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## Research article

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# Polycladida (Platyhelminthes, Rhabditophora) from Cape Verde and related regions of Macaronesia

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**Abstract.** The systematics and distribution of the order Polycladida within the Macaronesian archipelagos are analysed. New species (*Marcusia alba* sp. nov., *Prostheceraeus crisostomum* sp. nov., *Parviplana sodade* sp. nov., *Euplana claridade* sp. nov., *Stylochus salis* sp. nov. and *Distylochus fundae* sp. nov.), new variety (*Pseudoceros rawlinsonae* var. *galaxy*), new records and records of shared species among different archipelagos are studied to compare the marine flatworm biodiversity of each island. The complex of archipelagos known as Macaronesia (including Madeira, Selvagens Islands, Canary Islands, Azores and Cape Verde) share a volcanic origin and European political influence. The five archipelagos are located along the eastern coast of the Atlantic Ocean and are subject to similar trade winds, streams (like the Gulf Stream) and cold currents. The term Macaronesia has suffered several changes throughout the years and it still is a topic of discussion in present times. The new delimitation of Macaronesia is mainly based on systematic studies on the invertebrate fauna of the islands. The resulting analyses shed new light on the differences and similarities among these archipelagos. In addition, molecular analyses employing 28S nuclear gene sequences are compared to verify relationships among anatomically similar species of marine polyclads.

**Keywords.** Flatworms, distribution, Acotylea, Cotylea, 28S.

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## Introduction

From a political and biogeographic point of view, the complex of archipelagos known as Macaronesia is a mosaic of ecological and political factors, with two common denominators. One is that they are of volcanic origin and the other is that they are mostly European. Azores, Madeira and Selvagens Islands are Portuguese, the Canary Islands Spanish and Cape Verde independent, but with great European influence.

The five archipelagos are located along the eastern region of the Atlantic Ocean between latitude 15° N (Cape Verde) and 40° N (Azores) (Fig. 1) and are influenced by trade winds, the eastern branch of the Gulf Stream and the cold currents of the Canary Islands. Nonetheless, the biogeographic limits and definition of Macaronesia have undergone several changes over the years. Sunding (1979) included as part of Macaronesia a land strip ranging from Agadir (Morocco) to South Western Sahara (northern coast of the African continent), the so-called Macaronesian continental enclave. Sunding's inclusion of North Africa was the last addition to Macaronesia.

According to De Nicolas *et al.* (1989), Cape Verde belongs to Macaronesia due to its volcanic origin, but the presence of coral barriers, a tropical climate and specific vegetation make this archipelago notably different. Nonetheless, Kunkel (1993) subdivided Macaronesia into three subregions including Cape Verde: 1. Great Macaronesia (“Gross-Makaronesien”), which comprises the five archipelagos and the African and Iberian continental enclaves; 2. Central Macaronesia with Madeira, Selvagens Islands and Canary Islands and 3. Lauri-Macaronesia including Madeira, Selvagens Island, Canary Islands, Azores and the southwest region of Portugal (Algarve) (Fig. 1D), where some subregions overlap.

Médail & Quézel (1999), after an exhaustive study on the vegetation of southwestern Morocco and the Canary Islands, concluded that North Africa should not be part of Macaronesia and included it in the Mediterranean region as the Agadirensis province (Galán de Mera *et al.* 2003) or Agadiro-Ifniense region (Rivas-Martínez 2009; Salas & Naranjo 2015).

Macaronesia should be included in the Mediterranean hotspot as both biogeographical regions share similar fauna, flora and climatology (Myers *et al.* 2000). In recent studies (Spalding *et al.* 2007), two marine ecoregions were established in relation to Macaronesia, namely the Lusitanian-Macaronesia region, formed by the archipelagos of Azores, Madeira, Selvagens Islands and Canary Islands, and the West African Transition region, which only includes Cape Verde. These considerations are shared also by Freitas *et al.* (2019) who proposes that Cape Verde must be considered a unique biogeographic region not included in the Macaronesia complex.

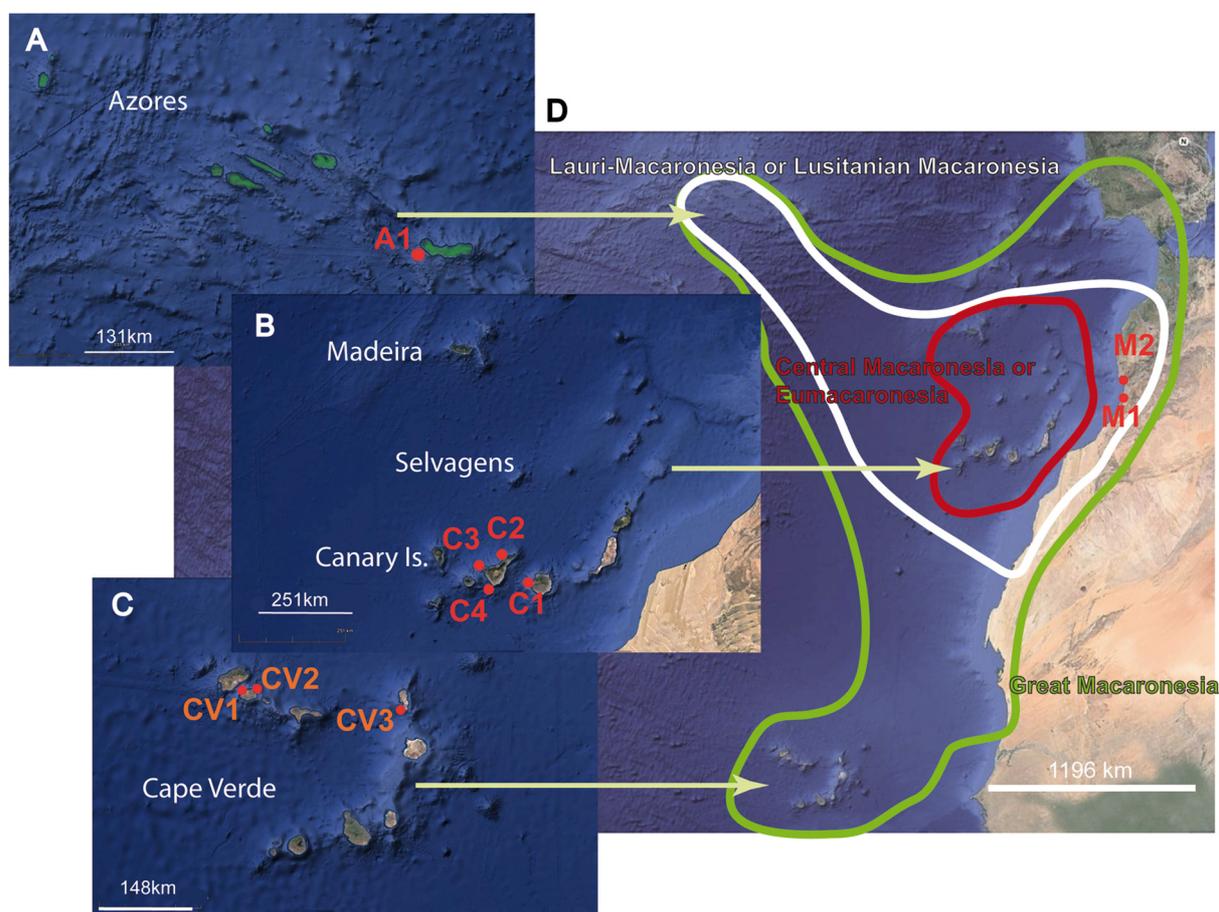
Today, most scientists accept that the term ‘Macaronesia s. str.’ is no longer valid as a biogeographical concept (Salas & Naranjo 2015), but some archipelagos have conserved the Macaronesian idea: Madeira, Selvagens Islands and Canary Islands are considered ‘Eumacaronesia’ (Fig. 1) within the Canary-Madeira sub-region (Rivas-Martínez 2009) or ‘Webbnesia’ after Freitas *et al.* (2019).

In the past as well as today, the interest on these archipelagos remains due to their ecological conditions and its strategic position along marine routes. However, some islands are much better studied and explored than others. The best known, geologically and biologically, are the Canary Islands, Cape Verde and Madeira, followed by the Azores. Selvagens Islands are practically unknown.

In the present study, the systematics and distribution of the Polycladida Lang, 1881 order within this archipelagos complex are analysed. New species, new records and records of shared species are considered to study the biodiversity between the archipelagos. The Macaronesian polyclad species are mainly known from the detailed studies conducted by Plehn (1896) and Laidlaw (1903, 1906) in Cape Verde and the Azores. According to Laidlaw (1906), the species of Cape Verde have a markedly Mediterranean character, but it should be noted that not all the regions and coasts bathed by the Atlantic waters are known.

Additionally, the morphological results were studied alongside the analyses of the 28S nuclear gene to verify relationships among anatomically similar and/or similarly distributed species (Table 1).

In summary, new concepts of the Macaronesia are based mainly in systematic studies on the invertebrate fauna (echinoderms, molluscs, decapod crustaceans, or polychaete annelids), fishes and algae of the islands. The present study on the systematics and distribution of the order Polycladida sheds new light



**Fig. 1.** Location of sampling points, distribution of the Macaronesian archipelagos and limitation of regions: Lauri-Macaronesia or Lusitanian Macaronesia (white); Central Macaronesia or Eumacaronesia (bordeaux) and Great Macaronesia (green). Abbreviations: A1 = San Miguel Island, Azores; C1 = Pasito Blanco, Gran Canaria, Canary Islands; C2 = Tenerife, Canary Islands; C3 = Garachico, Tenerife, Canary Islands; C4 = Amarilla Golf, Tenerife, Canary Islands; CV1 = Astillero Mindelo, São Vicente Island, Cape Verde; CV2 = Bahia das Gatas, São Vicente Island, Cape Verde; CV3 = Calheta Funda, Sal Island, Cape Verde; M1 = Ifni, South Morocco; M2 = Agadir, South Morocco.

**Table 1** (continued on next three pages). Species, localities and accession numbers of the material studied in the present work. Species sequenced for this study are highlighted in blue.

	Species	Localities	GenBank code
<b>Outgroup</b>	<i>Prorhynchus stagnalis</i>		KC869866
<b>ACOTYLEA</b>	<i>Callioplana marginata</i>	Japan	LC100082
	<i>Imogine zebra</i>	Massachusetts	AF342800
	<i>Imogine refertus</i>	Brazil	KY263694
	<i>Stylochus</i> sp.	Peru	KY263743
	<i>Paraplanocera oligoglana</i>	Hawaii	KC869849
	<i>Imogine ijimai</i>	Japan	LC100079
	<i>Leptostylochus gracilis</i>	Japan	LC100078
	<i>Latocestus plehni</i>	Cape Verde Is.	<b>MK299376</b>
		Canary Is.	<b>MK299377</b>
	<i>Hoploplana villosa</i>	Japan	LC100076
	<i>Hoploplana californica</i>	California	KC869850
	<i>Hoploplana divae</i>	Brazil	KY263692
		Brazil	KY263693
	<i>Paraplanocera</i> sp.	Greece	KY263699
	<i>Planocera multitentaculata</i>	Japan	LC100081
	<i>Planocera pellucida</i>	Canary Is.	<b>MK299355</b>
		Cape Verde Is.	<b>MK299356</b>
		Canary Is.	<b>MK299363</b>
	<i>Amemiyaia pacifica</i>	Japan	LC100077
	<i>Adenoplana evelinae</i>	Brazil	KY263647
	<i>Phaenocelis medvedica</i>	Brazil	KY263701
		Brazil	KY263702
	<i>Discocelis tigrina</i>	Canary Is.	<b>MK299370</b>
		Barcelona	AF022744
	<i>Ilyella gigas</i>	Japan	LC100080
	<i>Leptoplana tremellaris</i>	Spain	KY263695
		Spain	KY263696
	<i>Armatoplana leptalea</i>	Brazil	KY263648
		Brazil	KY263649
	<i>Notoplana</i> sp.	Brazil	KY263651
		Brazil	KY263650
	<i>Notoplana delicata</i>	Japan	LC100088
	<i>Koinostylochus elongatus</i>	Japan	LC100083
	<i>Pseudostylochus obscurus</i>	Japan	LC100084
	<i>Notoplana australis</i>	Australia	AY157153
	<i>Notocomplana humilis</i>	Japan	LC100085
	<i>Notocomplana</i> sp.	Japan	LC100089

**Table 1** (continued).

<b>Species</b>	<b>Localities</b>	<b>GenBank code</b>
	Spain	KY263689
<i>Cycloporus gabriellae</i>	Brazil	KY263656
	Brazil	KY263658
<i>Eurylepta cornuta</i> var. <i>melobesiarum</i>	Cape Verde	<b>MK299350</b>
<i>Eurylepta</i> sp.	Martinica Is.	<b>MK299372</b>
<i>Pseudoceros harrisi</i>	Panama	EF514802
<i>Pseudoceros astrorum</i>	Brazil	KY263737
<i>Pseudoceros atropurpureus</i>	Japan	LC100098
<i>Pseudoceros hancockanus</i>	Japan	LC100100
<i>Pseudoceros velutinus</i>	Greece	KY263740
	Spain	KY263741
	Japan	LC100095
	Japan	LC100095
<i>Pseudoceros velutinus</i>	Cape Verde Is.	<b>MK299360</b>
	Cape Verde Is.	<b>MK299380</b>
	Canary Is.	<b>MK299381</b>
<i>Pseudoceros rawlinsonae</i>	Brazil	KY263731
	Brazil	KY263733
	Florida	GQ398102
	Bahamas	GQ398101
	Virgin Is.	EF514803
<i>Pseudoceros rawlinsonae</i> var. <i>galaxy</i>	Cape Verde Is.	<b>MK299357</b>
	Cape Verde Is.	<b>MK299358</b>
<i>Pseudoceros nipponicus</i>	Japan	LC100096
<i>Pseudoceros bicolor</i>	Brazil	KY263730
	Panama	GQ398096
	Florida	GQ398097
	Jamaica	GQ398100
<i>Pseudoceros contrarius</i>	Papua NG	KY263728
<i>Yungia</i> sp.	Florida	HQ659018
<i>Yungia aurantiaca</i>	Cádiz (Spain)	<b>MK299386</b>
<i>Pseudobiceros nigromarginatus</i>	Japan	LC100097
<i>Pseudobiceros pardalis</i>	Brazil	KY263723
	Panama	EF514807
	Panama	EF514808
<i>Pseudobiceros wirtzi</i>	Senegal	KY263725
<i>Pseudobiceros bedfordi</i>	Papua NG	KY263715
<i>Maiazon orsakii</i>	Papua NG	KY263697
<i>Phrikoceros mopsus</i>	Brazil	KY263707

**Table 1** (continued).

	<b>Species</b>	<b>Localities</b>	<b>GenBank code</b>
	<i>Notocomplana japonica</i>	Japan	LC100087
	<i>Notocomplana koreana</i>	Japan	LC100086
<b>COTYLEA</b>	<i>Cestoplana rubrocinta</i>	Cyprus	<b>MK299367</b>
		Cyprus	<b>MK299368</b>
		Cyprus	<b>MK299369</b>
	<i>Cestoplana salar</i>	Brazil	KY263653
	<i>Cestoplana techa</i>	Brazil	KY263652
		Brazil	KY263654
		Brazil	KY263655
	<i>Pericelis</i> sp.	Cape Verde	<b>MK299353</b>
		Cape Verde	<b>MK299354</b>
	<i>Pericelis byerleyana</i>	Martinica	<b>MK299374</b>
	<i>Pericelis cata</i>	Brazil	KY263700
		Canary Is.	<b>MK299351</b>
		Cape Verde	<b>MK299352</b>
		Cape Verde	<b>MK299373</b>
	<i>Boninia divae</i>	Panama	KC869846
	<i>Chromyella</i> sp.	Panama	KC869848
	<i>Theama</i> sp.	Panama	KC869845
	<i>Anonymus ruber</i>	Canary Is.	<b>MK299347</b>
		Cape Verde	<b>MK299348</b>
		Cape Verde	<b>MK299364</b>
		Cape Verde	<b>MK299365</b>
		Cape Verde	<b>MK299366</b>
	<i>Enchiridium evelinae</i>	Brazil	KY263682
		Brazil	KY263683
	<i>Enchiridium magec</i>	Canary Is.	<b>MK299349</b>
		Canary Is.	<b>MK299371</b>
	<i>Prosthlostomum grande</i>	Japan	LC100090
	<i>Prosthlostomum vulgare</i>	Japan	LC100091
	<i>Cycloporus variegatus</i>	Brazil	KY263657
		Spain	KY263659
	<i>Cycloporus japonicus</i>	Japan	LC100092
	<i>Maritigrella fuscopunctata</i>	Indo-Pacific	KU674837
		Indo-Pacific	KU674838
	<i>Prostheceraeus vittatus</i>	Sweden	AJ315647
	<i>Maritigrella crozieri</i>	Florida	KY263686
		Florida	KY263687
	<i>Prostheceraeus roseus</i>	Spain	KY263688

**Table 1** (continued).

Species	Localities	GenBank code
	Brazil	KY263709
	Brazil	KY263711
<i>Monobiceros</i> sp.	Cape Verde	<b>MK299359</b>
<i>Monobiceros langi</i>	Spain	KY263710
	Greece	KY263738
	Spain	KY263713
<i>Thysanozoon alagoensis</i>	Brazil,	KY263747
	Martinica Is.	<b>MK299383</b>
	Martinica Is.	<b>MK299384</b>
	Martinica Is.	<b>MK299385</b>
<i>Pseudobiceros</i> sp.	Santa Helena Is.	KY263724
<i>Pseudobiceros caribbensis</i>	Florida	EF514805
	Jamaica	EF514806
	Martinica Is.	<b>MK299378</b>
	Martinica Is.	<b>MK299379</b>
	Cadiz (Spain)	<b>MK299387</b>
<i>Pseudobiceros flavomarginatus</i>	Japan	LC100099
<i>Pseudobiceros evelinae</i>	Brazil	KY263716
	Brazil	KY263717
<i>Pseudobiceros splendidus</i>	Florida	HQ659016
<i>Thysanozoon brocchii</i>	Brazil	KY263744
	Sicily	KY263745
	Australia	HQ659017
	Japan	LC100093
	Cape Verde	<b>MK299361</b>
	Canary Is.	<b>MK299382</b>
<i>Thysanozoon japonicum</i>	Japan	LC100094
<i>Acanthozoon aranfaibo</i>	Canary Is.	<b>MK299362</b>
<i>Thysanozoon raphaeli</i>	Panama	EF514809
	Belize	EF514810

on the differences and similarities among these archipelagos and provides new evidence to reach key conclusions about their biogeography and fauna.

## Material and methods

### Localities, sampling sites and material processed

#### Azores (Fig. 1A)

The Azores is the northernmost archipelago (37°44' N, 25°40' W) and includes nine islands: São Miguel, Pico, Terceira, São Jorge, Faial, Flores, Santa Maria, Graciosa and Corvo.

### **Canary Islands** (Fig. 1B)

The Canary archipelago (28°06' N, 15°04' W) includes two island complexes or provinces: Santa Cruz de Tenerife with four large islands (El Hierro, La Gomera, La Palma and Tenerife) and Las Palmas with three main islands and some small ones (Fuerteventura, Gran Canaria, Lanzarote and Isla Lobos). The Chinijo archipelago is part of Las Palmas and includes five small islands (La Graciosa, Alegranza, Montaña Clara, Roque del Este and Roque del Oeste).

### **Madeira** (Fig. 1B)

The Madeira archipelago (32°45' N, 17°00' W) consists of five islands; two inhabited, Madeira and Porto Santo, and three small, uninhabited isles, the Desertas. Madeira enjoys a Mediterranean climate, like the Canary and Selvagens Islands, which is characterized by 3 months of dryness and high temperatures.

### **Cape Verde** (Fig. 1C)

Cape Verde (15°07' N, 23°37' W) is the southernmost archipelago of Macaronesia and is divided into two groups of islands, Ilhas de Barlovento and Ilhas de Sotavento. Cape Verde comprises ten large islands: Santo Antão, São Vicente, Santa Luzia, São Nicolau, Sal, Boa Vista (Barlovento Island) and Maio, Santiago, Fogo, and Brava (Sotavento Island) as well as five minor isles Raso, Branco, Grande, Cima and Carneiro.

### **Southwestern Morocco** (Fig. 1B)

Although actually northern Morocco may not be part of Macaronesia, we include the species found in Agadir due to the similar ecological conditions and geographical proximity to the Canary Islands.

The study material from the Canary Islands, Cape Verde, Azores, Agadir and Ifni (Morocco) was collected by hand while scuba diving from rocks, the sea bottom, cave surfaces and macro-algae samples. Additional specimens were collected by taking rock and algae samples from the field to the laboratory and waiting for oxygen to be depleted, forcing animals to come out of hiding.

Exhaustive information about the external features was carefully recorded with notes, photographs and drawings. Information about pigmentation, color patterns, movement, size, and presence or absence of tentacles or eyes was gathered. Dorsal structures like papillae, warts or any type of epithelial or dermal formations were compiled.

Most of the photographs were taken on a black background with transmitted light using a Nikon D300 camera fitted with a Micro Nikon 60 mm lens, a Kenko extension tube and two wireless R1 speed lights.

### **Histological processing**

For fixation, the individuals were previously anesthetized with seawater/magnesium chloride (7%). A small piece of tissue was removed for molecular analysis and the whole individual was fixed in Bouin solution (0.8 gr of picric acid in 80 ml, 20 ml of formaldehyde and 2 ml of acetic acid) for histological studies. Histological sagittal series from 6 to 12 micrometres thick were stained with AZAN trichrome. The histological preparations of the studied specimens were deposited in the collections of the Nacional Museum of Natural Sciences (MNCN), Madrid.

For the definitive identification of genus/species internal anatomic reconstructions, particularly of the reproduction apparatus, were made using a Zeiss Axio Scope A1 microscope.

### **DNA extraction, amplification and sequencing**

Tissues for molecular studies were fixed in absolute ethanol. Total genomic DNA was extracted from each sample following the phenol-chloroform protocol (Chen *et al.* 2010). DNA concentration and

purity of the extraction was measured using a NanoDrop Fluorospectrometer (Thermo Fisher Scientific). Sequences of the ribosomal gene 28S of the investigated Polycladida species were studied. All PCRs were performed using Taq DNA polymerase of Mastermix (Invitrogen, Carlsbad, CA) following the manufacturer's protocol in a total volume of 25 µl.

Sequences of approximately 1100 bp of the 28S gene were amplified with degenerated primers designed *de novo* by the first two authors: forward primer (5'-AGCCCAGCACCGAATCCT3-') and reverse (5'-GCAAACCAAGTAGGGTGTTCGC-3'). The PCR consisted in an initial denaturation step at 95°C (3 min), followed a pre-cycle of 5 cycles of denaturation at 96°C (30 sec), annealing at 55°C (30 sec) and extension at 72°C (1 min), followed by 40 cycles of denaturation at 95°C (30 sec), annealing at 59°C (30 sec) and extension at 72°C (1 min), with a final extension of 10 min at 72°C.

Finally, the sequences obtained from forward and reverse primers were combined using the program Geneious R6 (ver. 6.1.5) (<http://www.geneious.com>; Kears *et al.* 2012).

### Sequence alignment and phylogenetic analyses

A comparative analysis with both newly obtained sequences and those obtained from the NCBI GenBank database was carried out. A total of 147 sequences were aligned and edited using Geneious R6 (ver. 6.1.5). Forty-one of them were new sequences (NCBI accession numbers in Table 1).

The newly obtained sequences of 1100 bps were adapted to the length of those gathered from GenBank. The alignment was generated using the program MAFFT ver. 7 (Kato & Standley 2013). Ambiguously aligned and variable regions were recognized and excluded using the program Gblocks ver. 0.91b (Castresana 2000) with relaxed parameters (smaller final blocks, gap positions within the final blocks, and less strict flanking positions allowed). Thus, a matrix of 744 bp was obtained.

Maximum likelihood (ML) was implemented through IQ-TREE (Trifinopoulos *et al.* 2016), using the evolutionary model BIC: TIM2+I+G4. The consensus tree of 1000 bootstrap pseudoreplicates was selected and edited with iTOL ver. 3.1.1 (Letunic & Bork 2016).

Bayesian inference (BI) analyses were carried out using MrBayes ver. 3.2.2 (Ronquist *et al.* 2012) (-mset option). Two independent runs of 1 000 000 generations and four chains (one cold, three heated) were run. Trees were sampled every 1000 generations. Convergence of chains was diagnosed using a deviation of standard frequencies below 0.05 and of the 1001 sampled trees, 250 trees were discarded as burn-in. A majority-rule consensus tree was constructed from the remaining 751 trees to approximate posterior probabilities.

### Abbreviations used in the figures

- af = female atrium
- am = male atrium
- b = brain
- ce = cerebral eyes
- cg = cement glands
- cga = common genital atrium
- cp = cement glands pouch
- e = eyes
- ed = ejaculatory duct
- fg = female gonopore
- i = intestine
- lv = Lang's vesicle

m = mouth  
me = marginal eyes  
mg = male gonopore  
mu = multiple uterine vesicles  
p = penis papilla  
pb = penis bulb  
ph = pharynx  
ps = pseudotentacles  
pt = pseudotentacles  
pv = prostatic vesicle  
s = stylet  
su = sucker  
sv = seminal vesicle  
t = tentacles  
te = tentacular eyes  
u = uteri  
v = vagina

#### **Institutional acronyms for collections**

MNCN = Museo Nacional de Ciencias Naturales, Madrid, Spain

RCCN = Research Collection of Carolina Noreña, Museo Nacional de Ciencias Naturales, Madrid, Spain

#### **Results**

Table 2 lists the complete register of the Polycladida from the Macaronesian Archipelagos. New species and records are described as follows.

AZORES (Fig. 1A). Currently, only six species are recorded for the Azores: *Enchiridium* cf. *magec* Cuadrado, Moro & Noreña, 2017, *Thysanozoon* cf. *brocchii* (Risso, 1818), *Prostheceraeus giesbrechtii* Lang, 1884, *Prostheceraeus* cf. *roseus* Lang, 1884 and the new records *Prostheceraeus moseleyi* Lang, 1884 and *Stylochus* sp. (Table 2) (Wirtz 1994; Wirtz & Debelius 2003).

CANARY ISLANDS (Fig. 1B). The species of the Canary Islands were listed and described recently in Cuadrado *et al.* (2017); therefore, only new records are described in this study: *Discocelis tigrina* (Blanchard, 1847) and *Gnesioceros sargassicola* (Mertens, 1833).

MADEIRA (Fig. 1B). *Pseudoceros wirtzi* (Bahia & Schrödl, 2016), *Pseudoceros* cf. *maximus* Lang, 1884 and *Prostheceraeus giesbrechtii* (Wirtz & Debelius 2003; Bahia & Schrödl 2016) are the three polyclad species currently recorded for Madeira. The new record *Planocera pellucida* (Mertens, 1833) is recorded in this study.

CAPE VERDE (Fig. 1C). 31 species are described for Cape Verde. The new records described in this study include *Pericelis cata* Marcus & Marcus, 1968, *Anonymus ruber* Cuadrado, Moro & Noreña, 2017, *Eurylepta cornuta* var. *melobesiarum* Lang, 1884, *Cycloporus gabriellae* Marcus, 1950, *Pseudoceros velutinus* (Blanchard, 1847), *Pseudoceros mororum* Cuadrado, Moro & Noreña, 2017, *Monobiceros langi* Faubel, 1984 and *Stylochus pillidium* (Götte, 1881). Additionally, a new variety *Pseudoceros rawlinsonae* var. *galaxy* and six new species *Marcusia alba* sp. nov., *Prostheceraeus crisostomus* sp. nov., *Stylochus salis* sp. nov., *Distylochus fundae* sp. nov., *Euplana claridade* sp. nov. and *Parviplana sodade* sp. nov. are described.

**Table 2** (continued on next two pages). Species of the insular complex of Macaronesia s. lat. Locations and bibliographical references. Species analyzed and described in this study are highlighted in blue.

Species	Azores	Madeira	Canary Islands	Cape Verde	Morocco	References
<i>Cestoplana rubrocincta</i>			Tenerife	Boa Vista; São Vicente		Laidlaw 1906; Cuadrado <i>et al.</i> 2017; <b>This study</b>
<i>Pericelis cata</i>			Tenerife	São Vicente		Cuadrado <i>et al.</i> 2017; <b>This study</b>
<i>Traufjelsia elongata</i>				Cape Verde		Laidlaw 1906
<i>Marcusia alba</i> sp. nov.				São Vicente		<b>This study</b>
<i>Anonymus virilis</i>			Tenerife	São Vicente		Laidlaw 1906; Cuadrado <i>et al.</i> 2017
<i>Anonymus ruber</i>			Tenerife	São Vicente		Cuadrado <i>et al.</i> 2017; <b>This study</b>
<i>Enchiridium magec</i>	Faial		Tenerife			Wirtz & Debelius 2003; Cuadrado <i>et al.</i> 2017
<i>Prosthlostomum dohrnii</i>				São Vicente		Laidlaw 1906
<i>Eurylepta cornuta</i> var. <i>melobesiarum</i>				São Vicente, Sal		<b>This study</b>
<i>Eurylepta guayota</i>			El Hierro			Cuadrado <i>et al.</i> 2017
<i>Cycloporus papillosus</i>				Santiago		Laidlaw 1906
<i>Cycloporus gabriellae</i>			Fuerteventura	São Vicente		<b>This study</b>
<i>Oligocladus sanguinolentus</i>				São Vicente		Laidlaw 1906
<i>Prostheceraeus giesbrechitii</i>	Faial	Porto Santo	La Gomera			Wirtz & Debelius 2003; Cuadrado <i>et al.</i> 2017
<i>Prostheceraeus moseleyi</i>	São Miguel					<b>This study</b>
<i>Prostheceraeus roseus</i>			Lanzarote			Cuadrado <i>et al.</i> 2017

Table 2 (continued).

Species	Azores	Madeira	Canary Islands	Cape Verde	Morocco	References
<i>Prostheceraeus rubropunctatus</i>				Cape Verde		Laidlaw 1906
<i>Prostheceraeus cristosomum</i> sp. nov.				Sal		<b>This study</b>
<i>Phrikoceros mopsus</i>			Canary Islands			Cuadrado <i>et al.</i> 2017
<i>Pseudoceros maximus</i>		Madeira	Fuerteventura			Wirtz & Debelius, 2003; Cuadrado <i>et al.</i> 2017
<i>Pseudoceros velutinus</i>			Lanzarote, Tenerife	São Vicente		Cuadrado <i>et al.</i> 2017; <b>This study</b>
<i>Pseudoceros mororum</i>			Gran Canaria	São Vicente		Cuadrado <i>et al.</i> 2017; <b>This study</b>
<i>Pseudoceros rawlinsonae</i> var. <i>galaxy</i>				São Vicente		<b>This study</b>
<i>Pseudoceros</i> sp.			La Gomera			Cuadrado <i>et al.</i> 2017
<i>Pseudobiceros wirtzi</i>		Madeira	Fuerteventura	Santo Antão		Bahia & Schrödl 2016; Cuadrado <i>et al.</i> 2017
<i>Monobiceros langi</i>				São Vicente		<b>This study</b>
<i>Thysanozoon brocchii</i>	Faial		Tenerife	São Vicente		Laidlaw 1906; Wirtz & Debelius 2003; <b>This study</b>
<i>Acanthozoon aranfaibo</i>			El Hierro			Cuadrado <i>et al.</i> 2017
<i>Yungia aurantiaca</i>			Fuerteventura			Cuadrado <i>et al.</i> 2017
<i>Multisepta fengari</i>			El Hierro			Cuadrado <i>et al.</i> 2017
<i>Zygantriplana verrilli</i>				São Vicente		Laidlaw 1906; <b>This study</b>
<i>Cryptocelis loveni</i>					Agadir	Beauchamp 1951

Table 2 (continued).

Species	Azores	Madeira	Canary Islands	Cape Verde	Morocco	References
<i>Planocera pellucida</i>		Madeira	El Hierro	Boa Vista; São Vicente		Laidlaw 1906; Cuadrado <i>et al.</i> 2017; Faubel 1984; <b>This study</b>
<i>Stylochus alexandrinus</i>					Agadir	Prudhoe 1989
<i>Stylochus neapolitanus</i>				Cape Verde	Ifni	Laidlaw 1906,
<i>Stylochus</i> sp.	São Miguel					<b>This study</b>
<i>Stylochus salis</i> sp. nov.				Sal		<b>This study</b>
<i>Stylochus piliidum</i>				São Vicente	Agadir	<b>This study</b>
<i>Imogine mediterranea</i>					Temara	Prudhoe 1989
<i>Distylochus fundae</i> sp. nov.				Sal		<b>This study</b>
<i>Stylochoplana graffi</i>				Deep Sampling, Boa Vista		Laidlaw 1906
<i>Latocestus plehmi</i>			Tenerife	São Vicente		Laidlaw 1906; <b>This study</b>
<i>Polyphalloplana bocki</i>					Agadir	Beauchamp 1951
<i>Gnesioceros sargassicola</i>			Gran Canaria; Tenerife			<b>This study</b>
<i>Discocelis tigrina</i>			Gran Canaria			<b>This study</b>
<i>Notoplana alcinoi</i>				São Vicente		Laidlaw 1906
<i>Emprostopharynx pallida</i>				Boa Vista		Laidlaw 1906
<i>Euplana claridade</i> sp. nov.				São Vicente		<b>This study</b>
<i>Parviplana sodade</i> sp. nov.				São Vicente		<b>This study</b>

SOUTHWESTERN MOROCCO (Fig. 1B). Four Polycladida species are known from the south coast near Agadir (Morocco), three species recorded by Beauchamp (1951): *Polyphalloplana bocki* Beauchamp, 1951, *Stylochus alexandrinus* Steinböck, 1937 and *Cryptocelis loveni* Bergendal, 1890, and *Stylochus mediterraneus* Galleni, 1976 by Prudhoe (1989). In the present study, two new records are described from Morocco, *Stylochus neapolitanus* (Delle Chiaje, 1841-1844) and *Stylochus pilidium* (Götte, 1881) (Table 2).

### ***New species and varieties***

Order Polycladida Lang, 1881

Suborder Cotylea Lang, 1884

Family Diposthidae Woodworth, 1898 sensu Litvaitis *et al.*, 2019

Genus *Marcusia* Hyman, 1953

***Marcusia alba*** sp. nov.

urn:lsid:zoobank.org:act:DE4AF1C8-D784-4AB7-B536-A1E88524115B

Figs 1C, 2

### **Etymology**

The name of the new species, *Marcusia alba*, comes from the Latin ‘*albus*’ (white), and refers to the ivory white coloration this species shows.

### **Material examined (3 specs)**

#### **Holotype**

CAPE VERDE • São Vicente Island, Mindelo; 16°53'46.54" N, 24°59'32.93" W (Fig. 1C V1); 6 May 2017; Leopoldo Moro leg.; MNCN 4.01/2620 to 2683 (64 slides). One sagittally sectioned specimen stained with AZAN.

#### **Additional material**

CAPE VERDE • 2 specs; São Vicente Island, Baía das Gatas; 16°54'09.33" N, 24°54'25.25" W (Fig. 1C V2); 6 May 2017; Leopoldo Moro leg.; RCCN.

### **Description**

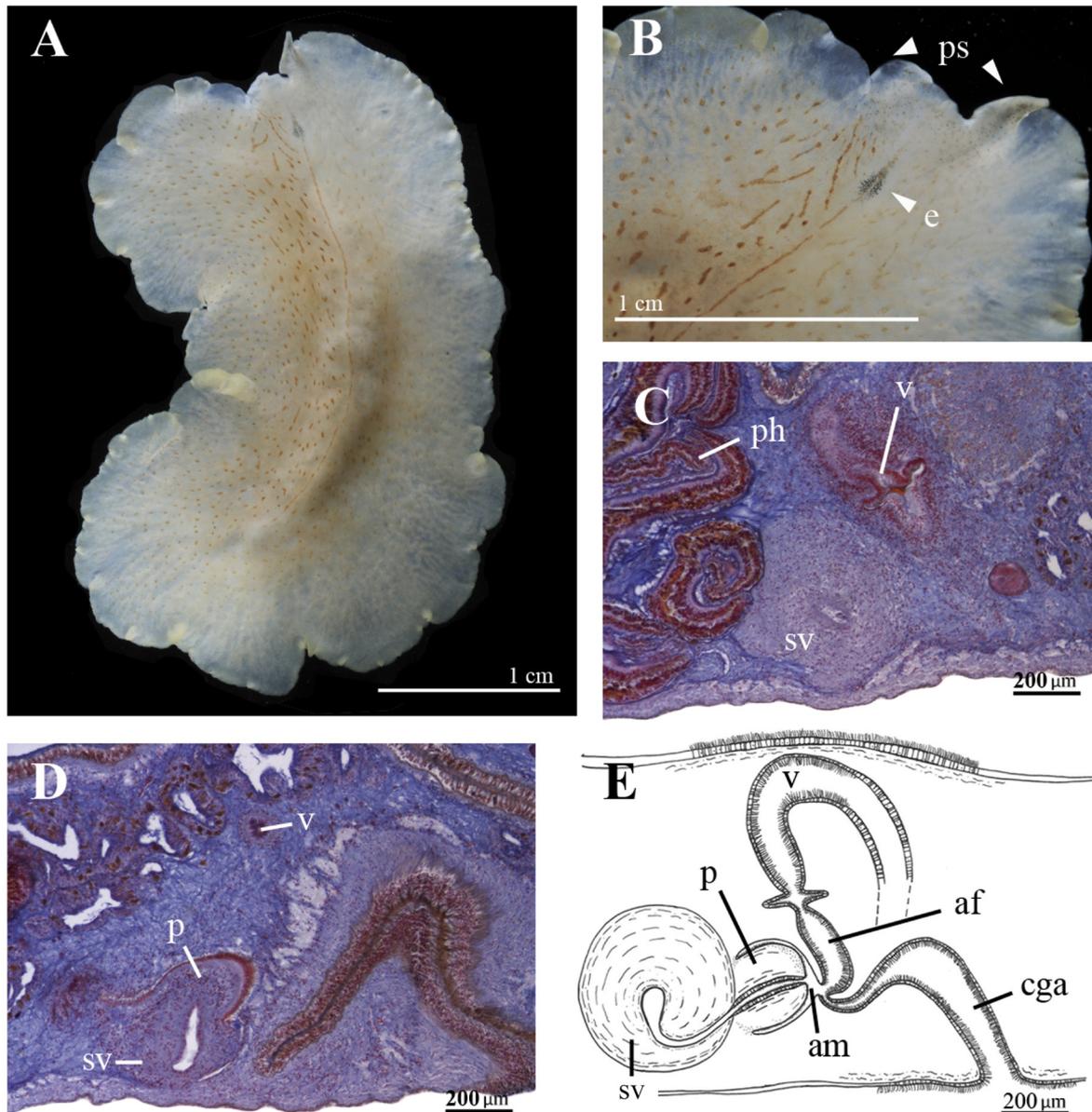
**BODY.** Shape oval. Length 2.6 cm. Smooth dorsal surface. Background pigmentation ivory white, darker along the middle dorsal region of the body. Amber dots and thin brushstroke-like lines garnish the dorsal surface. A thin, dark stripe, sometimes interrupted, extends along the central dorsal line (Fig. 2A). Two delicate marginal folds, separate and pointed, could be interpreted as pseudotentacles. Tentacular eyes scarce and widely dispersed over the tentacles. Cerebral eyes anterior drop-shaped and crossed by the central midline. Marginal eyes only at the anterior part (Fig. 2B). Ruffled pharynx, in the middle of the body with a central oral pore. Ventral sucker at the posterior part of the body. Male and female genital pore lead in a common genital atrium (Fig. 2D–E) that opens in the posterior body region after the pharynx (Fig. 2C).

**MALE REPRODUCTIVE SYSTEM.** Male copulatory organ backwards oriented, with a muscular penis papilla, very muscular seminal vesicle (Fig. 2C, E) and without a prostatic vesicle, instead a simple glandular epithelium leads into the penis papilla (Fig. 2D–E). Seminal vesicle rounded, frontal oriented and with thick muscular walls, opening into the ejaculatory duct. Short ejaculatory duct opens into the penis papillae. The male atrium is small and thin, connected to the common atrium.

FEMALE REPRODUCTIVE SYSTEM (Fig. 2C, E). The vagina runs from the common genital atrium and continues dorsally into a narrowed duct that widens into a chamber, the cement pouch. The vagina continues dorsally, then curves posteriorly and ventrally and ends with the entry of the oviducts.

**Remarks**

*Marcusia alba* sp. nov. belongs to the genus *Marcusia* due to the presence of cerebral, frontal and marginal eyes, male copulatory organ enclosed in a muscular bulb, the absence of prostatic vesicle and the common male and female atrium genital, as well as the common gonopore.



**Fig. 2.** *Marcusia alba* sp. nov. (MNCN 4.01/2620 to 2683). **A.** Whole live animal, dorsal view. **B.** Anterior region with cerebral eyes cluster and pseudotentacles (white arrows). **C.** Histological section of the male copulatory organ stained with AZAN. **D.** Histological section of the female copulatory organ stained with AZAN. **E.** Sagittal reconstruction of the reproduction system. Abbreviations: see Material and methods.

The genus *Marcusia* contained only one species, *Marcusia ernesti* Hyman, 1953, known from the coast of the Gulf of California (Hyman 1953). *Marcusia ernesti* and *M. alba* sp. nov. can be easily distinguished by their coloration patterns. *Marcusia ernesti* is black or grey with darker splotches and dotted with white spots, only visible in preserved individuals after Hyman (1953), *M. alba* sp. nov. is ivory white with brownish dots and stripes. The penis papilla is spherical in *M. alba* and elongated in *M. ernesti*, with the male atrium being tube-like and longer in the Californian species.

Another difference lies in the eyes' presence and distribution. *Marcusia ernesti* presents marginal, frontal and cerebral eyes as well as two characteristic eye clusters with diagnostic value (Hyman 1953). *Marcusia alba* sp. nov., on the other hand, has cerebral, marginal and tentacular eyes, but not frontal eyes or eye clusters.

The differences listed are enough to consider *M. ernesti* and *M. alba* sp. nov. as two different species of the same genus. Furthermore, the molecular analyses show the genus *Marcusia* (represented in this case by *Marcusia alba* sp. nov.) as a genus closely related to *Pericelis* Laidlaw, 1902 within the family Anonymidae Lang, 1884, but as a clearly independent genus.

Family Euryleptidae Lang, 1884  
Genus *Prostheceraeus* Schmarda, 1859

***Prostheceraeus crisostomum*** sp. nov.  
urn:lsid:zoobank.org:act:EC7A7E2B-99FD-447D-A189-676144875AC2  
Figs 1C, 3A–D

### Etymology

The name of the new species, *Prostheceraeus crisostomum*, is dedicated to the little cat, Crisostomo, roommate during the description of this species.

### Material examined

#### Holotype

CAPE VERDE • Sal Island, Calheta Funda; 16°39'03.34" N, 22°56'42.94" W (Fig. 1C V3); 8 Jul. 2018; Leopoldo Moro leg.; MNCN 4.01/2684 to 2698 (15 slides). One sagittally sectioned specimen stained with AZAN.

### Description

**BODY.** Shape elongated. Length 0.5 cm. Smooth dorsal surface; background pigmentation ivory white, darker along the middle dorsal region of the body between the cerebral eyes and the posterior end of the body. In the posterior middle end it shows a large conspicuous black spot. Small black dots on the entire dorsal surface (Fig. 3E). Two marginal tentacles, separate. Tentacular eyes scarce and widely dispersed between the tentacles. Cerebral eyes arrow-shaped located by the central midline (Fig. 3F). Bell-shaped pharynx located in the first body half. Ventral sucker in the middle of the body. Male and female genital pores well separated and located after the pharynx (Fig. 3G–H).

**MALE REPRODUCTIVE SYSTEM.** Male copulatory organ oriented forward. The muscular prominent penis papilla houses a conical, elongated stylet of pseudosclerotized nature. The rounded and well developed prostatic vesicle joins transversally with the sperm duct and lies over the penis papilla (Fig. 3H). Muscular seminal vesicle oval, dorsally located and caudo-frontally oriented. The *vasa deferentia* join at the ventro-caudal region of the vesicle and the sperm duct open ventro-frontally. Seminal and prostatic vesicles open together into the proximal region of the developed penis papillae. The male atrium surrounded the penis papillae and opens near the posterior end of the pharynx.



Three other species of *Prostheceraeus* show a color pattern free of lines or bands: *P. albocinctus* Lang, 1883, *P. moseleyi* and *P. rubropunctatus* Lang, 1884. These three species, together with *P. crisostomum* sp. nov., have a dotted pattern, but the background colors are different in the four species: caramel brown background with white or whitish spots and white marginal line in *P. albocinctus*, blue-gray or cream background with black dots and yellow marginal band in *P. moseleyi* and finally *P. rubropunctatus* with a pink to reddish background color, white dots and without marginal band. The base coloration of *P. crisostomum* is similar to *P. albocinctus*, but much clearer and almost ivory; the dorsal points are black like in *P. moseleyi* and lacks a marginal line or band similar to *P. rubropunctatus*. All these differences delimit *P. crisostomum* sp. nov. as a new species of the genus *Prostheceraeus*.

Family Pseudocerotidae Lang, 1884

Genus *Pseudoceros* Lang, 1884

*Pseudoceros rawlinsonae* var. *galaxy* var. nov.

Figs 1C, 3E–I

### Etymology

The name ‘galaxy’ comes from the pattern of the small white spots on the dorsal surface, which resemble a star galaxy.

### Material examined (2 specs)

#### Holotype

CAPE VERDE • São Vicente Island, Baía das Gatas; 16°54′09.33″ N, 24°54′25.25″ W (Fig. 1C V2); 5 May 2017; Leopoldo Moro leg.; MNCN 4.01/2729 to 2798 (70 slides). One sagittally sectioned specimen stained with AZAN.

#### Additional material

CAPE VERDE • 1 spec.; same collection data as for holotype; RCCN.

### Description

**BODY.** Shape oval. Length 2.3 cm. Smooth dorsal surface. Background color dark to velvety brown with white dots and spots that draw a longitudinal line along the body axis. One thin bright yellow external line and another internal and black line surround the entire body margin. The yellow one is interrupted at the pseudotentacles level (Fig. 3E–F). Marginal body edges lined with transversal whitish elongated drops (Fig. 3F). Ventral coloration dark grey to black. Ventral sucker in the middle of the body (Fig. 3G). Pseudotentacles constitute two simple folds that present each of them a small cluster of tentacular eyes in their margin. Round cluster of cerebral eyes present and surrounded by a spot of white pigment. Pharynx ruffled, butterfly-shaped and located at the anterior third of the body. Oral pore, female and male gonopore close to each other and located at the anterior end (Fig. 3G). Male and female genital pores located after the pharynx in the anterior half of the body (Fig. 3H–I).

**MALE REPRODUCTIVE SYSTEM.** Male genital pore between the posterior lobes of the ruffled pharynx. Male copulatory organ dorso-ventrally orientated consists in a prostatic vesicle and a very muscular seminal vesicle, as well as a penis papilla armed with a stylet (Fig. 3H–I). *Vasa deferentia* open separately into the seminal vesicle. Seminal vesicle rounded, frontally oriented and lined with a thick muscular wall. Prostatic vesicle rounded, muscular and smaller than the seminal vesicle. Sperm duct muscular and long, extends frontally to join the prostatic duct inside the proximal end of the conical stylet. The short ejaculatory duct appears surrounded by the stylet cone and the penis sheath. The male atrium is wide and tetra-folding (fork-like) as characteristic of the genus (Fig. 3I).

FEMALE REPRODUCTIVE SYSTEM. With a short muscular vagina, backwards oriented and surrounded by cement glands.

### Remarks

The genus *Pseudoceros* comprises approximately 89 species with similar copulatory organs, but bright and unique coloration patterns. However, within these patterns some taxa share evident similarities. *Pseudoceros rawlinsonae* var. *galaxy* shares with *P. bicolor* Verrill, 1902, *P. mororum* and *P. rawlinsonae* Bolaños, Quiroga & Litvaitis, 2007 the brown background and one whitish, broad marginal band, but in *P. bicolor* the marginal band is wide with inner waves (Litvaitis *et al.* 2010: fig. 4a–i); *P. rawlinsonae* shows, in addition to the wide band, a thin orange line (Litvaitis *et al.* 2010: fig. 4j–p); in *P. mororum* the whitish band is interrupted and drop-shaped and additionally, two orange marginal stripes border the entire body (Cuadrado *et al.* 2017: fig. 6a–b); finally, the Cape Verdean species shows, together with the drop-shaped white band, two black and orange thin lines (Fig. 3E–F).

Although the four previously mentioned species can be clearly differentiated due to their coloration, this is not the case in the molecular analysis (Fig. 8). In both the Bayesian and Maximum Likelihood analyses, individuals from Cape Verde appear closely related to *P. rawlinsonae*, so much so that the separation of both populations (the Cape Verdean population and the Caribbean population) is only possible at the level of variety, not of species. Therefore, we determined the individuals from Cape Verde as a variety within the species *P. rawlinsonae*.

Nonetheless, we want to emphasize that the decision to maintain this population (organisms) as a ‘variety’ of the species *P. rawlinsonae* is the sole and exclusive responsibility of the authors. We are aware that ‘variety’ is not a taxonomic category (according to ICZN) and that therefore it will remain a non-existent species until molecular analyses allow us to consider it as such.

Suborder Acotylea Lang, 1884  
Superfamily Leptoplanoidea Faubel, 1984  
Family Leptoplanidae Stimpson, 1857  
Genus *Parviplana* Hyman, 1953

***Parviplana sodade* sp. nov.**

urn:lsid:zoobank.org:act:10171C70-7431-41EF-B39D-AC3982D24143

Figs 1C, 4A–C

### Etymology

The name of the new species, *Parviplana sodade*, comes from ‘sodade’ the Cape Verdean expression for saudade and regional song with rhythms of ‘coladeira’.

### Material examined

#### Holotype

CAPE VERDE • São Vicente Island, Mindelo; 16°53'46.54" N, 24°59'32.93" W (Fig. 1C V1); 24 Nov. 2017; Leopoldo Moro leg.; MNCN 4.01/2699 to 2708 (10 slides). One sagittally sectioned specimen stained with AZAN.

### Description

BODY. Shape oval elongated. Length 0.8 cm. Smooth dorsal surface. Background pigmentation light white, transparent where the intestinal braches can be appreciated (Fig. 4A–B). Four clusters of cerebral eyes, two anterior with few eyes and more elongated than the posterior two. In sum around 50 cerebral eyes (Fig. 4B). Ruffled pharynx. Male and female genital pores located in the posterior half of the body.

**MALE REPRODUCTIVE SYSTEM.** Directed backwards and with a dorso-ventrally oriented penis papilla. With elongated prostatic vesicle, tall granular lining included in the muscular penis bulb (Fig. 4C). The *vasa deferentia* enter the seminal vesicle separately. Seminal vesicle rounded, below the penis bulb and connected with a sort seminal duct to the prostatic vesicle (Fig. 4C). The male atrium is small and thin, with an internal fold that surrounds the distal part of the penis bulb like a penis sheath (Fig. 4C).

**FEMALE REPRODUCTIVE SYSTEM.** With a vagina bulbosa (Fig. 4C) and backwards oriented. Cement and shell glands open in a pouch located within the vaginal complex. Lang's vesicle present.

### Remarks

*Parviplana sodade* sp. nov. belongs to the genus *Parviplana* due to the absence of tentacles, presence of seminal vesicle, and prostatic vesicle with a tall granular lining with prostatic functions. Female apparatus with vagina bulbosa and Lang's vesicle.

*Parviplana* comprises 3 species, *P. hymani* Faubel, 1983, *P. jeronimo*i Pérez-García, Noreña & Cervera, 2018 and *P. lynca* (Du Bois-Reymond Marcus, 1958). *Parviplana lynca* can be easy and clearly distinguished from the other two species by the presence of nuchal tentacles, exclusive of this species.

*Parviplana hymani* can be distinguished from *P. sodade* sp. nov., by the *vas deferens* which opens together into the seminal vesicle, and the prostate vesicle not included into de penis bulb.

*Parviplana sodade* sp. nov. possesses more similarities with *P. jeronimo*i. Both species share the penis sheath and more than 25 cerebral eyes, but clear differences separate them. *Parviplana jeronimo*i has a fleshy appearance and amber pigmentation. The size is also noticeable different, *P. jeronimo*i can reach lengths of 20 mm, while *P. sodade* in full mature state does not reach 8 mm. *Parviplana jeronimo*i also presents *vasa deferentia* joined in a single *vas deferens*, a small female atrium and a corrugated surface between the two genital pores, characteristics not present in *P. sodade*.

Family Euplanidae Marcus & Marcus, 1966

Genus *Euplana* Girard, 1893

***Euplana claridade*** sp. nov.

urn:lsid:zoobank.org:act:C7E211FD-32AF-4FA4-A3EB-ACA1737DF2EE

Figs 1C, 4D–H

### Etymology

The name of the new species, *Euplana claridade*, comes from “Claridade”, a journal of literary review that revolutionized Cape Verdean culture during the first half of the twentieth century.

### Material examined

#### Holotype

CAPE VERDE • São Vicente Island, Mindelo; 16°53'46.54" N, 24°59'32.93" W (Fig. 1C V1); 23 Nov. 2017; Leopoldo Moro leg.; MNCN 4.01/2709 to 2718 (10 slides). One sagittally sectioned specimen stained with AZAN.

### Description

**BODY.** Shape oval. Length 1.1 cm. Smooth dorsal surface. Background pigmentation ivory white, denser along the middle dorsal region of the body and in the intestinal braches (Fig. 4D). Two clusters of 16 cerebral eyes each (Fig. 4E). Ruffled pharynx. Male and female genital pores located in the second half of the body close behind the posterior end of the pharynx.

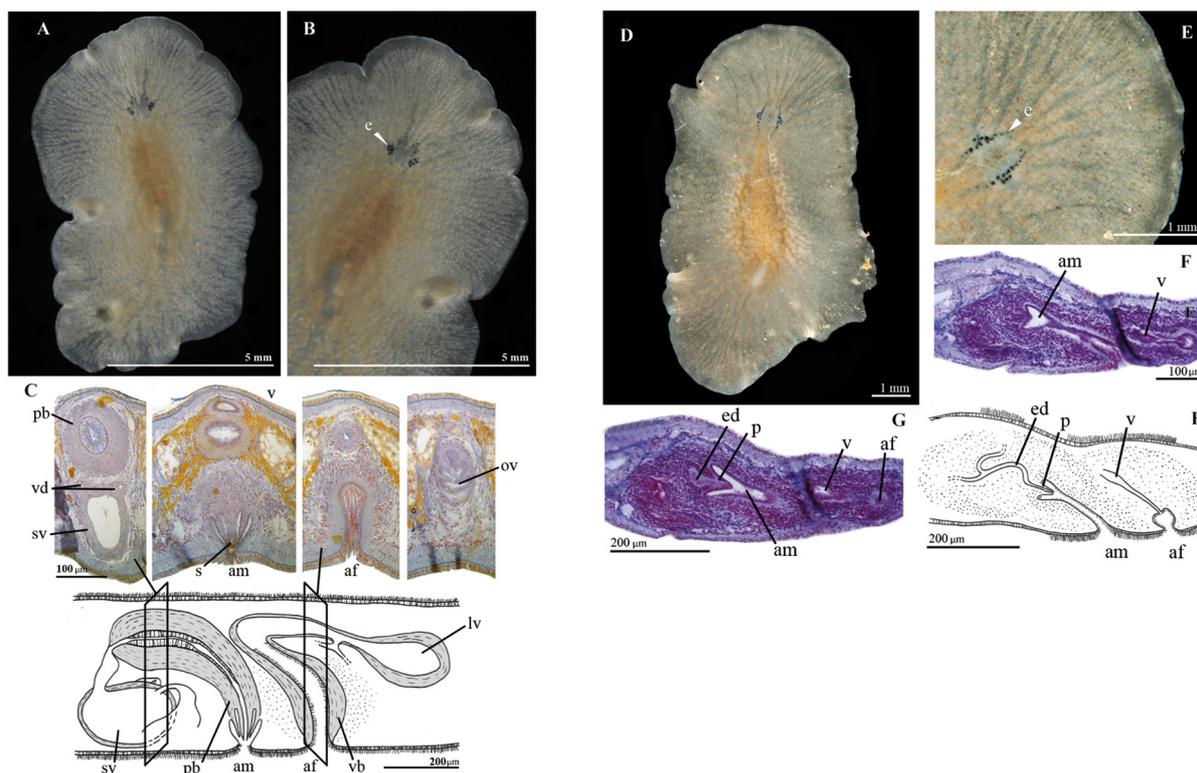
**MALE REPRODUCTIVE SYSTEM.** Male copulatory organ backwards oriented, englobed in a muscular bulb with a small penis papilla. *Vas deferens* opens proximally into the ejaculatory duct (Fig. 4F–H). Without prostatic or seminal vesicle. Male atrium deep and thickened in the point of union with the penis (Fig. 4F–H).

**FEMALE REPRODUCTIVE SYSTEM.** Rounded female atrium (Fig. 4F–H). Vagina backwards oriented, surrounded by muscular fibres. Without Lang’s vesicle.

### Remarks

*Euplana claridade* sp. nov. belongs to the genus *Euplana* due to the absence of tentacles, prostatic vesicle and Lang’s vesicle, and presence of a true seminal vesicle and elongated coiling ejaculatory duct.

The genus *Euplana* encompasses 3 species, *E. carolinensis* Hyman, 1940, *E. gracilis* Girard, 1853 and *E. hymanae* Marcus, 1947. The three species can be differentiated through the eyes number and disposition of them. *E. gracilis* and *E. carolinensis* present four cluster of eyes, two cerebral and two tentacular; *E. hymanae* and *E. claridade* sp. nov. only show two groups of cerebral eyes.



**Fig. 4.** A–C. *Parviplana sodade* sp. nov. (MNCN 4.01/2699 to 2708). A. Whole live animal, dorsal view. B. Anterior region with eyes cluster and tentacles (white arrows). C. Histological cross sections of male and female copulatory organ stained with AZAN and sagittal reconstruction of the reproductive system. – D–H. *Euplana claridade* sp. nov. (MNCN 4.01/2709 to 2718). D. Whole live animal, dorsal view. E. Anterior region with cerebral eyes cluster and tentacles (white arrows). F. Histological section of female copulatory organ stained with AZAN. G. Histological section of male copulatory organ stained with AZAN. H. Sagittal reconstruction of reproductive system. Abbreviations: see Material and methods.

Within the reproductive system another differences can have founded, *E. gracilis* and *E. hymanae* present a small, short male atrium and a backwards oriented vagina externa, while *E. carolinensis* and *E. claridade* sp. nov. share a long tubular male atrium and a forward directed vagina externa.

Both species differ mainly in the male copulatory organ. The ejaculatory duct in *E. carolinensis* is wide and straight and by *E. claridade* sp. nov. is narrow and sinuous. Furthermore, the penis papilla is very short in *E. carolinensis*, while in *E. claridade* sp. nov. it is longer in comparison. On the other hand, the seminal vesicle is practically non-existent in *E. claridade* sp. nov., represented by the confluence of the two vasa deferentia, while in *E. carolinensis* it is a well-developed seminal vesicle, surrounded by circular muscles and in which the vas deferens empties proximally.

Superfamily Stylochoidea Poche, 1926

Family Stylochidae Stimpson, 1857

Genus *Stylochus* Ehrenberg, 1831

***Stylochus salis*** sp. nov.

urn:lsid:zoobank.org:act:8A640FC8-D74C-4617-9766-F0FB9806BD4B

Figs 1C, 5A–D

### Etymology

The name of the new species, *Stylochus salis* refers to the type locality, Sal, a Cape Verdean Island.

### Material examined (2 specs)

#### Holotype

CAPE VERDE • Sal Island, Calheta Funda; 16°39'03.34" N, 22°56'42.94" W (Fig. 1C V3); 12 Nov. 2018; Leopoldo Moro leg.; MNCN 4.01/2719 to 2723 (5 slides). One sagittal sectioned specimen stained with AZAN.

#### Additional material

CAPE VERDE • 1 spec.; Sao Vicente Island, Mindelo (Fig. 1C V1); 16°53'46.54" N, 24°59'32.93" W; 6 May 2017; Leopoldo Moro leg.; RCCN.

### Description

**BODY.** Shape elongated. Length 0.4 cm. Smooth dorsal surface. Background pigmentation cream white with an orange internal outline, sometimes interrupted, along the body margin and bordered by a white/creamy outer band (Fig. 5A). Few cerebral and marginal eyes, scattered between the tentacles and anterior end (Fig. 5C). Two small nuchal tentacles with abundant basal eyes (Fig. 5B). Ruffled pharynx in the middle of the body and the oral pore in the end of the pharynx pouch and close to the gonopores. Male and female gonopores located close together in the posterior end of the body.

**MALE REPRODUCTIVE SYSTEM.** Male copulatory organ backwards oriented and provided with an inconspicuous unarmed penis papilla. Prostatic vesicle muscular with granular lining (polyglandular-type after Bulnes *et al.* 2005) (Fig. 5D). Seminal vesicle elongated, empties at the distal end of the prostatic vesicle. The short penis papilla and ejaculatory duct emerge in a small male atrium (Fig. 5D).

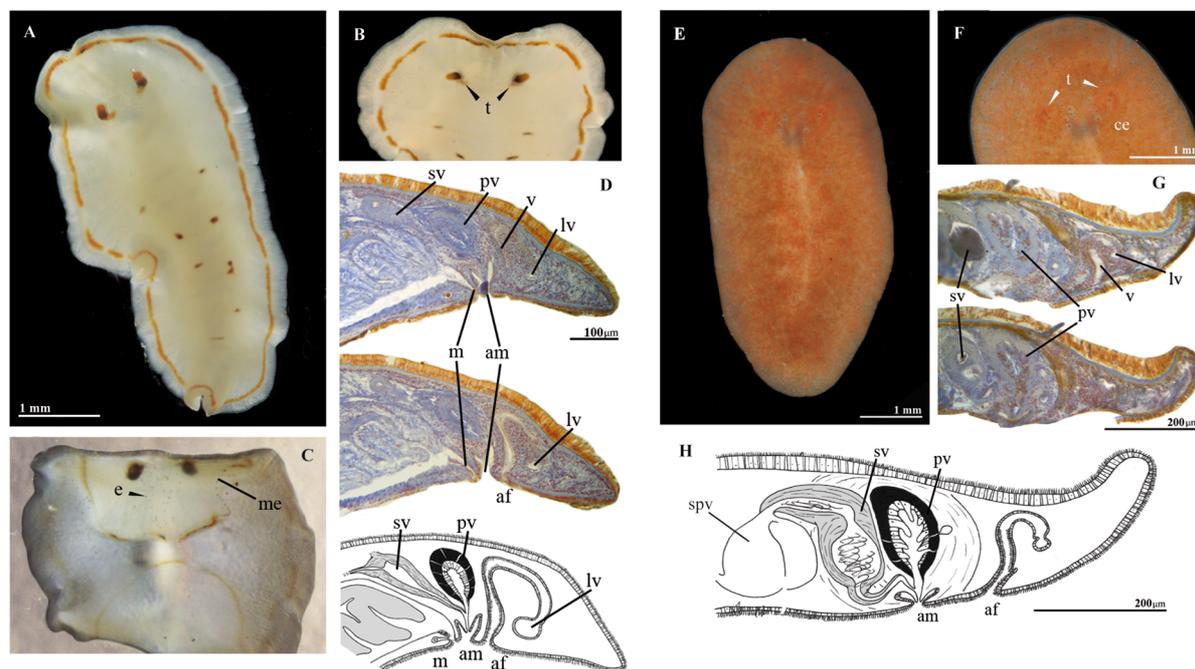
**FEMALE REPRODUCTIVE SYSTEM.** Shows the characteristic configuration of the genus. A tubiform canal with s-shaped ending in a small widening.

## Remarks

*Stylochus salis* sp. nov. belongs to the genus *Stylochus* due to the presence of gonopores separate and arranged in the second body half. With large and much ruffled pharynx. Tentacular, cerebral, marginal, and often frontal eye-spots present. Male copulatory apparatus with seminal vesicle and papillate penis. Lang's vesicle lacking (after Faubel 1983).

*Stylochus salis* sp. nov. clearly differs from other known species of *Stylochus* Ehrenberg, 1831 by its peculiar cream pigmentation bordered with the internal orange outline and the white/creamy outer band. The color of the eastern Atlantic known species (*S. alexandrinus*, *S. castaneus* Palombi, 1939, *S. neapolitanus*, *S. plessissii* Lang, 1884, and *S. suesensis* Ehrenberg, 1831) varies between brown, light brown, reddish or beige and spotted as in *S. neapolitanus*. None of them present a continuous (or discontinuous) marginal line.

The most conspicuous anatomical feature is the location of the oral pore, very close to the gonopore, clearly different from the central position of the oral pore in this genus. The peculiar location of the oral and genital pores distinguishes *S. salis* sp. nov. from the remaining species. Such a close location of the pores could only be found in the genus *Latocestus* Plehn, 1896 (Latocestidae, Stylochoidea).



**Fig. 5.** A–D. *Stylochus salis* sp. nov. (MNCN 4.01/2719 to 2723). A. Whole live animal, dorsal view. B. Anterior region with eyes cluster and tentacles (black arrows). C. Whole live animal ventral view. D. Histological sagittal sections of the reproductive system and sagittal reconstruction. – E–H. *Distylochus fundae* sp. nov. (MNCN 4.01/2724 to 2725). E. Whole live animal, dorsal view. F. Anterior region with cerebral eyes and tentacles (white arrows). G. Histological sagittal section of the reproductive system. H. Sagittal reconstruction of reproductive system. Abbreviations: see Material and methods.

Genus *Distylochus* Faubel, 1983

*Distylochus fundae* sp. nov.

urn:lsid:zoobank.org:act:27AE0619-D25F-48A0-8345-31D38765F732

Figs 1C, 5E–H

### Etymology

The name of the new species, *Distylochus fundae* sp. nov. refers to the type locality Calheta Funda in the Island of Sal.

### Material examined

#### Holotype

CAPE VERDE • Sal Island, Calheta Funda; 16°39'03.34" N, 22°56'42.94" W (Fig. 1C V3); 14 Nov. 2018; Leopoldo Moro leg.; MNCN 4.01/2724 to 2725 (5 slides). One sagittally sectioned specimen stained with AZAN.

### Description

**BODY.** Shape elongated. Length 0.4 cm. Smooth dorsal surface. Background pigmentation orange-garnet (Fig. 5E). Cerebral and tentacular eyes, scattered between the small tentacles (Fig. 5F). Ruffled pharynx, well developed, extending along  $\frac{2}{3}$  of the body. Male and female genital pores located in the posterior half of the body, together, but clearly separated.

**MALE REPRODUCTIVE SYSTEM.** Male copulatory system backwards oriented, with a small penis papilla. Prostatic vesicle surrounded by muscular layers and lined with fingered granular lining, most probably polyglandular (Fig. 5G–H). Seminal vesicle divided into two sections. A muscular and elongated proximal section, and a more rounded distal section provided with a thin wall. Both regions separated by muscle narrowing (Fig. 5H). The distal section leads to the seminal duct that opens into the prostatic duct and forms a short ejaculatory duct. The two *vasa deferentia* dilated to form spermatic vesicles, open into the proximal section. Male atrium small, englobing a short penis papilla (Fig. 4H).

**FEMALE REPRODUCTIVE SYSTEM.** Apparatus simple and backwards oriented. Comprises an elongated tube without clear differentiation between external and internal vagina and ends in a small widening, without Lang's vesicle.

### Remarks

The new species belongs to the genus *Distylochus* due to the presence of few scattered marginal, tentacular and cerebral eyes. Gonopores together and are located near the posterior end. Male apparatus with a short papilla and unarmed. Seminal vesicle configured in two regions, following the “double-vesicle-system” after Faubel (1983) and female apparatus simple, without Lang's vesicle.

There are currently only three known species for the genus *Distylochus*: *D. pusillus* (Bock, 1913) recorded for Hong Kong, *D. martae* (Marcus, 1947) in Brazil and *D. isifer* (Du Bois-Reymond, 1955) also from Brazil. These species were described on fixed specimens, therefore the colors are unknown, but apparently and after the original descriptions, they have pale pigmentation that contrast sharply with the orange-vermilion colors of the new species.

The most conspicuous difference of the new species is the disposition of female and male gonopore. In *Distylochus fundae* sp. nov. the gonopores are clearly separated, while in the Brazilian species are common and in the Chinese species are very close together.

### ***New records***

Following the known species that are captured in the study area. All of them have been studied through photographs and histological sections, currently in RCCN.

Suborder Cotylea Lang, 1884  
Family Euryleptidae Lang, 1884  
Genus *Eurylepta* Ehrenberg, 1831

*Eurylepta cornuta* var. *melobesiarum* (Schmidtlein, 1880)

Figs 1C, 6

### **Material examined**

CAPE VERDE • 1 spec.; São Vicente Island, Baía das Gatas; 16°54'09.33" N, 24°54'25.25" W (Fig. 1C V2); May 2017; Leopoldo Moro leg.; RCCN.

### **Distribution**

Ireland (Thompson 1845); Norway (Müller 1776); northwest France (Keferstein 1868); United Kingdom (Gamble 1893).

### **New record**

São Vicente Island, Cape Verde.

### **Description**

Body shape oval. Length 0.5 cm. Smooth dorsal surface; background color red-orange, with white dots scattered over the dorsal surface (Fig. 6A–C). Ventral sucker in the posterior half of the body (Fig. 6F). Small tentacles. Few tentacular eyes distributed frontally and at the base of the tentacles. Cerebral eyes fused in a single elongated oval cluster (Fig. 6B). Ruffled pharynx located at the anterior third of the body. Oral pore posterior to the cerebral ganglion.

The reproductive system coincides with the original description, presenting the characteristic fold in front of the female genital pore mentioned by Lang (1884) for some specimens (Fig. 6D–F).

Genus *Cycloporus* Lang, 1884

*Cycloporus gabriellae* Marcus, 1950

Figs 1C, 7B

### **Material examined**

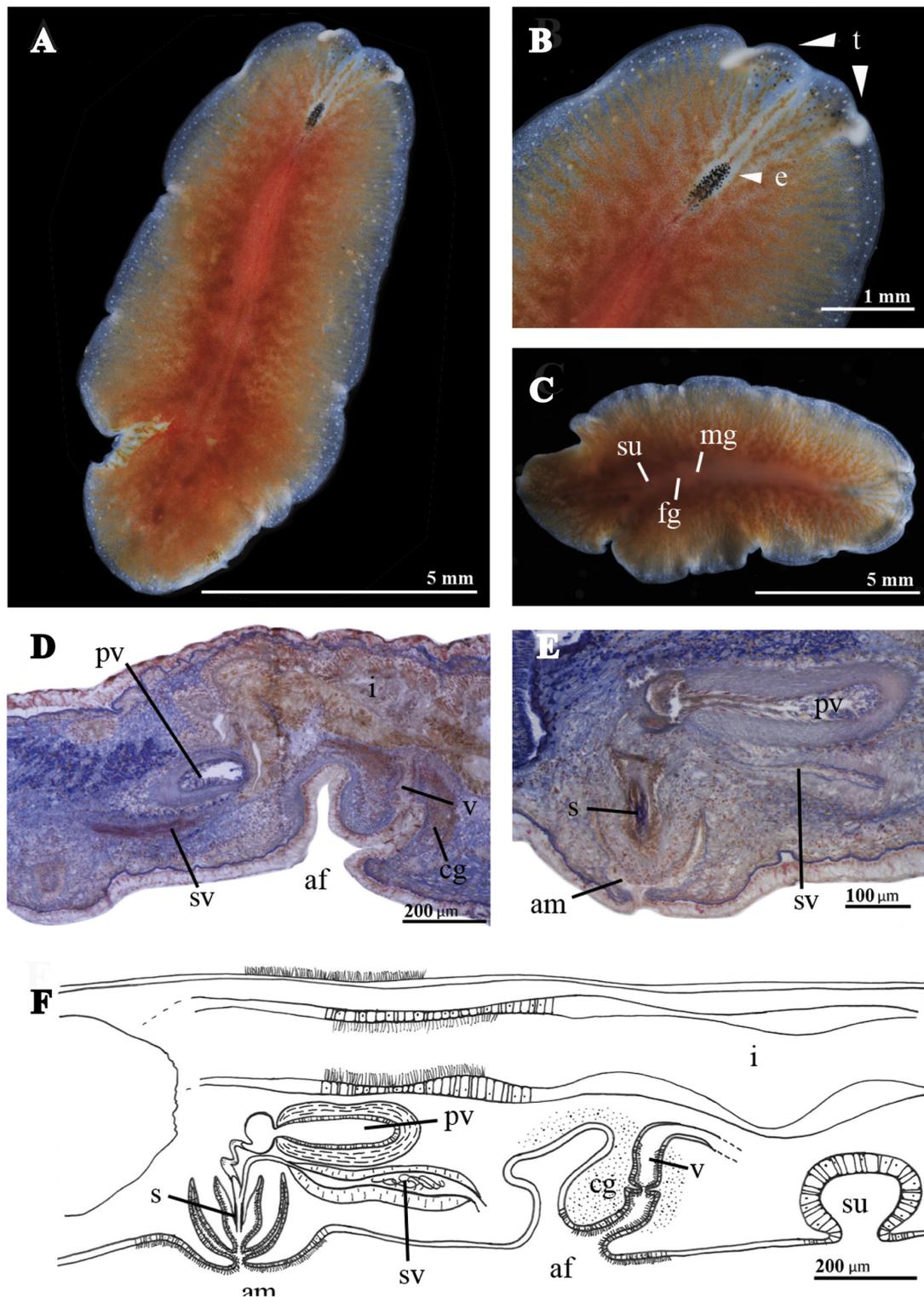
CAPE VERDE • 1 spec.; São Vicente Island, Mindelo; 16°53'46" N, 24°59'32.93" W (Fig. 1C V1); May 2017; Leopoldo Moro leg.; RCCN.

### **Distribution**

São Sebastião Isle, Brazil (Marcus 1950); São Paulo, Brazil (Marcus 1952); Antigua and Barbuda; Curaçao, Netherlands Antilles; Isla de Aves, Venezuela (Marcus & Marcus 1968).

### **New record**

Mindelo, São Vicente Island, Cape Verde.



**Fig. 6.** *Eurylepta cornuta* var. *melobesiarum* (Schmidtlein, 1880) (RCCN). **A.** Whole live animal, dorsal view. **B.** Anterior region with eyes and tentacles (white arrows). **C.** Whole live animal, ventral view. **D.** Histological sagittal section of female copulatory organ. **E.** Histological sagittal section of male copulatory organ. **F.** Sagittal reconstruction of reproductive system. Abbreviations: see Material and methods.

### Remarks

The specimens of *Cycloporus gabriellae* captured in Cape Verde summarize the original description of *C. gabriellae* published by Marcus (1950), but differ externally and in coloration from *C. gabriellae* of Cabo Frio, Brazil (Bahia *et al.* 2014). This is the first record in the eastern region of the Atlantic Ocean for the species.

Genus *Prostheceraeus* Schmarda, 1859

*Prostheceraeus moseleyi* Lang, 1884

Figs 1A, 7C

### Material examined

AZORES • 1 spec.; São Miguel Island; 37°44'59.23" N, 25°37'12.12" W (Fig. 1A 1); 22 Jul. 2001; Leopoldo Moro leg.; RCCN.

### Distribution

Tyrrhenian Sea (Lang 1884); Ria de Arosa, Spain (Noreña *et al.* 2015). This species has also been cited by DORIS (Données d'Observations pour la Reconnaissance et l'Identification de la faune et la flore Subaquatiques) for the English Channel and the North Sea, from the south of the United Kingdom to the Bay of Biscay (Spain) (<http://doris.ffessm.fr/Especies/Prostheceraeus-moseleyi-Planaire-tachetee-716>).

### New record

São Miguel Island, Azores.

### Remarks

The specimen collected in the Azores presents similar coloration to the specimen photographed by Wirtz & Debelius (2003) and determined as Euryleptidae sp.

Family Anonymidae Lang, 1884

Genus *Anonymus* Lang, 1884

*Anonymus ruber* Cuadrado, Moro & Noreña, 2017

Figs 1C, 7A

### Material examined

CAPE VERDE • 3 specs; São Vicente Island, Baía das Gatas; 16°54'09.33" N, 24°54'25.25" W (Fig. 1C V2); May 2017; Leopoldo Moro leg.; RCCN.

### Distribution

Tenerife Island, Canary Islands (type locality) (Cuadrado *et al.* 2017).

### New record

Baía das Gatas, São Vicente Island, Cape Verde.

### Remarks

*Anonymus ruber* is characterized by reddish brown tones, but some individuals from Cape Verde are paler and presented cream tonalities. From an anatomical (internal or external) and molecular point of view, the species found in Cape Verde does not differ from the species in the Canary Islands (Fig. 8).

Family Pseudocerotidae Lang, 1884

Genus *Pseudoceros* Lang, 1884

*Pseudoceros velutinus* (Blanchard, 1847)

Figs 1C, 7D

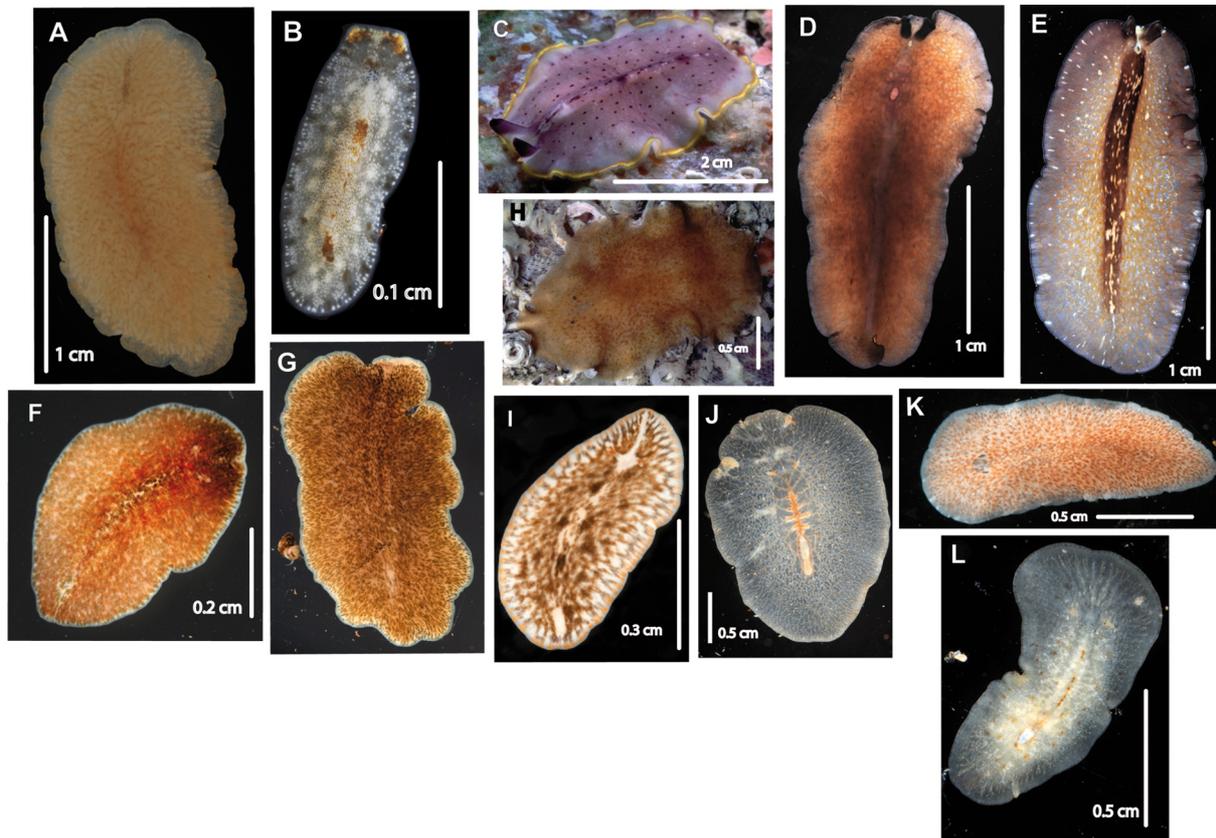
*Proceros velutinus* Blanchard, 1847: 273, pl. 8, fig. 2; pl. 9, fig. 1.

*Eurylepta velutina* – Diesing 1850: 210; 1862: 548. — Stimpson 1857: 2. — Schmarda 1859: 26. — Diesing 1862: 548.

*Pseudoceros velutinus* – Lang 1884: 538–539, pl. 5, fig. 4.

### Material examined

CAPE VERDE • 1 spec.; São Vicente Island, Baía das Gatas; 16°54'09.33" N, 24°54'25.25" W (Fig. 1C V2); 5 May 2017; Leopoldo Moro leg.; RCCN.



**Fig. 7.** A. *Anonymus ruber* Cuadrado, Moro & Noreña, 2017 from Cape Verde. B. *Cycloporus gabriellae* Marcus, 1950 from Cape Verde. C. *Prostheceraeus moseley* Lang, 1884 from Cape Verde. D. *Pseudoceros velutinus* (Blanchard, 1847) (transmitted light) from Cape Verde. E. *Monobicerus langi* Faubel, 1984 from Cape Verde. F. *Stylochus pilidium* (Götte, 1881) from South Morocco. G. *Stylochus pillidium* (Götte, 1881) from Azores. H. *Stylochus* sp. from South Morocco. I. *Stylochus neapolitanus* (Delle Chiaje, 1841–1844) from Madeira. J. *Planocera pellucida* (Mertens, 1833) from Canary Islands. K. *Discocelis tigrina* (Blanchard, 1847) from Canary Islands. L. *Gnesioceros sargassicola* (Mertens, 1833) from Canary Islands.

### Distribution

Gulf of Genova, Italy (type locality) (Blanchard 1847); Suez Canal, Egypt (Palombi 1928); Rovigno, Croatia (Vátova 1928); Canary Islands (Cuadrado *et al.* 2017).

### New record

Baía das Gatas, São Vicente, Cape Verde.

### Remarks

*Pseudoceros velutinus* is known for its velvety black pigmentation, but the coloration varies with transmitted or reflected light. Fig. 7D shows the appearance of *P. velutinus* with transmitted light.

Genus *Monobiceros* Faubel, 1984

*Monobiceros langi* Faubel, 1984

Figs 1C–D, 7E

*Pseudoceros maximus* Lang, 1884 (in part): 270–271, 543, pl. 30, fig. 17.

*Monobiceros langi* Faubel, 1984: 215.

### Material examined

CAPE VERDE • 1 spec.; São Vicente Island, Baía das Gatas; 16°54'09.33" N, 24°54'25.24" W (Fig. 1C V2); May 2017; Leopoldo More leg.; RCCN.

MOROCCO • 3 specs; Sidi Ifni; 29°22'58.00" N, 10°10'37.88"O (Fig. 1D M2); 2 Sep. 2016; Leopoldo Moro leg.; RCCN.

### Distribution

Gulf of Naples, Italy (Lang 1884); Cádiz, Spain (Bahia *et al.* 2017); Crete, Greece (Bahia *et al.* 2017).

### New records

Baía das Gatas, São Vicente Island, Cape Verde. Sidi Ifni, Morocco. These are the first records outside the Mediterranean Sea.

### Description

Body oval and elongated sometimes pear-shaped, with wavy margins and thickened dorsal midline. Length 2.3 cm. The background color varies with transmitted or reflected light. With reflected light, the dorsal surface is chocolate brown, speckled with white patches. Under transmitted light, the pigmentation looks milky brown with dark margins and a dark dorsal midline that delimited the characteristic bulge (Fig. 7E). Dorsal surface smooth. Tentacles formed by simple folds and cone-like in shape. Cerebral eyes form a single large rounded cluster behind the tentacles; frontal eyes scattered between the tentacles. Compact ruffled pharynx located directly behind the cerebral eyes.

Suborder Acotylea Lang, 1884

Superfamily Stylochoidea Poche, 1926

Family Stylochidae Stimpson, 1857

Genus *Stylochus* Ehrenberg, 1831

*Stylochus pilidium* (Götte, 1881)

Figs 1C–D, 6E, I, 7F–G

*Stylochopsis pilidium* Götte, 1881: 189.

*Planaria neapolitana* – Götte 1878: 75–76.

*Stylochus pilidium* – Götte 1882: 1–56, pls I–II. — Lang 1884: 320–321, 325, 329, 341–343, 351–353, 357, 401, pl. 36. fig. 19, pl. 37, figs 1, 16–17, 22.

#### Material examined

CAPE VERDE • 3 specs; São Vicente Island, Mindelo; 16°53'46.54" N, 24°59'32.93" W (Fig. 1C V1); May 2017; Leopoldo Moro leg.; RCCN.

MOROCCO • 3 specs; Sidi Ifni; 29°22'58.00" N, 10°10'37.88" W (Fig. 1D M2); 2 Sep. 2016; Leopoldo Moro leg.; RCCN.

#### Distribution

Gulf of Naples, Mediterranean Sea (type locality) (Götte 1881; Lang 1884; Lo Bianco 1888); Gulf of Venice (Bock 1925); Black Sea (Rzhepishevskii 1979); Vietnam (Dawydoff 1952); Somalia (Laidlaw 1903); Zanzibar (Meixner 1907).

#### New records

São Vicente Island, Cape Verde (Fig. 6E). Sidi Ifni, Morocco (Fig. 6I).

*Stylochus* sp.  
Figs 1A, 7H

#### Material examined

AZORES • 1 spec.; São Miguel Island, Ponta Delgada; 37°44'59.23" N, 25°37'12.12" W (Fig. 1A A1); 22 Jul. 2001; Leopoldo Moro leg.; RCCN.

#### New record

São Miguel Island, Azores. This is the first record for the genus *Stylochus* in the Azores. No molecular data.

*Stylochus neapolitanus* (Delle Chiaje, 1841–1844)  
Figs 1D, 7I

*Planaria neapolitana* Delle Chiaje, 1841: vol. III: 133 (text); vol. V: 112 (description); pl. 109, figs 13–15, 22.

*Stylochus neapolitanus* – Lang 1884: 447–449, pl. 1, fig. 7.

#### Material examined

MOROCCO • 2 specs; Sidi Ifni; 29°22'58.00" N, 10°10'37.88" W (Fig. 1D M1); 2 Sep. 2016; Leopoldo Moro leg.; RCCN.

#### Distribution

Western Mediterranean Sea, Sicily (type locality) (Delle Chiaje 1841); Naples, Italy (Lang 1884); Catalonia, Spain (Novell 2003); Mar Menor, Murcia, Spain (Marquina *et al.* 2014); Ría de Arousa, Galicia, Spain (Noreña *et al.* 2015); Cape Verde (Laidlaw 1906); Rufisque, Senegal (Palombi 1939).

#### New record

Sidi Ifni, Morocco.

Family Planoceridae Lang, 1884  
Genus *Planocera* Blainville, 1828

***Planocera pellucida*** (Mertens, 1833)  
Figs 1B, 7J

*Planaria pellucida* Mertens, 1833: 8–13, pl. 1

*Stylochus pellucidus* – Ehrenberg 1836: 67. — Diesing 1850: 216. — Claparède 1861: 143. — Moseley 1877: 23.

*Planocera pellucida* – Örsted 1844: 48.

*Gnesioceros pellucidus* – Diesing 1862: 571.

#### Material examined

MADEIRA • 1 spec.; 32°41'18.52" N, 16°58'57.36" W (Fig. 1B); 2000; Leopoldo Moro leg.; RCCN.

#### Distribution

Atlantic Ocean, between Newfoundland and Ireland (type locality) (Mertens 1833); Pacific Ocean (Graff 1892; Woodworth 1894; Plehn 1896); Canary Islands (De Vera *et al.* 2009; Cuadrado *et al.* 2017); Vietnam (Dawydoff 1952); the North Sea (Prudhoe 1982); Japan (Kato 1938); the Mediterranean Sea (Lang 1879, 1884; Riedl 1959; Marquina *et al.* 2014); pelagic fauna of the Atlantic Ocean (Graff 1892; Plehn 1896); Cape Verde and Ascension islands (Plehn 1896; Laidlaw 1903);

#### New record

Madeira.

#### Remarks

*Planocera pellucida*, together with *Pseudoceros wirtzi*, *Pseudoceros cf. maximus* and *Prostheceraeus giesbrechtii* are the three polyclad species currently recorded for Madeira. As can be seen in Fig. 7J. The specimens of *Planocera pellucida* from Madeira do not differ molecularly from those captured in the Canary Islands.

Superfamily Leptoplanoidea Faubel, 1984  
Family Discocelidae Laidlaw, 1903  
Genus *Discocelis* Ehrenberg, 1836

***Discocelis tigrina*** (Blanchard, 1847)  
Figs 1B, 7K

*Polycelis tigrinus* Blanchard, 1847: 271–272, pl. 8, fig. 1.

*Leptoplana tigrina* – Diesing 1850: 195; 1862: 527.

*Elasmos tigrinus* – Stimpson 1857: 3.

*Discocelis tigrina* – Lang 1884: 467–469, pl. 3, fig. 3; pl. 4, fig. 1; pl. 2, fig. 6.

#### Material examined

CANARY ISLANDS • 1 spec.; Gran Canaria, Pasito Blanco; 27°44'50.46" N, 15°37'31.85" W (Fig. 1B C1); 9 Aug. 2016; Leopoldo Moro leg.; RCCN.

### Distribution

Río de Oro peninsula, Western Sahara (Palombi 1939); Naples, Italy (Lang 1884); Catalonia, Spain (Novell 2003); Asturias, Spain (Marquina *et al.* 2014); Galicia, Spain (Noreña *et al.* 2015); Mauritania (Palombi 1939).

### New record

Pasito Blanco, Gran Canaria, Canary Islands.

Family Gnesiocerotidae Marcus & Marcus, 1966  
Genus *Gnesioceros* Diesing, 1862

***Gnesioceros sargassicola*** (Mertens, 1833)  
Figs 1B, 7L

*Planaria sargassicola* Mertens, 1833: 13–14, pl. I, figs 4–6.

*Stylochus Mertensi* Diesing, 1850: 216.

*Stylochus sargassicola* – Ehrenberg 1836: 67. — Claparède 1861: 143.

*Planocera sargassicola* – Örsted 1844: 48.

*Gnesioceros sargassicola* – Diesing 1862: 571.

*Gnesioceros Mertensi* – Diesing 1862: 572.

*Stylochus Mertensi* – Moseley 1877: 23.

### Material examined

CANARY ISLANDS – **Gran Canaria Island** • 1 spec.; Pasito Blanco; 27°44'50.46" N, 15°37'31.85" W (Fig. 1B C1); 4 Jan. 2017; Leopoldo Moro leg.; RCCN. – **Tenerife Island** • 1 spec.; 28°24'35.71" N, 16°18'25.31" W (Fig. 1B C2); 7 Jun. 2011; Leopoldo Moro leg. RCCN.

### Distribution

Bermuda Islands (Hyman 1939); Sargasso Sea, Caribbean Sea, Atlantic Ocean (Faubel 1983); Gulf of Mexico (Hyman 1954); Atlantic Ocean (Moseley 1877); Boa Vista Island, Cape Verde (Laidlaw 1903); Santa Marta, Colombia (Quiroga 2008); Netherlands, Puerto Rico, Florida, USA, Sargasso Sea, Atlantic Ocean (Marcus & Marcus 1968).

### New records

Pasito Blanco, Gran Canaria, and Tenerife, Canary Islands.

### Remarks

*Gnesioceros sargassicola* was limited to the Antilles and the Caribbean Sea until the record of Laidlaw (1903) for the Cape Verde Islands. The new record of *G. sargassicola* for the Canary Islands shows a progressive 'colonisation' of the Atlantic east coast.

### Molecular analysis

The main purpose of the 28S analysis was to confirm the determinations made from the morphological study and verify the relationships among similar species.

The recovered topology by both trees, Bayesian Inference (BI) as well as Maximum Likelihood (ML) strongly supports the monophyly of the suborders Acotylea and Cotylea (Fig. 8; Acotylea: BPP (Bayesian

posterior probabilities) = 1 and BS (Bootstrap values of ML analysis) = 73, Cotylea: BPP = 0.98, BS = 100).

Within Acotylea, the monophyly of Leptoplanoidea (BPP = 1, BS = 97) and Stylochoidea (BPP = 1, BS = 73) is well supported. *Callioplana marginata* Stimpson, 1857 considered within the superfamily Stylochoidea appears isolated with the highest support (BS = 100).

As a sister group of Leptoplanoidea, there is a clade of species: *Ilyella gigas* (Schmarda 1859), *Discocelis tigrina* (Blanchard, 1847), *Adenoplana evelinae* Marcus, 1950, *Amemiyaia pacifica* Kato, 1944 and *Phaenocelis medvedica* Marcus, 1952 showing low support and not clearly grouped (Fig. 8).

The main group of Leptoplanoidea encloses the family Leptoplanidae, with the genera *Leptoplana* Ehrenberg, 1831 and *Armatoplana* Faubel, 1983, and the family Notoplanidae Marcus & Marcus, 1966 with the genera *Notoplana* Laidlaw, 1903 and *Notocomplana* Faubel, 1983. *Pseudostylochus* Yeri & Kaburaki, 1918 and *Koinostylochus* Faubel, 1983 appear together and belong to the family Pseudostylochidae Faubel, 1983.

Within Stylochoidea, four main clades are recovered: family Stylochidae with the genera *Stylochus*, *Imogine* Girard, 1853 and *Paraplanocera* Laidlaw, 1903 (see the Discussion); family Latocestidae Laidlaw, 1903 with *Leptostylochus* Bock, 1925 and *Latocestus*; family Hoploplanidae Stummer-Traunfels, 1933 with *Hoploplana* Laidlaw, 1902 and finally family Planoceridae with *Paraplanocera* Laidlaw, 1903 and *Planocera*. *Callioplana marginata* can be considered a sister group of Stylochoidea.

Within the suborder Cotylea, the families Cestoplanidae Lang, 1884, Pericelidae Laidlaw, 1902 and Anonymidae represented by the genera *Cestoplana* Lang, 1884 (BPP = 1, BS 100), *Pericelis* (BPP = 1, BS 100) and *Anonymus* (BPP = 1, BS 100), respectively are presented as isolated groups. While *Boninia* Bock, 1923, *Chromyella* Correa, 1958 and *Theama* Marcus, 1949 form a well-supported group (BPP = 1, BS = 100), although currently belonging to separate families and not specifically related to each other.

After Anonymidae, we find a well-supported branch (BPP = 0.9, BS = 95) that encompasses the families Prosthiosomidae Lang, 1884, Euryleptidae and Pseudocerotidae. This branch is, in turn, divided into two main branches (both supported by maximum values: BPP = 1, BS = 100) where Prosthiosomidae is separated from Euryleptidae and Pseudocerotidae, families with a clear relation.

## Discussion

After the present study and the results obtained, it is evident that the knowledge on polyclad biodiversity in Macaronesia is clearly uneven. Polyclads from Cape Verde, Madeira and Canary Islands are mostly well studied, while information from the Azores and Selvagens Islands is irregular. Consequently, a comparison of the biodiversity of the different archipelagos is biased, but not impossible.

The 6 new species of Cape Verde could currently be considered endemic from Cape Verde until new or future studies show an expansion of its distribution. On the other hand, from the 50 species considered in the present study, 31 belong to Cape Verde, of which 11 species are also present in the Canary Islands and 2 species in South Morocco. These species are mostly considered cosmopolitan: *Thysanozoon brocchi*, *Cestoplana rubrocincta* (Grube, 1850) and *Planocera pellucida*. Other species like *Pseudobiceros wirtzi* and *Pseudoceros mororum* are recorded from Madeira, Canary Islands and Cape Verde. *Pseudoceros velutinus* and *Anonymus virilis* Lang, 1884 show a known broad distribution along the Mediterranean (Tyler *et al.* 2006–2020). The four remaining species *Anonymus ruber*, *Latocestus plehni* Laidlaw, 1906, *Cycloporus gabriellae* and *Pericelis cata*, are currently only recorded for Canary Islands and also known for the coasts of Brazil.



*Stylochus neapolitanus* is described for the Mediterranean and has also been recorded for Cape Verde by Laidlaw (1906). Considered by the same author as doubtful because according to this author the species of this genus (*Stylochus*) are difficult to distinguish (Laidlaw 1906: 707).

This group of common species shows Cape Verde as an independent archipelago with unique biological and ecological characteristics and therefore, fauna. Regardless, it is still under the influence of other regions. As such, our results confirm the hypotheses of Spalding *et al.* (2007) and Freitas *et al.* (2019) who propose Cape Verde as an independent archipelago outside of Macaronesia with its own ecological and biological history and as a specific hotspot (Freitas *et al.* 2019).

Most of the studied species of Cape Verde belong to the island of São Vicente (Laidlaw 1906) and only 9 species were collected in Boa Vista, Santo Antão and Santiago. In the present study, samples from the island of São Vicente were examined, but only two species coincide with those recorded by Laidlaw (1906).

The second best studied archipelago is the Canary Islands (Cuadrado *et al.* 2017). In the present study, *Discocelis tigrina* and *Gnesioceros sargassicola* are new records added to the biodiversity of the archipelago and the distribution of *Pseudoceros mororum*, to date limited to the island of Gran Canaria, has been extended to Tenerife.

While the focus of this study doesn't lie in the analysis of the Macaronesian species from a molecular point of view, such analyses were carried out to verify the previous morphological determination and to add another source of proof to the delimitation of new species.

The latest molecular analyses have contributed to improve the resolution of the systematics and configuration of families and superfamilies of the order Polycladida. The studies carried out by Aguado *et al.* (2016, 2017), Bahia *et al.* (2017), Dittmann *et al.* (2019), Kenny *et al.* (2019) and Litvaitis *et al.* (2019) are of particular relevance. As the last authors discuss the previous studies, we will mainly compare our results with their analyses. In general, the phylogenetic trees (both ML and BI) obtained in the present study show an almost identical topology at the family level to those presented by Litvaitis *et al.* (2019).

Regarding the newly sequenced and analysed material (Bordeaux in Fig. 8), *Latocestus plehni*, within Acotylea, shows the same close relationship with *Leptostylochus gracilis* Kato, 1934 as mentioned by Litvaitis *et al.* (2019), therefore we include the genus *Leptostylochus* together with *Latocestus* in the family Latocestidae.

The genus *Notoplana* certainly presents a problem in our analysis. While some species from Brazil (*Notoplana* sp.) appear within the family Notoplanidae, others from Japan are closely related to the family Stylochoplanidae Faubel, 1983. Finally, *Notoplana australis* (Laidlaw, 1904) is included within the family Notocomplanidae Litvaitis, Bolaños & Quiroga, 2019. This shows the variety of the genus *Notoplana*, at least with respect to the 28S gene. With new morphological analyses and the use of more molecular markers, perhaps we will obtain more satisfactory results to resolve the relationship of the genus *Notoplana*.

On the other hand, and within Leptoplanoidea, *Pseudostylochus* and *Koinostylochus* are considered synonymous and belong to the family Pseudostylochidae (Oya & Kajihara 2020).

**Fig. 8** (on the opposite page). Polycladida 28S rDNA Maximum Likelihood phylogenetic tree at the right and Bayesian Inference tree at the left. Species highlighted in bordeaux: own material.

*Planocera pellucida*, regardless of the origin location (Canarias or Cape Verde) seems to represent the same species despite differences in populations and well framed within the family Planoceridae. The same happens with *Discocelis tigrina*, although there are differences between the populations of the Atlantic (Canary Islands) and the Mediterranean (Catalonia).

Within Cotylea, *Cestoplana rubrocincta* is included together with *Cestoplana salar* Marcus, 1949 and *Cestoplana techa* Du Bois-Reymond Marcus, 1957 within the family Cestoplanidae. Next, the family Diposthidae shows a clear relationship between the genera *Pericelis* and *Marcusia*, but in both analyses (ML and BI) the two genera are clearly differentiated and confirmed as valid genera.

*Anonimus ruber* within Anonimidae, presents the same condition as *Planocera pellucida* (Acotylea), where no differences appear between the populations of the Canary Islands and Cape Verde.

*Prostheceraeus roseus* and *Enchiridium magec*, both species captured in the Canary Islands, are well defined and included within their respective families, Euryleptidae and Prosthiostomidae. On the other hand, *Eurylepta cornuta* var. *melobesiarum* shows clear differences with *Eurylepta* sp., an immature specimen from Martinique, possibly *E. cornuta* but could not be confirmed.

Within the family Pseudocerotidae the relationships are more complex. In general, the species of the genus *Pseudoceros* are well framed within a clade, while the rest of the genera appear scattered. *Pseudobiceros* Faubel, 1984, *Thysanozoon* Grube, 1840, *Monobiceros*, *Phrikoceros* Newman & Cannon, 1996 and *Yungia* Lang, 1884 appear closely related and well separated from the genus *Pseudoceros*. The first three genera share a double male copulatory organ while in *Phrikoceros* and *Yungia* it is single. *Monobiceros* and *Phrikoceros* are grouped in a single clade in our analysis, but they are morphologically different.

Close relationships among diverse morphotypes are already mentioned by Lang (1884), which includes clearly different morphotypes under the species *Pseudoceros maximum*. According to Lang (1884) this species could present externally three coloration patterns: brown with dark striations (Form A; Lang 1884: pl. 9, fig 1), dark with white splatters (Form B; Lang 1884: pl. 9, fig. 2) or beige with lighter, rounded spots (Form C; Lang 1884: pl. 9, fig. 3). Internally, *Pseudoceros maximum* could present one (type a) or two (type b) male copulatory organs, both types with a single gonopore. Faubel (1984) separates both morphotypes into *Pseudoceros maximum*, with a male copulatory organ type a, and in *Monobiceros langi* with a type b, but no reference to the coloration pattern of these two species is made.

As such, species with one of the different color patterns described for *P. maximum* by Lang (1884) are: *Phrikoceros mopsus* (Marcus, 1952) (form C, type a) and *Pseudobiceros caribbensis* Bolaños, Quiroga & Litvaitis, 2007 (form A; type b, but with 2 gonopores) from the Caribbean. In our analysis, *Phrikoceros mopsus* and *Monobiceros langi* (form B, type b) appear together, while *Pseudobiceros caribbensis* appears grouped in a single isolated branch. These results indicate the close relationship between *P. mopsus* and *M. langi*, which seem to support the different morphotypes of Lang's *P. maximum*. Unfortunately, without sequences of *Pseudoceros maximum* (form A, type a) the resolution of the complex of species (or morphotypes) '*P. maximum*' remains for a future study.

The species whose sequences were obtained from GenBank (NCBI) appear in the same positions described in other works (Bahia *et al.* 2017; Litvaitis *et al.* 2019) but some showcase new relationships that should be discussed.

*Leptostylochus gracilis*, which appears as closely related to *Latocestus* and *Idioplana* in Litvaitis *et al.* (2019), appears as a sister group to *Latocestus* in our analyses, a relationship that indicates a clear belonging to the family Latocestidae and not family Stylochidae.

The Hawaiian *Paraplanocera oligoglana* (Schmarda, 1859) is found within Stylochidae and clearly related to *Stylochus* and *Imogine*, genera which present little differences from one another. These results indicate that *Stylochus* and *Imogine* are possibly one genus (Dittman *et al.* 2019), to which the Hawaiian species belongs.

*Amemiyaia pacifica* does not seem to belong to the Cryptocelidae Laidlaw, 1903 as it appears in the results obtained by Litvaitis *et al.* (2019), but it is closely related to this family. *Adenoplana evelinae*, in comparison, appears in both analyses (ML and BI) within Cryptocelidae and not within Discocelidae, a fact that looks to corroborate the results of Bahia *et al.* (2017).

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