lakshadweep Island and the Jaluit Atoll (Read and Fauchald 2021), as well as~~. *Marphysa corallina* has also been recorded~~ in South Africa from Sodwana Bay to East London in KwaZulu-Natal, and there is also ~~and~~ an isolated population in Witsand ~~on the~~(south coast of ~~the~~ Western Cape) ~~,that where it~~ is used as bait, like ~~and~~ probably~~ssibly~~ in all other South African locations~~the rest of its distribution~~ (Kara 2015; Simon et al. 2019c; Simon et al. 2021a Simon et al. 2021b). Despite the South African specimens were attributed to *M. corallina* due to the distribution of the branchiae and the presence of compound falcigers and bidentate subacicular hooks (Day 1953, 1967), there are no formal taxonomic studies. Indeed, they are morphologically closer to *M. haemasona*. Thus~~The presence of this species has not been confirmed in South Africa, they probably through taxonomic studies and it most likely~~ represents an equivocal reports,~~ly cosmopolitan species~~ as demonstrated for the other species of *Marphysa* listed ~~species~~ above. ~~South African specimens identified as *M. corallina* are morphologically very similar to *M. haemasona*, and i~~Identifying characters allowing ~~to~~ distinguishing them in the field, as well as designating common names, ~~is~~are ~~important~~ imperative for management. ~~Thus, it is imperative that guidelines are provided (i.e., designate a common name and identify features that separate similar species apart in the field) so that we can~~to understand their usage as a bait resource, and to prevent overexploitation ~~as either species can be mistaken for one another~~.

In this study we investigated the occurrence of ~~whether *Marphysa*~~ *M. corallina* ~~occurs in~~ South Africa by examining the type ~~of~~ material ~~of *M. corallina*~~ from Hawaii and specimens from throughout its known distribution in South Africa, ~~and~~ including molecular comparisons when~~re~~ possible. ~~Our findings show that~~As a result, we prove that *M. corallina* is not present in South Africa and that specimens identified as such are new species to science ~~of~~ belonging to a different genus, *Treadwellphysa*, which we fully describe and illustrate. ~~Finally, w~~We also ~~provide a~~ redescribeprion ~~for~~ *M. corallina* ~~using~~ basd on the holotype~~, a description of new *Marphysa* species to science~~ and provide a taxonomic key to all species of *Marphysa* ~~species~~ from the region.

Material and Methods

Sample collection

Marphysa-like specimens were collected from fringing intertidal zones of eight open-coast sites along the KwaZulu-Natal and Eastern Cape coasts from 2013 – to 2014 (*n* = 32) and in 2019 (*n* = 29) (Fig 1), including a. An isolated population was collected from rock crevices on the muddy banks of the Mzingazi canal in Richards Bay (Fig 1). Whole specimens were preserved in 96% ethanol for further taxonomic and morphological analyses. The cCollection of specimens was approved by the Department of Forestry, Fisheries and the Environment in South Africa under permit numbers RES2013/13, RES2014/06 issued to Angus Macdonald and RES2019/49 issued to Carol Simon.

Morphological examination

The type material of *Marphysa M. corallina* (SMNH-Type-429) was examined and compared to related South African specimens that conformed to the general description of this species from South Africa infollowing Day (1967) based on . For the type material and South African specimens, characters such asshape of the prostomium, peristomium, anterior body region of the body, maxillary apparatus, frequency of branchiae with total number of filaments, shape of parapodia and pygidium, as well as the frequency of branchiae with total number of filaments, were examined, and conventions and measurements were recorded according to Molina-Acevedo & Carrera-Parra (2015). Images were taken with a Leica S9i dissecting and light ICC50W microscope both equipped with built-in cameras and. Images were edited in Adobe Photoshop 2022 and included in descriptions. Specimens used for this investigated are deposited at the Iziko South African Museum under accession numbers: (MB-A095266 – MB-A095297).

Molecular methodsanalyses

DNA was extracted from tissue samples of all fresh specimens using the ZR Genomic DNA Tissue MiniPrep Kit (Zymogen) according tofollowing the manufacturer's protocol. DNA was amplified using the universal COI primers (LCO1490 and HCO2198) from (Folmer et al. (1994). Polymerase Chain Reaction (PCR) amplifications were carried out accordingfollowed to Kara et al. (2020) for all fresh specimens collected from South Africa. Amplicons were sequenced at the Central Analytical Facility at Stellenbosch University using only the forward primer (LCO1490). All rRaw sequences were subjected to quality controlled methods to check for any sequencing errors using BioEdit (v7.2.6) (Hall, 1999).

GenBank sequences from *Molecular analyses*

Sspecies belonging toof *Marphysa, Paucibranchia, Eunice, Palola* and *Leodice* were included in the analysis as ingroups taxa, whilst *Hyalinoecia* sp. was used as the outgroup to root the tree and were downloaded from GenBank (Table 1), together with. A total of 32 newly generated sequences were generated in this study (Table 1). The COI data set was trimmed and aligned using the ClustalW multiple alignment method (Thompson et al. 1994) in BioEdit. A nexus file was compiled using Dna-SP v5 (Librado & Rozas, 2009). A best-fit evolution model of evolution was calculated using PAUP (Swofford, 2003) and MrModelTest v2.3 (Nylander, 2004). Using the Aikaike Information Criterion (AIC), the SYM+G model was

~~selected for our data set and was~~ used to ~~model~~ build the phylogenetic relationships using Bayesian Inference (BI) implemented in MrBayes 3.1.2 (Ronquist et al. 2012). Trees were calculated using four Markov Chains of 5 million generations with every 1000th tree sampled. The first 25% ~~of trees~~ were discarded as burn-in and the resulting trees were used to build a 50% majority-rule consensus tree. Convergence of runs was assess~~ed by examin~~ing the average standard deviation of split frequencies (≤ 0.01) using Tracer v1.5 (Rambaut 2012). The plot of likelihood versus the sampled trees and the effective sample sizes (ESS > 200) were analysed to verify the mixing quality of all parameters, both of which were satisfied. Trees were visualised using FigTree v1.4.4 (Rambaut, 2012) and edited in Photoshop. This analysis wias designed to check species identi~~f~~ty ~~species~~, not to ~~understand~~ assess their phylogenetic relationships, which are not discussed here, particularly because ~~, in addition~~ nodal support for more inclusive clades ~~were~~ is poor~~, thus phylogenetic relationships between species will not be discussed in detail~~.

Inter- and intraspecific genetic differences were computed using the Kimura 2-parameter model (K2P) in MEGA-X (Kumar et al. 2018) and run for 100 000 bootstrap replicates with complete deletion of gaps.

The sequences generated in this study are available on GenBank ~~under accession numbers:~~ (OQ836443 – OQ836473).

Results

Morphological and molecular comparisons

Thorough morphological comparisons indicate that *M.~~arphysa~~ corallina* does not occur in South Africa, where it ~~and~~ had been misidentified ~~locally~~. Instead, the specimens correspond~~Not~~ ontoly this is a new indigenous record ~~for the region, but thorough morphological examination also revealed that the species~~ belongs ~~to one of the newly erected genera for the family,~~ *Treadwellphysa* Molina-Acevedo and Carrera-Parra 2017, which ~~and~~ represents the first record of this genus ~~for the region~~in South Africa. Diagnostic characters are ~~Specimens had~~ the characteristic curvature of the inner base on maxilla I and the elevated inner base of maxilla II together with the ventral cirrus as transverse welt with rounded tip~~, that has been diagnosed for the genus~~ (Molina-Acevedo and Carrera-Parra, 2017). Indeed, they revealed to belong to a new species,~~*Treadwellphysa izinqa* sp. nov. differs from other species within the genus by the colour of~~characterised by ~~the~~ having reddish/golden subacicular hooks, ~~consistent length of falcigers throughout the body, shape of the~~ postchaetal lobe varying from digitiform to ear-shaped to inconspicuous, and tridentate falcigers (first recorded for the genus) of consistent length throughout the body~~importantly, the presence of tridentate falcigers, which is a character recorded for the first time for the genus~~.

COI sequences ~~were not available for~~ from Hawaiian *M. corallina* were not available ~~from~~ its type locality (Hawaii) ~~and thus could not be compared molecularly~~. Nonetheless, ~~T. izinqa sp. nov.~~our new species formed a distinct ~~species~~ clade (intraspecific variation: <1%~~,) with strong Bayesian posterior probability support (~~BS>0.95), which) and~~ differ~~s~~ed genetically from other species of *Marphysa* ~~species~~ by 21%–25% (Fig. 2)~~, thus confirming that it is indeed an independent species~~.

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~~The specimens collected included a~~Our samples revealed another distinct morpho-species, characterised by having ~~distinct from all other *Marphysa* species recorded for the region. Distinct features include the presence of~~ red eyes, six branchial filaments ~~that~~ extending to ~~the~~ posterior body end ~~of the body~~, ~~the colour of~~ aciculae varying from black/amber to golden, ~~the shape and colour of~~ subacicular hooks blunt or weakly bidentate and yellow or brown/golden, and ~~the presence of similar sized~~ spinigers of similar size ~~throughout the length of~~all along the body. Accordingly, they correspond to ~~As such,~~ a new species, ~~which also was described, *Marphysa mzingazia* sp. nov. The morphological distinctness of the species was corroborated molecularly as species~~ formsed an independent clade (intraspecific variation: < 1%,~~) with strong Bayesian posterior probability support~~ (BS>0.95) (Fig. 2) ~~and~~ that differsed genetically from other species of *Marphysa* ~~species~~ by 9–~~%~~ 21%.

Systematics

~~Order~~ Eunicida Dales, 1962

~~Family~~ Eunicidae Berthold, 1827

~~Genus~~ *Treadwellphysa* Molina-Acevedo & Carrera-Parra, 2017

***Treadwellphysa izinqa* sp. nov.**

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(Figs. 3–4)

Marphysa corallina – Day 1954:19, Day 1967: 400, fig. 17.7 f – j (*Non* Kinberg, 1865).

Marphysa cf. *corallina* – Simon, Kara, du Toit, van Rensburg, Naidoo, Matthee 2021:30–31, fig. 15.

Material examined*. Holotype.* One incomplete specimen (MB-A095291) in 96% ethanol, Umhlanga Rocks, KwaZulu-Natal, South Africa, -29.727550, 31.088894, coll. J.Kara, 19 September 2019. *Paratypes 1-4.* Four incomplete specimens (MB-A095512, MB-A095511, MB-A095290, MB-A095287) in 96% ethanol, Umhlanga Rocks, KwaZulu-Natal, South Africa, -29.727550, 31.088894, coll. J.Kara, 19 September 2019. *Non-type material.* Three specimens, two incomplete, one complete (SAMC-A20577), Umhlali Shore station, KwaZulu-Natal, South Africa, det. J.H.Day. Nine specimens (MB-A095280 - MB-A095289) in 96% ethanol, Umhlanga Rocks, KwaZulu-Natal, South Africa, -29.727550, 31.088894, coll. J.Kara, 19 September 2019. Fourteen specimens (MB-A095266 – MB-A095279) in 96% ethanol, Port Shepstone, KwaZulu-Natal, South Africa, (-30.740956, 30.459572, coll. J.Kara, 30 September 2019. Five specimens (MB-A095537- MB-A095541) in 96% ethanol, Mabibi, KwaZulu-Natal, South Africa, (-27.416198, 32.712154), coll. J.Kara, 29 April 2014. Six specimens (MB-A095517 – MB-A095522) in 96% ethanol, Adlams, KwaZulu-Natal, South Africa, (-27.624539, 32.656256), coll. J.Kara, 30 March 2014. Four specimens (MB-A095513 – MB-A095516) in 96% ethanol, Ballito, KwaZulu-Natal, South Africa, (-29.539767, 31.223861), coll. J.Kara, 31 January 2014. Six specimens (MB- A095527 – MB-A0995532) in 96 ethanol, Reunion Rocks, KwaZulu-Natal, South Africa, (-29.986525, 30.964167), coll. J.Kara, 13 June 2013. Four specimens (MB-A095533 – MB-A095536) in 96% ethanol, Green Point, KwaZulu-Natal, South Africa, (-30.250169, 30.782197), coll. J.Kara, 23 June 2013. Four specimens (MB-A095523 –

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Mb-A095526), in 96% ethanol, Umgazana, KwaZulu-Natal, South Africa (-31.705366, 29.413664), coll. J.Kara, 10 July 2013.

Comparative material examined. *Marphysa corallina* (Kinberg, 1865), holotype, one specimen in poor condition, SMNH-Type-429, Hawaiian Islands, Oahu, Honolulu, 21°19' N, 157°52' W, Eugenie Epx. 1851–53 (three vials each with parapodia, and one vial with the maxillary apparatus).

Description ~~***of Treadwellphysa izinqa* sp. nov.***~~ Holotype incomplete (~~currently incomplete because~~ posterior end ~~was cut off~~ used for molecular analyses) with up to 151 chaetigers, L10= 9mm, W10= 7mm, TL= 80mm. Transversal b~~B~~ody section rounded anteriorly, dorsoventrally flattened from ~~18th~~ chaetiger 18 to ~~the~~ posterior end (Fig. 3A–C); widest at chaetiger 35–40 (0.35 mm). Body ~~colour~~ light brown anteriorly (Fig. 3A, D), cream ~~coloured~~ from middle to posterior end (Fig. 3B–C), iridescent throughout. Palps, lateral and median antennae olive green/dark brown with white conical tips (Fig. 3A, D–E).

Prostomium bilobed (0.1 mm long, 0.3 mm wide), lobes frontally rounded, ventral sulcus deep (Fig. 3A, D–E). Prostomial appendages in semicircle (Fig. 3D), median antenna slightly isolated by a gap. Palps reaching ~~the~~ second peristomial ring, lateral and median antenna reaching ~~1st and 2nd~~ chaetigers 1 and 2, respectively. Palpophores and ceratophores thick, short, ~~and~~ ring shaped~~, ;~~ palpostyles and ceratostyles thick, cylindrical, with conical ends. ~~Pair of~~Two black reniform eyes at ~~the~~ base of lateral antennae (Fig. 3A, red arrow).

Peristomium (0.1 mm long, 0.25 mm wide) with first ring twice as long as second, clearly~~. S~~separated~~ation between the two peristomial rings distinct~~ on all sides (Fig. 3A, D-E).

Maxillary apparatus sclerotized~~.,~~ ~~with~~ MF= 1+1, 3+3, 4+0, 3+5, 1+1 (Fig. 3F). MI 2.4 times longer than maxillary carriers, ~~3~~ three times longer than closing system, MI forceps-like, ~~and~~ slightly extended~~ed~~ but rounded, with curved basal inner edge (Fig. 3F). MII wide, with triangular teeth directed laterally, ~~3~~ three times longer than cavity opening, and ~~base a~~with small elevation at base, fitting ~~the~~ inner edge of maxillae I (Fig. 3F). MIII short, with blunt triangular teeth and~~,~~ irregular attachment lamella ~~irregular, situated in~~ at centre of posterior edge in relation to ~~the~~ maxilla (Fig. 3F). Right MIV with distal tooth longest~~,~~ and strongly sclerotized rectangular attachment lamella ~~rectangular, situated~~at 2/3 along posterior edge of maxilla~~, strongly sclerotized~~. Right MIV with three teeth, distal tooth longest and sclerotized circular ~~,~~ attachment lamella ~~circular,~~ along posterior edge of maxilla~~, sclerotized~~. MV rectangular, longer than wide, with single tooth (Fig. 3F). Mandibles brown, with transparent cutting plates and ~~6~~ six growth rings.

Branchiae pectinate, with up to three long filaments, starting from chaetiger 26 to posterior end (Fig. 4C), with~~.~~ a single filament at ~~C~~chaetigers 26 ~~to~~ – 37 ~~as a single filament, ; chaetigers 38–97 as~~ three long filaments from 38–97, ~~reducing to~~ two filaments ~~in chaetigers~~from 98–120 and ~~; reducing to~~ one filament from ~~chaetigers~~ 121 to posterior end (Fig. 3B, C, 4C-D). Branchial filaments longer than dorsal cirri (Fig. 4C–D).

First three parapodia smallest (Fig. 4A, D–E). Dorsal cirri digitiform in anterior chaetigers, longer than chaetal, pre- and postchaetal lobes (Fig. 4A); in median-posterior chaetigers conical, 1/2 length of chaetal lobe (Fig. 4C), in posterior chaetigers 1/3 longer than chaetal lobe (Fig. 4D). Ventral cirri digitiform in first three parapodia, longer than chaetal, pre- and postchaetal lobes (Fig. 4A); with oval swollen base and rounded tip from chaetiger 4 ~~to~~ – 8; with transverse welt with a rounded tip from chaetiger 9 ~~to~~ – 97; reducing to small swollen

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base with round tip from chaetiger 98 to posterior end (Fig. 4B–D). Prechaetal lobe as a transverse fold in all chaetigers (Figs. 4A–D). Chaetal lobe rounded in anterior chaetigers (Fig. 4A–B); triangular in median to posterior chaetigers, longer than post- and prechaetal lobes with aciculae emerging from midline (Fig. 4C–E). Postchaetal lobe developed in first 38 chaetigers, and longer than chaetal lobe; digitiform in first 2 two chaetigers; ear-shaped from chaetigers 30 to – 37, progressively smaller and inconspicuous from chaetiger 38 to posterior end (Fig. 4B–E).

Acicula with black shafts and amber blunt tip in all chaetigers (Fig. 4E, I), . Usually up two 2 aciculae in from anterior to median chaetigers, reducing to one in posterior chaetigers (Figs. 4A–E, I). Limbate capillaries of two lengths in supracicular position in all chaetigers (Fig. 4F). Four types of pectinate chaetae: ; in anterior chaetigers, 4–6 isodont symmetrical pectinate chaetae, with wide blade, thin shaft, up to 26–28 short and slender teeth (Fig. 4G); in median to posterior chaetigers, 1–2 isodont asymmetrical pectinate chaetae, with wide blade, thick shaft and up to 21 long and slender teeth (Fig. 4H), 1–4 anodont pectinate chaetae, with wide blade, , thick shaft and up to 8–10 short and thick teeth (Fig. 4I), 1–2 anodont pectinate chaetae, with wide blade, thick shaft and up to 8–10 long and thick teeth (Fig. 4J). Compound spinigers absent. Compound falcigers tridentate; in anterior parapodia, with triangular proximal tooth bigger than rounded distal teeth, ; in median to posterior chaetigers, compound falcigers with blades of similar length, shorter than anterior falcigers, with rounded distal teeth shorter than triangular proximal tooth (Fig. 4K–L). Subacicular hook bidentate with rounded guard, reddish basally and golden distal end; with triangular teeth, proximal tooth larger than distal tooth (Fig. 4E); starting from 28th 30th chaetiger 28–30, usually one per parapodium, present in all chaetigers.

Pygidium observed from holotype (examined before DNA extractions), with two pairs of pygidial cirri emerging ventrally, dorsal pair as long as last five chaetigers; ventral pair 1/3rd as long as length of the dorsal pair (Fig. 3C).

Variation. Paratypes incomplete, with up to 145–176 chaetigers, L10= 7–8mm, W10= 5–6mm and TL= 75–88mm. Palps reaching between the first and second peristomial rings. lateral and median antenna reaching between the first and third chaetigers 2 and 3. Start of subacicular hooks starting in chaetigers 28–30. Non-type material: L10 = 0.6–1.5 mm, W10= 0.2–0.55 mm; palps reaching between the first peristomial ring and the second chaetiger 2, lateral antenna reaching between the first peristomial ring and third chaetiger 3, median antenna reaching between the first peristomial ring and fifth chaetiger 5. Branchiae starting from chaetiger 22–42, maximum number of branchial filaments from 49th to 55th chaetiger 49–55. Subacicular hooks starting from the 25th chaetiger 25–to 40th. Postchaetal lobe well-developed from chaetiger one 1–to 38. Maxilla formula: MII 2+3, MIV 3+5.

Habitat. Found in mucous-sand burrows in sediment under algal beds, under worm rocks and, rocks, and in crevices on the fringing intertidal rocky shores at low tide.

Distribution. Northern KwaZulu-Natal (Mabibi) to Eastern Cape (Umgazana), Witsand in the Western Cape (Simon et al. 2021b).

DNA barcode. Umhlanga Rocks, KwaZulu-Natal, South Africa. Holotype (MB-A095291), 575 bp COI fragment with GenBank accession number OQ836467. 575 bp fragment isolated with the universal mitochondrial cytochrome oxidase subunit 1 gene, primer pair LCO1490, HCO2198 (Folmer et al. 1994).

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Etymology. The isiZulu (the native language of the KwaZulu-Natal people) word "izinqa" translates to buttocks and refers to the round prostomial lobes that are separated by a distinctive butt-like appearance deep ventral sulcus, a of the prostomiumdistinctive butt-like appearancetah, which can be seen with the naked eye. isiZulu is the native language of the people in the KwaZulu-Natal province in South Africa and is where this species is found in high abundances.

Common name. Brown wonderworm. Living *T. izinqa* sp. nov. This species can be distinguished from the closely related *Marphysa M. haemasona*, the blood wonderworm also that is commonly used as bait in the Western Cape (Simon et al. 2021), by thein having a solid brown body colour and the white tips of the antennae and palps (see Fig. 15A in Simon et al. 2021b), instead of . *Marphysa haemasona* on the other hand when alive, is more reddish/deep violet body with a speckled anterior dorsum, has and the antennae tips with brown and white bands on tips of antennae and is called the blood wonderworm (Simon et al. 2021, Simon et al. 2022).

Remarks. Day (1953, 1967) initially identified the specimens in South Africa as *Marphysa corallina* (Hawaii) due to the distribution of the branchiae, the presence of compound falcigers and the bidentate subacicular hook. However, after a thorough examination of the type material of *M. corallina* (Fig. 5A–L), we found that the South African specimens have distinct characters that are more related with the diagnosis of *Treadwellphysa* genus, such as the curvature in the inner base of maxilla I, and elevation on the inner base of maxilla II from maxillary apparatus, and the ventral cirrus as transverse welt with a rounded tip throughout most of the body. The specimens examined here are a new species to science and the first record of the genus in South Africa and this includes specimens that Day (1954, 1967) identified as *M. corallina*.

Marphysa cf. *corallina* recorded from Witsand (Simon et al. 2021b) matches the description of *Treadwellphysa T. izinqa* sp. nov., particularly in having a small elevation at the inner base of maxilla II fitting in the inner curved base of maxilla I (Fig. 15F, Simon et al. 2021c),Additionally, morphology of the maxillary apparatus, considered a stable character (Molina-Acevedo and Carrera-Para, 2017), conform to that of *T. izinqa* sp. nov. with having the small elevation at the inner base of maxilla II fitting in the inner, curved base of maxilla I (Fig. 15F, Simon et al. 2021c). but with a few minor differences. Specimens from Witsand are 40 mm longer than *T. inzinqa* sp. nov.; have two – four2–4 more branchial filaments and branchiae that start between 9 –13 chaetigers later. However, the a low intraspecificgenetic distances (<lower than 1%) between specimens collected from Witsand and the KwaZulu-Natal coast confirms that they are a singlebelong to the same species. Additionally, morphology of the maxillary apparatus, considered a stable character (Molina-Acevedo and Carrera-Para, 2017), conform to that of *T. izinqa* sp. nov. with having the small elevation at the inner base of maxilla II fitting in the inner, curved base of maxilla I (Fig. 15F, Simon et al. 2021c). Thus, specimens from Witsand are considered here as *Treadwellphysa izinqa* sp. nov.

Treadwellphysa izinqa sp. nov. resembles *T. rizzoae* Molina-Acevedo, 2019, *T. villalobosi* Molina-Acevedo, 2019, *T. languida* (Treadwell, 1921), *T. veracruzensis* (de León-González and Díaz-Castañeda, 2006) due to the presence ofin having only compound falcigers throughout the body. However, Furthermore, *T. rizzoae* has a poorly developed postchaetal lobe, which is (rounded in most of chaetigers where present. In *T. izinqa*. sp. nov.), the a well-developed postchaetal lobe, is well-developed, digitiform in first chaetigers and auricular in

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following ones (XXXXX in *T. izinqa*. sp. nov.), and ~~.~~ ~~Also, *T. rizzoae* has bidentate compound falcigers (tridentate while *T. izinqa* n. sp.) has tridentate falcigers, the first description of tridentate falcigers in this genus.~~ *T. readwellphysa villalobosi, T. languida* and *T. veracruzensis* have translucent subacicular hooks (reddish basally and translucent distally in *T. izinqa* sp. nov.)~~,~~ and compound falcigers with blades of various lengths in anterior region~~,~~ (with ~~whereas in *T. izinqa* sp. nov. the subacicular hook is reddish basally and translucent distally, and the falcigers~~ only ~~present~~ one length in anterior chaetigers in *T. izinqa* sp. nov.). ~~Furthermore, *T. rizzoae* has a poorly developed postchaetal lobe, which is rounded in most of chaetigers where present. In *T. izinqa*. sp. nov. the postchaetal lobe is well-developed, digitiform in first chaetigers and auricular in following ones. Also, *T. rizzoae* has bidentate compound falcigers while *T. izinqa* n. sp. has tridentate falcigers, the first description of tridentate falcigers in this genus.~~

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Genus *Marphysa* de Quatrefages, 1865

***Marphysa corallina* (Kinberg, 1865)**

(Fig. 5)

Material examined. *Marphysa corallina* (Kinberg, 1865), holotype, one specimen in poor condition, SMNH-Type-429, Hawaiian Islands, Oahu, Honolulu, 21°19' N, 157°52' W, Eugenie Epx. 1851–53 (three vials each with parapodia, and one vial with the maxillary apparatus).

Redescription. Holotype incomplete, ~~in poor condition,~~ with 104 chaetigers~~,~~ (fragments: ~~broken in six parts,~~ anterior = ~~first fragment with~~ 9 chaetigers, second and third ~~ones with~~= 19 each, fourth ~~one =with~~ 2, fifth ~~one with~~= 70, sixth ~~one with~~= 3), L10= 6.1 mm, W10= 3.4 mm. Anterior region with dorsum convex, flat venter; widest at chaetiger 13, tapering after chaetiger 48. Prostomium bilobed (0.9 mm long, 2.4 mm wide), sulcus anteriorly shallow, dorsally inconspicuous and ventrally deep (Fig. 5A–B). Prostomial appendages in a semicircle, median antenna slightly isolated by a gap. Palps reaching second peristomial ring; lateral antennae reaching first chaetiger; median antenna reaching second chaetiger (Fig. 5A). Palpophores and ceratophores short, thick; palpostyles and ceratostyles digitiform, slender, without articulations (Fig. 5A–B). Eyes brown, between palps and lateral antennae.

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Peristomium (1.5 mm long, 3.6 mm wide) with first ring two times longer than second ring. Separation between rings distinct on all sides. Inferior lip dissected (Fig. 5A–B).

Maxillary apparatus in poor condition (Fig. 5C), with MIII, MIV and MV lost; FM= 1+1,4+4, ?+? ?+?, ?+? MI with falcal arch angular shaped and with straight outer edge (Fig. 5C). MI forceps-like, 2.5 times longer than length of maxillary carriers. MII wide, left distal teeth shorter, directed laterally, other teeth recurved; cavity opening oval, MII 3.8 times longer than length of cavity opening (Fig. 5C).

Branchiae pectinate ~~with up to 5 long filaments~~, from chaetigers 17L–18R to last chaetiger ~~of the fragment~~ (Fig. 5F, G), with up to five long filaments,~~branchial filaments~~ longer than dorsal cirri.

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Dorsal cirri digitiform in anterior chaetigers, conical in following ones; longer than ventral cirri in first chaetigers, similar in length to ventral cirri in median-posterior ones (Fig.

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5D–G). Ventral cirri tongue-shaped in first ~~4~~ four chaetigers; from chaetiger~~s~~ 5, with an elongated oval swollen base. Prechaetal lobes in first chaetigers with dorsal edge longer than ventral, as transverse fold in following chaetigers (Fig. 5D–G); chaetal lobes rounded in anterior chaetigers, triangular in following ones (Fig. 5D–G); postchaetal lobes well developed in first 58 chaetigers; tongue-shaped in first ~~4~~ four chaetigers, ear-shaped from chaetiger 5, progressively smaller in following ones (Fig. 5D–G).

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Aciculae blunt, reddish basally, amber distally, up to ~~3~~ three aciculae per parapodia (Fig. 5D–G). Limbate chaetae of two lengths in same chaetiger. Two types of pectinate chaetae: ~~in anterior chaetigers, with~~ 2–3 isodont in anterior chaetigers, ~~pectinate chaetae~~ with wide blade, thin shaft, and up to 11 long, slender teeth (Fig. 5H); ~~in median chaetigers,~~ 2–3 ~~pectinate chaetae~~ isodont in median chaetigers, with narrow blade, thin shaft, and with up to 20–21 short, slender teeth (Fig. 5I~~);~~ ~~).~~ ~~p~~Pectinate chaetae from posterior region not ~~observed~~seen. Compound falcigers bidentate, with blade of similar lengths in all parapodia, with triangular teeth in anterior region (Fig. 5J)~~,~~ and blunt teeth in posterior parapodia (Fig. 5K). Subacicular hooks bidentate, translucent~~,~~; starting in chaetigers 30R–32L, distributed continuously in each parapodia throughout the body, always ~~two or three~~2–3 per parapodia; with blunt teeth; both teeth of similar sizes (Fig. 5L).

***Marphysa mzingazia* sp. nov.**

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(Fig. 6)

Material examined. *Holotype*. One incomplete specimen (MB-A095294) in 96% ethanol, Richards Bay harbour, KwaZulu-Natal, South Africa, -28.785998, 32.082154, coll. J. Kara and R. Kara, 1 September 2019. *Paratypes 1-3.* Three incomplete specimens (MB-A095293, MB-A095510, MB-A095292) in 96% ethanol, Richards Bay harbour, KwaZulu-Natal, South Africa, -28.785998, 32.082154, coll. J. Kara and R. Kara, 1 September 2019. *Non-type material*. Three incomplete specimens (MB-A095295 – MB-A095297) in 96% ethanol, Richards Bay harbour, KwaZulu-Natal, South Africa, -28.785998, 32.082154, coll. J. Kara and R. Kara, 1 September 2019.

Description. Holotype complete with a maximum of 205 chaetigers, L10= 4 mm, W10= 3 mm, TL= 55 mm. Body rounded anteriorly, becoming dorsoventrally flattened from chaetiger 7–8 (Fig. 6A-C) to posterior end~~.~~ ~~W~~widest ~~part of the body~~ at chaetiger 25 (2.49 mm)~~.~~ ~~Body colour when alive:~~ blood red with white antennae when alive; ~~. Body colour when preserved:~~ iridescent throughout, chaetigers 1–8 purple/brown, from segment 9 till posterior, cream (Fig. 6A-B), middle to posterior segments, red midline on segments and pinkish-peach pigment on dorsum (Fig. 6B) when preserved.

Prostomium bilobed (0.71 mm long, 1.48 mm wide), lobes frontally rounded, ventral sulcus deep (Fig. 6A, C). Prostomial appendages in semicircle, median antenna slightly isolated by a gap. (Fig. 6A, C). Palps reaching 2nd peristomial ring, lateral antennae reaching ~~2nd~~ chaetiger 2, median antenna reaching ~~3rd~~ chaetiger 3. Palpophores and ceratophores thick, short and ring shaped; palpostyles and ceratostyles thick, rounded with conical ends. Pair of red eyes as subdermal patches between palps and lateral antennae (Fig. 6C, black arrows).

Peristomium (1.8 mm long, 5.2 mm wide), first ring two times longer than second ring (Fig. 6A); separation between the two peristomial rings distinct on all sides (Fig. 6A, C).

Maxillary apparatus sclerotized, with MF= 1+1, 6+ 6, 4+0, 4+8, 1+1 (Fig. 6D). MI 3 three times longer than maxillary carriers, 3.5 times longer than closing system, forceps-like, falcal arch slightly extended with inner base slightly rounded. MII wide, with triangular teeth directed laterally (Fig. 6D, E); 3 three times longer than cavity opening. MIII short, slightly curved, blunt triangular teeth, attachment lamella irregular, situated only in center of anterior edge of maxilla, slightly sclerotized (Fig. 6E). Left MIV with distal tooth longest, attachment lamella semicircle, wide, better developed in central portion, situated 2/3 along anterior edge of maxilla, strongly sclerotized. Right MIV with distal teeth bigger; attachment lamella semicircle, wide, better developed in central portion, situated 2/3 along anterior edge of maxilla, slightly sclerotized. MV rectangular, longer than wide, with rounded tooth (Fig. 6E). Mandibles brown with transparent cutting plates.

Branchiae palmate with up to six short filaments, from chaetigers 18 to posterior chaetigers end (Fig. 6G-I). First 7 seven chaetigers with one branchial filament, two from chaetigers 26–28 as double filaments, chaetiger four from 29–59 as four filaments, five from chaetigers 60–90 as five filaments, maximum of six filaments infrom chaetigers 91 to posterior end. Branchial filaments slightly longer than dorsal cirri in anterior chaetigers and double length in posterior chaetigers.

First three parapodia smallest, best developed in chaetigers 7 to –31, becoming gradually smaller (Fig. 6A). Dorsal cirri conical in most chaetigers, longer than ventral cirri in anterior, best developed in chaetigers 1– to 25 (Fig. 6F). Ventral cirri digitiform in first two parapodia (Ffig. 6F); with short swollen base and rounded tip from chaetiger 3 to middle (Fig. 6B); becoming triangular with pointed tip in following chaetigers (Fig. 6G-I). Prechaetal lobe as a transverse fold in all chaetigers (Figs. 6F-I). Chaetal lobe conical in anterior chaetigers (Fig. 6F); triangular in middle to posterior chaetigers, with aciculae barely emerging from midline (Figs. 6G-I). Postchaetal lobe well developed and longer than chaetal lobe in first 42 chaetigers; digitiform in chaetigers 4–32; auricular from chaetiger 33– to 42, progressively smaller from chaetiger 43 onwards (Fig. 6G-I).

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Aciculae with black shafts and amber blunt tip from anterior to middle chaetigers (Figs. 6F-H), becoming golden in posterior (Fig. 6I).. Usually up to 2 two aciculae in anterior to middle chaetigers, reducing to one in posterior chaetigers (Figs. 6F-I). Limbate chaetae only in supracicular position. Four types of pectinate chaetae: 1) 5–6 isodont symmetrical pectinate chaetae in anterior parapodia, with narrow blades, thin shaft and 11–13 short, slender teeth (Fig. 6J), in anterior region; 2) 5–6 isodont asymmetrical pectinates with wide blades, thick shaft, 20–21 short and slender teeth (Fig. 6K), from anterior to middle region; 3) 4–5 anodont pectinates, asymmetrical with wide blades, thick shaft 13–14 medium-coarse short teeth (Fig. 6L) in middle to posterior chaetigers; 4) 1–2 anodont with asymmetrical, wide blades, thick shafts, 5–7 long and thick teeth and wide blades (Fig. 6M), in posterior parapodia. Compound spinigers with blades of two lengths in all chaetigers, longer blade more abundant (Fig. 6N). Compound falcigers absent. Subacicular hooks blunt or weakly bidentate;—, yellow or brown/golden (Fig. 6O-P), starting from 25th chaetiger 25, two hooks per parapodium in anterior chaetigers, reducing to one in posterior region, hook present in all the parapodia.

Variation. Complete specimens with up to 201 chaetigers, L10= 3–5 mm, W10= 3–4 mm, TL= 23– 65 mm. Palps reaching from 2nd peristomial ring to 3rd chaetiger 3, lateral antenna reaching

from to 2nd – 4th chaetiger 2–4, median antenna up to 3rd – 4th chaetiger 3–4. MF varies: MII: 4–5 + 5–6, MIII: 6–7, MIV: 3–4 + 6–8. Start of branchiae from chaetigers 18– 26. Number of branchial filaments maximum at 73 – 97 chaetiger 73–97 s. Start of subacicular hook from 23 – 36th chaetiger 23–36. Postchaetal lobe development from first to 1 – 43 chaetiger 1–43s.

Habitat. Found in crevices of muddy bank rocks on the banks of the Mzingazi canal leading to the Richards Bay waterfront.

Distribution. Localised population in the Mzingazi Canal, leading to the Richards Bay waterfrontRichards Bay, KwaZulu-Natal, South Africa.

DNA barcode. Richards Bay, KwaZulu-Natal, South Africa. Holotype: MB-A095294, GenBbank accession number OQ836470. 575 bp fragment isolated with the universal mitochondrial cytochrome oxidase subunit 1 gene, primer pair LCO1490, HCO2198 (Folmer et al. 1994).

Etymology. The specific epithet es *mzingazia* was named after therefers to the type location it was found in, i.e., the Mzingazi Ccanal in the Richards Bay harbour system.

Remarks. *Marphysa capensis* (Schmarda, 1861), *M. durbanensis, M. haemasona, M. sherlockae,* and *Marphysa M. mzingazia* sp. nov. inhabit the South African coasts and have in common the presence of branchiae throughout the body. *Marphysa. capensis* is different from the rest by havinghas only compound falcigers present in all parapodia, while all compared to the others speci es have alsoin which compound spinigers are present. Furthermore, *Marphysa. durbanensis* and *M. haemasona* have long branchial stems (, while *M. mzingazia* sp. nov. has a short in *M. mzingazia* sp. nov.)branchial stem. Also, *Msrphysa. durbanensis* and *M. sherlockae* have reddish subacicular hooks while in the new species the hooks are (amber in *M. mzingazia* sp. nov.). Moreover, *M. mzingazia* sp. nov. has red subdermal eyes, whereas in *Marphysa. haemasoma* the has eyes are colourless subdermal eyes (red in *M. mzingazia* sp. nov.) and ovoid postchaetal lobe in first four chaetigers (triangular in *M. sherlockae*, digitiform in *M. mzingazia* sp. nov.). On other hand, the new species has a digitiform postchaetal lobe in first 4 chaetigers, while in *M. haemasona* it is ovoid and in *M. sherlockae* it is triangular (Day 1967; Kara et al. 2020b).

Marphysa mzingazia sp. nov. resembles *M. aransensis* Treadwell, 1939 (Texas), *M. brevibranchiata* Treadwell, 1921 (Bahamas), *M. fauchaldi* Glasby and Hutchings, 2010 (Australia), *M. gravelyi* Southern, 1921 (India), *M. hongkongensa* Wang, Zhang and Qiu, 2018 (China), *M. gaditana* Martin, Gil and Zanol in Martin et al. 2020 (Spain), and *M. kristiani* Zanol, da Silva and Hutchings, 2016 (Australia) by in having only compound spinigers present in all chaetigers, and amber subacicular hooks. However, *M. mzingazia* sp. nov. has four types of pectinate chaetae: INLS, IWSS, AWSS, AWLT; while *M. aransensis* (INLS, IWST, AWLT), *M. fauchaldi* (INLS, IWSS, AWLS), *M. gravelyi* (INSS, IWLS, AWLS), *M. kristiani* (INLS, IWLS, AWLT), and *M. gaditana* (INLS, IWLT, AWLT) have only three types of pectinate chaetae, and *M. hongkongensa* has five types of pectinates (NLSS, IWSS, IWLS, AWLT, AWLS). Furthermore, *M. mzingazia* sp. nov. has the postchaetal lobe as auricular form from chaetiger 4, (whilst rounded in *M. brevibranchiata*) the postchaetal lobe is rounded from the same chaetiger. and Finally, *M. mzingazia* sp. nov. has conical chaetal lobes in first chaetigers, while (rounded in *M. brevibranchiata*) the chaetal lobe is rounded in first chaetigers.

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Identification kKey to the *Marphysa* South African species of *Marphysa* from South Africa

1 With oOnly compound falcigers presented throughoutall along the body ……. *M. capensis* (Schmarda, 1861)

- With both cCompound falcigers and spinigers presented in anterior-median body regions of the body ……. ……*M. sherlockae* Kara, Molina-Acevedo, Zanol, Simon, Idris, 2020

- With oOnly compound spinigers throughout all along the body ……2

2(1) Branchiae palmate, with short filaments, just exceeding dorsal cirrithe length of…… the dorsal cirrus …… *M. mzingazia* sp. nov.

- Branchiae pectinate, with long filaments, at least four times longer than dorsal cirri in median body region of the body ……4

3(2) Postchaetal lobe digitiform in first three chaetigers, MII 5–6+6–8, and five types of pectinate chaetae: narrow isodont pectinate with long and slender teeth, wide isodont pectinate with short and slender teeth, wide isodont pectinate with short and thick teeth, wide anodont pectinate with short and slender teeth, and wide anodont with long and thick teeth …… *M. durbanensis* Day 1934

- Postchaetal lobe tongue-shaped (ovoid) in first three chaetigers, MII 4+4, and four types of pectinate chaetae: narrow isodont pectinate with long and slender teeth, wide isodont pectinate with short and slender teeth, wide anodont pectinate with short and slender teeth, and wide anodont with long and thick teeth …… *M. haemasona* de Quatrefages, 1866

Discussion

This study examined the final last unresolved southern Africa cosmopolitan species of *Marphysa*, species that was recorded for southern Africa. Not only did we find that *M. corallina*, revealing that 1) it has been historically misidentified and thus does not occur in South Africa, but also2) that the southern African specimens examined here does not belong to *Marphysa,* but instead has distinctive characters that place it within theto *Treadwellphysa*, which represents the first record of this genus in South Africa genus. ThusThe species, it wais herein described as *Treadwellphysa izinqa* sp. nov. and represents the first record of this genus in South Africa. Furthermore, a new species of *Marphysa* was found in Richards Bay (, KwaZulu-Natal) , andis newly described herein as *Marphysa mzingazia* sp. nov. Accordingly, The fact that the *Marphysa* and related genus in South Africa isare now represented solely by indigenous species, rather than predominantly by cosmopolitan species as previously thought, which, reflects the importance of conducting thorough taxonomic revisions, even in places

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regions whithere the theoretically well-resolved polychaete faunas is considered well-resolved (Griffiths et al. 2010; Hutchings and Lavesque 2020; Simon et al. 2022).

The extensive revisions of species belonging tofocusing on *Marphysa* have revealed additional several informative characters that are informative for species delimitation, such as the maxillary apparatus, the chaetae variation of chaetae along the bodylength of the body, the shape of parapodial lobes, the shape of ventral cirri and the number and type of pectinate chaetae (Carrera-Parra and Salazar-Vallejo 1998; Zanol et al. 2014; Molina-Acevedo, 2015; Zanol et al. 2017). In thisOur study, revealed that the previously considered *M. corallina* belongs to *Treadwellphysa* due to closer inspection of the shape of inner and outer base of MI, the small, rounded projection at the base of MII that fits into the base of MI, and the characteristic ventral cirri as a transverse welt, allowed us to determine that what was considered *M. corallina* actually belongs to the newly erected *Treadwellphysa* Molina-Acevedo & Carrera-Parra, 2017. But wWe also uncovered the presence of tridentate falcigers, not reported to date an additional chaetal type for this genus that was not reported previously i.e., tridentate falcigers.

The other southern African species clearly belongs to *Marphysa*, and is here considered as new due to In the case of *M. mzingazia* the shape of the branchiae, and the postchaetal lobes, a different combination of pectinate chaetae, and the colour of subacicular hooks are characters that justified the establishment as a new species from other local and worldwide species of *Marphysa*. The species rich subgroup *Ssanguinea* of within *Marphysa* is recognized for the presence ofby having only compound spinigers, but , it is rich in number of species, however, it is also known for its by having low morphological complexity and low number of distinctive and stable characters, apart which from the fact that the characters can also change with the growth of the organism. Despite this, we have found that the combinationg of different character forms in the characters producesgives rise to a unique morphological patterns for each species, which are characteristic at species level and can be comparable with the others and allow their clear species delimitation.

Incorporating The molecular data into thorough taxonomic revisions has contributed significantly to the knowledge on species delimitations and thus the DNA sequence has been included as an additional character for each species to helpcontribute to unambiguously define new species, as demonstrated for them unambiguously. For e.g., DNA data confirmed the presence of the alien *Marphysa victori* Lavesque, Daffe, Bonifacio & Hutchings, 2017 in France, whose introduction was the oysterand identified aquaculture and the vector for introduction (Lavesque et al. 2020). As such, molecular data greatly helped to uncover that an alien species was can be mistaken for a new indigenous species in the invaded range, and thus helpinged to stabilisinge the species name even when large morphological variations are noticeable within species. On the other hand, the two sympatric Iberian species *M. gaditana* and *Marphysa chirigota* Martin, Gil & Zanol in Martin et al. (2020) were initially traded as fish baits under the name of "*M. sanguinea*", while the morphological and molecular data revealed they were two different new species. However, whether they are native form the type locality or not is not yet clear, as the former had a wide distribution likely indicating introductions at some regions and the latter has also been found in the Mediterranean coasts of Tunisia (Chaibi et al, 2021).

The phylogenetic tree revealed two distinct species clades belonging for the twoto the new species described here, *Treadwellphysa izinqa* sp. nov. and *Marphysa mzingazia* sp. nov.

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Even though *Treadwellphysa*. *izinqa* sp. nov. occurred widely across South Africa, spanning three biogeographic provinces, i.e.,from the subtropical Natal inshore ecozone, and the tropical Delagoa inshore ecozone and to the Agulhas inshore ecozone. However, the intraspecific variation within this speciesis was significantly very low (0.05±0.00) indicating that it is a single, panmictic population. Moreover, the species prefers the warm east coast (Kara 2015). Interestingly, during field collectionswhile, *M. haemasona* was found to dominates along the cool-temperate stretch of the west coast (Simon et al. 2021b; Kara et al. *in prep.*), whereas *T. izinqa* preferred the warm east coast (Kara 2015), possibly suggesting that they might be segregated both species distributions could be limited by temperature. Similarly, this was demonstrated by Kara et al. (2020a), who found that two cryptic *Platynereis* species had temperature preferences where *P. entshonae* Kara, Santos, Macdonald & Simon, 2020 was found to be more abundant along the cooler west coast, while the cryptic morphorype and *Platynereis* sp. seemed to prefer the warmer southeast coast (Kara et al. 2020a). Unfortunately, samples were not collected fromFurther sampling in the southeastern coast between Witsand and Umgazana is required to, an dassess the presence of *T. izinqa* cannot be confirmed there.

It is importantCorrect identifications of that species used as bait are identified correctly so that we cankey to understand their usage so thatand to develop appropriate management practices allowingcan be adopted to preventing overexploitation (Simon et al. 2021b). Recent investigation ofThe common names applied to bait species by recreational and subsistence fisherman found that wonderworm wais widely used when referring to eunicid worms used as baits including (i.e., *Marphysa*, *Eunice* and *Lysidice*), ao it is highly and qualifying names were recommended qualifying names to facilitate distinguish among species (Simon et al. 2021b, Simon et al. 2002). We here proposed the name brown wonderworm for *T. izinqa* sp. nov., whose The characteristic brown body and white-tipped antennae of *T. izinqa* (brown wonderworm) will allow rangers to distinguishing it from *M. haemasona*, (known as blood wonderworm and being ; reddish/ deep purple colour with white flecks all over its body and white and brown tipped antennae, particularly to rangers) when inspecting bait harvested by fishermen. This will facilitate aallow better understanding of which species are being used more commonly used throughout their distribution ranges, as well as to understand how harvesting pressures impacts their populations along the coast and thusto improve resource management practices of these two resources (Simon et al. 2021b).

This Our study, like Kara et al. (2020b) has once again confirmsed that the previously underestimated the indigenous diversity of species previously identified as *Marphysa* (Kara et al. 2020b) and had been significantly underestimated. This study also provides evidence to supports the estimates in Simon et al. (2022) that more than 500 of the South African species recorded for the South African coast are hiding undescribed local species based on the proportion of unresolved cosmopolitan taxa hiding undescribed local species (Simon et al. 2022). Among them, *Marphysa* was ranked the 7th most important genus requiring taxonomic revisiong, including three unresolved cosmopolitan species with scores above 0.75 (Simon et al. 2022). This Our study has contributed to a better understanding of the diversity, distribution and biogeography of the indigenous species within the genus for the regionof *Marphysa*and related genera, with . Theour morphological findings from this study have contributed to improving our the knowledge on generic character the stability of generic characters within Eunicidae, such as the maxillary apparatus shape and morphology of the maxillary apparatus, the chaetae morphology and distribution of chaetae along the length of the body, the type and number of pectinate chaetate, and the morphology of parapodial features. All them which have

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provedn to be reliable at teasing apart species within genera in Eunicidae (Molina-Acevedo and Idris, 2021; Capa and Hutchings, 2021; Zanol et al. 2021). Lastly, the our newly generated sequences generated in this study will contribute greatly both to understanding the phylogenetic relationships between taxa at the family level and to developing more robust phylogenetic hypotheses that could be usefulfurther allowing in determining divergence times estimates and the evolution trends inof eunicid polychaetes (Zanol et al. 2021).

Acknowledgements

We sincerely thank Emma Sherlock (BMNH), Tarik Meziane (MNHN), Helmut Sattmann (NHMW), Albe Bosman (SAM) and Lena Gustavsson (SMNH) for making available some of the materials that made this study possible. We gratefully acknowledge the financial contribution from the Iziko South African Museums for the financial contribution for of publication fees. To Drs. Luis F. Carrera-Parra and Dr. Sergio I. Salazar-Vallejo for their advice and conversations about on the morphology of the Eunicidae family. To Biol. Humberto Bahena-Basave (ECOSUR, Mexico) for his advice on digital photography and editing and the. We also thank editors and reviewers, whose comments made a significant contribution to improving theis final version of the manuscript.

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Funding

Jyothi Kara was supported by a Postdoctoral Fellowship from the National Research Foundation (Grant Number: 116654) and Isabel C. Molina-Acevedo was supported by a scholarship from CONACyT (514117/298079). The Iziko South African Museums funded the publication fees for this manuscript.

Permits – field work

The collection of specimens was approved by the Department of Forestry, Fisheries and the Environment in South Africa under permit numbers RES2013/13, RES2014/06 issued to Angus Macdonald and RES2019/49 issued to Carol Simon.

Author contributions

• Jyothi Kara and Isabel C. Molina-Acevedo conceived and designed the experiments, performed the experiments, analyszed the data, prepared figures and/or tables, authored and reviewed drafts of the paper, and approved the final draft.

• Joana Zanol, Carol Simon and Angus Macdonald reviewed drafts of the paper, and approved the final draft.

• Angus Macdonald and Carol Simon paid for field trip and sequencing costs for this project.

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Table 1. GenBbank accession numbers (GB), type (TL) locality,and collection (CL) localitiesy and references information efor the mitochondrial cytochrome c oxidase subunit 1 (COI) sequences of taxa used in the molecular analyses

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Figure 1. Sampling localities of *Treadwellphysa izinqa* sp. nov., and *Marphysa mzingazia* sp. nov and *Marphysa haemasona* in South Africa.

Black symbols: *Treadwellphysa T. izinqa* sp. nov.; empty symbols:: Mabibi, Adlams, Ballito, Umhlanga Rocks, Reunion Rocks, Green Point, Port Shepstone, Umgazana and Witsand and *Marphysa M. mzingazia* sp. nov.: Mzingazi Canal, Richards Ba;y. discontinuous dots: *Marphysa*, *haemasona* (Kara et al. 2020; Simon et al. 2021c). Collection dates, : Ttriangles: represent material collected in 2013– and 2014; and circles: represent material collected in 2019. Discontinuos dots represent known distribution of *Marphysa haemasona* (Kara et al. 2020; Simon et al. 2021c).

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Figure 2. Bayesian tree based on mitochondrial cytochrome c oxidase subunit 1 sequences of the species of *Marphysa* sppand *Treadwellphysa*.

*: Bayesian posterior probabilities >95%; are denoted at each node by an *. Ppurple *Treadwellphysa*, *izinqa* sp. nov.; and green: clades refer to the newly named species: *Treadwellphysa izinqa* sp. nov. and *M. arphysa mzingazia* sp. nov.; The scalebar: represents the number of substitutions per site.

Figure 3. *Treadwellphysa izinqa* sp. nov.

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A: anterior region (dorsal), B: middle region (dorsal), C: posterior region (dorsal) (MB-A095291), D: anterior region (dorsal, red arrow: reniform eye), E: ventral view (MB-A095512), F: Maxillary apparatus (MB-A095511). Scale bars: a cA–C = 5 mm, d eD–E = 2 mm, Ff = 1 mm.

Figure 4. *Treadwellphysa izinqa* sp. nov., MB-A095290. Parapodial and chaetal morphology of *Treadwellphysa izinqa* sp. nov., MB-A095290.

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A: 2nd cChaetiger 2, B: 15th chaetiger 15, C: 56th chaetiger 56, D: pPosterior chaetiger, E: aciculum and bidentate subacicular hook. F: limbate capillaries. G: thin isodont pectinate chaetae, wide with fine short teeth, H: thick anodont pectinate chaetae, wide with short fine teeth, posterior chaetiger, I: thick anodont pectinate chaetae, wide with thick short teeth, posterior chaetiger, J: thick anodont pectinate chaetae, wide with short thick teeth, posterior chaetiger, K-L: compound tridentate falcigers, posterior chaetiger. Parapodia are in dorsal view. Scale bars: A: = 0.2 mm, B–D: = 0.5 mm, E–L: = 0.05 mm.

Figure 5. *Marphysa corallina* (Kinberg, 1865) holotype SMNH-type-429.

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A. Anterior end, dorsal view; B. Anterior end, ventral view; C. Maxillary apparatus, dorsal view; D. Chaetiger 3; E. Chaetiger 14; F. Chaetiger 54; G. Chaetiger 98; H. Isodont pectinate chaeta

with wide blade and short, slender teeth, chaetiger 54; I. Isodont pectinate chaeta with narrow blade and short, slender teeth, chaetiger 98; J, Compound falciger, chaetiger 14; K. Compound falciger, chaetiger 98; L. Subacicular hook, chaetiger 54. All chaetigers in anterior view. Scale bars: A, B = 1.5 mm; C, = 0.8 mm; D–F, = 0.3 mm; G, = 0.25 mm; H, = 10 µm; I, = 8.5 µm; J, L, = 12.3 µm.

Figure 6. *Marphysa mzingazia* sp. nov., MB-A095294.

A: anterior region (dorsal), B: middle region (dorsal), C: Prostomium (dorsal, black arrows=red eyes), D: Maxillary apparatus MI-MII, E: Maxillary apparatus MIII-MV, F: A: 2nd cChaetiger 2, G: 41st cChaetiger 41, H: 98th cChaetiger 98 (dorsal), I: 141st cChaetiger 141 (dorsal), J: Isodont pectin, 13th cChaetiger 13 (holotype), K: Isodont pectinate, posterior chaetiger, L: Isodont pectinate, posterior chaetiger, M: Anodont pectinate, posterior chaetiger, N: Spinigers, 141st chaetiger 141, O: Subacicular hook, weakly bidentate, P: Subacicular hook. Scale bars: A–B = 0.2 mm, C–D = 0.5 mm, E–G = 0.02 mm.

Commented [DM42]: also the photos here look dark and lack contrast. thso on subacicular hooks include too much balck tissues and the hooks are small. J is particularly unclear