Molecular Phylogenetics and Evolution 162 (2021) 107209

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Due South: The evolutionary history of Sub-Antarctic and Antarctic Tritoniidae nudibranchs

Juan Moles [a,b,*], Maria I. Berning [a], Yuri Hooker [c], Vinicius Padula [d], Nerida G. Wilson [e,f], Michael Schrödl [a,b]

[a] SNSB-Bavarian State Collection of Zoology, Section Mollusca, Münchhausenstrasse 21, D-81247 Munich, Germany
[b] Biozentrum Ludwig Maximilians University and GeoBio-Center LMU Munich, Germany
[c] Universidad Peruana Cayetano Heredia, Laboratorio de Biología Marina, Facultad de Ciencias y Filosofía, Lima, Peru
[d] National Museum, Federal University of Rio de Janeiro, UFRJ, Quinta da Boa Vista, São Cristóvão, Rio de Janeiro, RJ 20940-040, Brazil
[e] Collections & Research, Western Australian Museum, 49 Kew St, Welshpool, Perth, WA 6106, Australia
[f] School of Biological Sciences, University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia

ARTICLE INFO

Keywords:
Sea slugs
Tritonia
Tritoniella
Pseudocryptic speciation
Marine biodiversity
Southern Ocean

ABSTRACT

The Tritoniidae provides one of the most famous model species for neurophysiology and behaviour, yet a well-developed phylogenetic framework for this family is still incomplete. In this study, we explored the species-level taxonomy, phylogenetic relationships, and geographic distributions of the tritoniid nudibranchs. During numerous expeditions, specimens from southern South America, Sub-Antarctic Islands, and Antarctica were collected, documented alive, and fixed for anatomical descriptions and genetic sequencing. DNA from 167 specimens were extracted and sequenced for mitochondrial (COI, 16S) and nuclear (H3) markers. An additional 109 sequences of all available tritoniids plus additional outgroups were downloaded from GenBank for comparative purposes. Maximum Likelihood under the GHOST model of evolution and Bayesian inference using the GTR + GAMMA model produced congruent topologies from concatenated alignments. The results of ABGD, GMYC, bPTP, and mPTP species delimitation analyses suggest many separately evolving units that do not coincide with traditionally recognized species limits. Southern Ocean *Tritoniella* and *Tritonia* species split into several previously unrecognized species. This result is in accordance with the limited dispersal abilities of some southern tritoniids. Along with the most complete phylogeny of Tritoniidae to date, we also provided many taxonomic notes at the species and genus level. Tritoniidae species are yet another example of under-recognized diversity in the Southern Ocean.

1. Introduction

The family of marine slugs Tritoniidae Lamarck, 1809 received its name from Greek mythology, where Triton was the God of the sea. With over 80 species described (WoRMS Editorial Board, 2020), these nudibranchs are found in all oceans from shallow to deep-sea waters. One of its key species is *Tritonia diomedea* Bergh, 1894, currently accepted as *T. tetraquetra* (Pallas, 1788), a well-known model in neurophysiology and behavioural studies (e.g., Willows, 1976). Still, scarce phylogenetic hypotheses on this iconic family hamper our understanding of comparative biology, with molecular data on many representatives missing; this is especially true for the Southern Hemisphere (e.g., Schrödl, 2009) and Indo-Pacific regions (e.g., Gosliner et al., 2018). Preliminary phylogenies on Tritoniidae identified the presence of polyphyletic genera although these studies lacked comprehensive taxon sampling (Hulett et al., 2015; Valdés et al., 2018). This has recently been confirmed by Korshunova & Martynov (2020). Among the valid genera in the family, molecular data is now available for the most common and widespread genera *Duvaucelia* Risso, 1826, *Marionia* Vayssière, 1877, *Tritonia* Cuvier, 1798, *Tritonicula* Korshunova & Martynov, 2020, and *Tritoniopsis* Eliot, 1905, although little is available from the low diversity genera *Marianina* Pruvot-Fol, 1931, commonly found in the tropical Indo-West Pacific (Gosliner et al., 2018), *Tochuina* Odhner, 1963, a widespread boreal taxon (Behrens and Hermosillo, 2005), and *Tritoniella* Eliot, 1907, endemic to the Southern Ocean (Wägele, 1989a). For two other elusive and monotypic genera, i.e. *Paratritonia* Baba, 1949, and

* Corresponding author at: SNSB-Bavarian State Collection of Zoology, Section Mollusca, Münchhausenstrasse 21, D-81247 Munich, Germany.
E-mail address: moles.sanchez@gmail.com (J. Moles).

https://doi.org/10.1016/j.ympev.2021.107209
Received 6 February 2021; Received in revised form 3 May 2021; Accepted 19 May 2021
Available online 25 May 2021

Table 1
Species sequenced including voucher numbers, sampling details, and GenBank accession numbers.

Species	Voucher number	Locality	Collection date	Collecting gear	Lat_Lon	Depth (m)	COI	16S	H3
Marionia cucullata	ZSM20141000	Brazil: Rio de Janeiro, Baía de Guanabara	2-may.-07	Bottom trawl	22.812697 S 43.153634 W	10	MW444217	MW444395	MW453540
Tritonia antarctica	SIO-BIC M12579A	Antarctica: South Georgia	25-sep.-11	Blake trawl	53.892315 S 39.19146 W	310	MW444218	MW444396	MW453541
Tritonia antarctica	SIO-BIC M12579B	Antarctica: South Georgia	25-sep.-11	Blake trawl	53.892315 S 39.19146 W	310	MW444219	MW444397	MW453542
Tritonia antarctica	SIO-BIC M12580	Antarctica: Shag Rocks	22-sep.-11	Blake trawl	53.42398 S 42.017738 W	160	MW444220	MW444398	MW453543
Tritonia antarctica	SIO-BIC M12581A	Antarctica: Antarctic Peninsula, Bransfield Strait	25-oct.-11	Blake trawl	63.322833 S 59.851052 W	199	MW444221	MW444399	MW453544
Tritonia antarctica	SIO-BIC M12581C	Antarctica: Antarctic Peninsula, Bransfield Strait	25-oct.-11	Blake trawl	63.322833 S 59.851052 W	199	MW444222	MW444400	MW453545
Tritonia antarctica	SIO-BIC M12585A	Antarctica: South Sandwich	4-oct.-11	Blake trawl	56.70875 S 27.04858 W	116	MW444223	MW444401	MW453546
Tritonia antarctica	SIO-BIC M12585B	Antarctica: South Sandwich	4-oct.-11	Blake trawl	56.70875 S 27.04858 W	116	MW444224	MW444402	MW453547
Tritonia antarctica	SIO-BIC M12592	Antarctica: South Shetland Islands	27-oct.-11	Blake trawl	62.355853 S 60.730602 W	165	MW444225	MW444403	MW453548
Tritonia antarctica	SIO-BIC M12603	Antarctica: Antarctic Peninsula, Bransfield Strait	24-sep.-11	Blake trawl	62.752845 S 57.321675 W	292	MW444226	MW444404	MW453549
Tritonia antarctica	SIO-BIC M12605	Antarctica: Shag Rocks	24-sep.-11	Blake trawl	53.534252 S 41.633907 W	133	MW444227	MW444405	MW453550
Tritonia antarctica	SIO-BIC M12607	Antarctica: Shag Rocks	24-sep.-11	Blake trawl	53.7211 S 41.462667 W	180	MW444228	MW444406	MW453551
Tritonia antarctica	SIO-BIC M12611	Antarctica: South Georgia	29-sep.-11	Blake trawl	55.038813 S 35.44801 W	125	MW444229	MW444407	MW453552
Tritonia antarctica	SIO-BIC M12614	Antarctica: Elephant Island	22-oct.-11	Blake trawl	61.160927 S 54.212093 W	247	MW444230	MW444408	MW453553
Tritonia antarctica	SIO-BIC M12618A	Antarctica: Shag Rocks	22-sep.-11	Blake trawl	53.42398 S 42.017738 W	160	MW444231	MW444409	MW453554
Tritonia antarctica	SIO-BIC M12618C	Antarctica: Shag Rocks	22-sep.-11	Blake trawl	53.42398 S 42.017738 W	160	MW444232	MW444410	MW453555
Tritonia antarctica	SIO-BIC M12637	Antarctica: Discovery Bank	14-oct.-11	Blake trawl	60.124038 S 34.900757 W	391	MW444233	MW444411	MW453556
Tritonia antarctica	SIO-BIC M12938A	Antarctica: Shag Rocks	24-sep.-11	Blake trawl	53.724733 S 41.462667 W	180	–	MW444412	MW453557
Tritonia antarctica	SIO-BIC M12938B	Antarctica: Shag Rocks	24-sep.-11	Blake trawl	53.724733 S 41.462667 W	180	–	MW444413	MW453558
Tritonia antarctica	SIO-BIC M12956	Antarctica: South Georgia	25-sep.-11	Blake trawl	53.938103 S 39.200615 W	275	–	MW444414	MW453559
Tritonia antarctica	SIO-BIC M13056	Antarctica: Elephant Island	19-mar.-12	Blake trawl	61.334333 S 55.527333 W	149	MW444234	MW444415	MW453560
Tritonia antarctica	SIO-BIC M13481A	Antarctica: Shag Rocks	23-sep.-11	Blake trawl	53.45317 S 42.057903 W	186	MW444235	MW444416	MW453561
Tritonia antarctica	SIO-BIC M13481B	Antarctica: Shag Rocks	23-sep.-11	Blake trawl	53.45317 S 42.057903 W	186	MW444236	MW444417	MW453562
Tritonia antarctica	SIO-BIC M13806	Antarctica: Discovery Bank	15-oct.-11	Blake trawl	60.125 S 34.895 W	402	MW444237	MW444418	MW453563
Tritonia antarctica	SIO-BIC M12220 (NW26)	Antarctica: South Orkney Islands	22-feb.-09	Bottom trawl	61.20975 S 45.94017 W	240	–	MW546273	MW544053
Tritonia antarctica	YPM IZ 070059 (NW27)	Antarctica: South Orkney Islands	9-feb.-09	Bottom trawl	60.400807 S 46.646184 W	140–153	MW535727	MW546274	MW544054
Tritonia antarctica	YPM IZ 047930 (NW29)	Antarctica: South Orkney Islands	17-feb.-09	Bottom trawl	60.791611 S 44.455300 W	169–174	MW535728	MW546275	MW544055
Tritonia antarctica	SIO-BIC M12219 (NW32)	Antarctica: South Orkney Islands	22-feb.-09	Bottom trawl	61.20975 S 45.94017 W	240	–	MW546276	MW544056
Tritonia antarctica	SIO-BIC M12218 (NW33)	Antarctica: South Orkney Islands	22-feb.-09	Bottom trawl	61.20975 S 45.94017 W	240	MW535729	MW546277	MW544057
Tritonia antarctica	SIO-BIC SG-4B-12	Antarctica: South Georgia	17-apr.-13	Blake trawl	53.676833 S 37.246117 W	137	MW444238	MW444419	MW453564
Tritonia antarctica	SIO-BIC SG5-13	Antarctica: South Georgia	18-apr.-13	Blake trawl	54.289417 S 37.9168 W	170	MW444239	MW444420	MW453565
Tritonia antarctica	SIO-BIC SG5-14	Antarctica: South Georgia	18-apr.-13	Blake trawl	54.2968 S 37.9045 W	157	MW444240	MW444421	MW453566
Tritonia antarctica	SR-4-19	Antarctica: Shag Rocks	19-apr.-13	Blake trawl	53.560867 S 41.65425 W	129	MW444241	MW444422	MW453567
Tritonia antarctica	ZSM20012171	Antarctica: Weddell Sea, Kapp Norvegia	6-apr.-00	n.a.	71.125 S 11.463333 W	62	MW444242	MW444423	MW453568
Tritonia antarctica	ZSM20012236	Antarctica: Weddell Sea, Kapp Norvegia	6-apr.-00	n.a.	71.125 S 11.463333 W	62	MW444243	MW444424	MW453569
Tritonia antarctica	ZSM20020876	Antarctica: N Antarctic Peninsula	16-jul.-02	n.a.		301	MW444244	MW444425	MW453570

(*continued on next page*)

Table 1 (*continued*)

Species	Voucher number	Locality	Collection date	Collecting gear	Lat_Lon	Depth (m)	COI	16S	H3
					62.712333 S 55.369667 W				
Tritonia antarctica	ZSM20021038	Antarctica: South Orkney Islands	1-aug.-02	Agassiz trawl	60.9865 S 43.457001 W	402	MW444245	MW444426	MW453571
Tritonia challengeriana	ZSM20050396	Chile: Los Lagos, Huinay	17-feb.-05	SCUBA	42.379574 S 72.415795 W	30	MW444246	MW444427	MW453572
Tritonia challengeriana	ZSM20050582	Chile: Los Lagos, Huinay	17-feb.-05	SCUBA	42.379574 S 72.415795 W	30	MW444247	MW444428	MW453573
Tritonia challengeriana	ZSM20060871	Chile: Aysén, Farquhar Channel	13-jun.-06	SCUBA	48.577 S 74.3656 W	6	MW444248	MW444429	MW453574
Tritonia challengeriana	ZSM20060918	Chile: Aysén, Melinka	13-jun.-06	SCUBA	43.935001 S 73.818336 W	30	MW444249	MW444430	MW453575
Tritonia challengeriana	ZSM20061113	Chile: Aysén, Messier Channel	15-mar.-06	SCUBA	47.979168 S 74.679726 W	20	MW444250	MW444431	MW453576
Tritonia challengeriana	ZSM20070360	Chile: Magallanes, Channel Passage	29-mar.-07	SCUBA	50.463612 S 75.128334 W	10	MW444251	MW444432	MW453577
Tritonia challengeriana	ZSM20070378	Chile: Aysén, Fallos Channel, Bahia sin nombre	14-mar.-06	SCUBA	48.462502 S 75.051941 W	10	–	MW444433	MW453578
Tritonia challengeriana	ZSM20070446	Chile: Los Lagos, Huinay	2-mar.-07	SCUBA	42.379574 S 72.415795 W	18	MW444252	MW444434	MW453579
Tritonia challengeriana	ZSM20080808	Chile: Puerto Cisnes, Isla Faro	18-dec.-08	SCUBA	44.696111 S 72.698889 W	7	MW444253	MW444435	MW453580
Tritonia challengeriana	ZSM20090429-1	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444254	MW444436	MW453581
Tritonia challengeriana	ZSM20090429-3	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444255	MW444437	MW453582
Tritonia challengeriana	ZSM20090429-4	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444256	MW444438	MW453583
Tritonia challengeriana	ZSM20090429-5	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444257	MW444439	MW453584
Tritonia challengeriana	ZSM20090429-6	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444258	MW444440	MW453585
Tritonia challengeriana	ZSM20090429-7	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444259	MW444441	MW453586
Tritonia challengeriana	ZSM20090429-8	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444260	MW444442	MW453587
Tritonia challengeriana	ZSM20090429-9	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444261	MW444443	MW453588
Tritonia challengeriana	ZSM20090429-10	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444262	MW444444	MW453589
Tritonia challengeriana	ZSM20090429-11	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444263	MW444445	MW453590
Tritonia challengeriana	ZSM20090429-12	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444264	MW444446	MW453591
Tritonia challengeriana	ZSM20090429-13	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444265	MW444447	MW453592
Tritonia challengeriana	ZSM20090429-14	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444266	MW444448	MW453593
Tritonia challengeriana	ZSM20090429-15	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444267	MW444449	MW453594
Tritonia challengeriana	ZSM20090429-16	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444268	MW444450	MW453595
Tritonia challengeriana	ZSM20090429-17	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444269	MW444451	MW453596
Tritonia challengeriana	ZSM20090429-18	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444270	MW444452	MW453597

(*continued on next page*)

Table 1 (*continued*)

Species	Voucher number	Locality	Collection date	Collecting gear	Lat_Lon	Depth (m)	COI	16S	H3
Tritonia challengeriana	ZSM20090429-19	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444271	MW444453	MW453598
Tritonia challengeriana	ZSM20090429-20	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444272	MW444454	MW453599
Tritonia challengeriana	ZSM20090429-21	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444273	MW444455	MW453600
Tritonia challengeriana	ZSM20090429-22	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444274	MW444456	MW453601
Tritonia challengeriana	ZSM20090429-23	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444275	MW444457	MW453602
Tritonia challengeriana	ZSM20090429-24	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444276	MW444458	MW453603
Tritonia challengeriana	ZSM20090429-25	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444277	MW444459	MW453604
Tritonia challengeriana	ZSM20090429-26	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444278	MW444460	MW453605
Tritonia challengeriana	ZSM20090429-27	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444279	MW444461	MW453606
Tritonia challengeriana	ZSM20090429-28	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444280	MW444462	MW453607
Tritonia challengeriana	ZSM20090429-29	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444281	MW444463	MW453608
Tritonia challengeriana	ZSM20090429-30	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444282	MW444464	MW453609
Tritonia challengeriana	ZSM20090429-31	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444283	MW444465	MW453610
Tritonia challengeriana	ZSM20090429-32	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444284	MW444466	MW453611
Tritonia challengeriana	ZSM20090429-33	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444285	MW444467	MW453612
Tritonia challengeriana	ZSM20090429-34	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444286	MW444468	MW453613
Tritonia challengeriana	ZSM20090429-35	Chile: Los Lagos, Lilihuapi Island	23-jun.-09	SCUBA	42.158697 S 72.59704 W	6	MW444287	MW444469	MW453614
Tritonia challengeriana	ZSM20090525	Chile: Aysén, Melinka	13-jun.-06	SCUBA	43.935001 S 73.818336 W	30	MW444288	MW444470	MW453615
Tritonia challengeriana	ZSM20090569	Chile: Los Lagos, Cahuelmo, Huinay	28-feb.-09	SCUBA	42.3473 S 72.455481 W	30	MW444289	MW444471	MW453616
Tritonia challengeriana	ZSM20110264	Argentina: Tierra del Fuego, Beagle Channel, Islotes Gemelos	16-jun.-11	SCUBA	54.919417 S 67.363083 W	22	MW444290	MW444472	MW453617
Tritonia challengeriana	ZSM20110267	Argentina: Tierra del Fuego, Beagle Channel, Islotes Gemelos	17-jun.-11	SCUBA	54.919417 S 67.363083 W	13	MW444291	MW444473	MW453618
Tritonia challengeriana	ZSM20110430	Chile: Los Lagos, Caleta Gonzalo	5-jul.-11	SCUBA	42.546111 S 72.616944 W	20	MW444292	MW444474	MW453619
Tritonia challengeriana	ZSM20130448	Chile: Magallanes, Fuerte Bulnes	23-jan.-14	SCUBA	53.65 S 70.933333 W	18	MW444293	–	MW453620
Tritonia challengeriana	ZSM20130449	Chile: Magallanes, Fuerte Bulnes	23-jan.-14	SCUBA	53.65 S 70.933333 W	18	MW444294	MW444475	MW453621
Tritonia challengeriana	ZSM20130450	Chile: Magallanes, Fuerte Bulnes	23-jan.-14	SCUBA	53.65 S 70.933333 W	18	–	MW444476	MW453622
Tritonia challengeriana	ZSM20130451	Chile: Magallanes, Fuerte Bulnes	23-jan.-14	SCUBA	53.65 S 70.933333 W	18	MW444295	MW444477	MW453623
Tritonia challengeriana	ZSM20130453	Chile: Magallanes, Fuerte Bulnes	23-jan.-14	SCUBA	53.65 S 70.933333 W	18	–	–	MW453624
	ZSM20130479	Chile: Magallanes, Corbeta Island	23-jan.-14	SCUBA		16	MW444296	MW444478	MW453625

(*continued on next page*)

Table 1 (*continued*)

Species	Voucher number	Locality	Collection date	Collecting gear	Lat_Lon	Depth (m)	COI	16S	H3
Tritonia challengeriana					50.356302 S 75.333525 W				
Tritonia challengeriana	ZSM20130480	Chile: Magallanes, Cono Guarello	23-jan.-14	SCUBA	50.379241 S 75.37901 W	14	MW444297	MW444479	MW453626
Tritonia challengeriana	ZSM20130482	Chile: Magallanes, Cono Guarello	23-jan.-14	SCUBA	50.379241 S 75.37901 W	15	MW444298	MW444480	MW453627
Tritonia odhneri	ZSM20060892	Chile: Aysén, Bernardo Fjord	13-jun.-06	SCUBA	48.481968 S 74.095408 W	15	MW444299	MW444481	MW453628
Tritonia odhneri	ZSM20060926	Chile: Aysén, Guaiteca Island	13-jun.-06	SCUBA	43.882236 S 73.735583 W	25	–	–	MW453629
Tritonia odhneri	ZSM20060978	Chile: Aysén, Isla Castillo	26-jun.-06	SCUBA	45.316666 S 73.73333 W	35	MW444300	MW444482	MW453630
Tritonia odhneri	ZSM20061109	Chile: Magallanes, Copihue Channel	9-mar.-06	SCUBA	50.339722 S 75.377502 W	5	MW444301	–	MW453631
Tritonia odhneri	ZSM20061116	Chile: Aysén, Ofhidro Island	15-mar.-06	SCUBA	48.164444 S 74.396667 W	15	MW444302	MW444483	MW453632
Tritonia odhneri	ZSM20061117	Chile: Magallanes, Adalberto Channel	12-mar.-06	SCUBA	48.608055 S 74.898888 W	30	MW444303	MW444484	MW453633
Tritonia odhneri	ZSM20070576	Chile: Magallanes, Angostura Inglesa	16-apr.-07	SCUBA	48.988335 S 74.418892 W	25	MW444304	MW444485	MW453634
Tritonia odhneri	ZSM20130477	Chile: Magallanes, Cono Guarello	24-jan.-14	SCUBA	50.379241 S 75.37901 W	10	MW444305	MW444486	MW453635
Tritonia sp. 1	ZSM20070588	Chile: Aysén, Isla Castillo	17-apr.-07	SCUBA	45.316666 S 73.73333 W	28	MW444306	MW444487	MW453636
Tritonia sp. 2	SIO-BIC M13413	Falkland Islands: Burdwood Bank East	24-apr.-13	Blake trawl	54.548667 S 56.734967 W	109	MW444307	MW444488	MW453637
Tritonia sp. 3	CZA-104	Peru: Paracas, La Mina	1-jul.-09	SCUBA	13.91205 S 76.317623 W	10	MW444308	MW444489	MW453638
Tritonia sp. 3	CZA-175	Peru: Piura, Foca Island	14-jun.-12	SCUBA	5.21203 S 81.206262 W	10	MW444309	MW444490	MW453639
Tritonia sp. 3	ZSM20100720	Peru: Ancash, Santa Island	18-jan.-06	SCUBA	9.028839 S 78.680507 W	10	MW444310	MW444491	MW453640
Tritonia vorax	SIO-BIC M12579C	Antarctica: South Georgia	25-sep.-11	Blake trawl	53.892315 S 39.19146 W	310	MW444311	MW444492	MW453641
Tritonia vorax	SIO-BIC M13014	Antarctica: Shag Rocks	22-sep.-11	Blake trawl	53.42398 S 42.017738 W	160	MW444312	MW444493	MW453642
Tritonia vorax	SIO-BIC M13395	Falkland Islands: South	3-may.-13	Blake trawl	52.4789 S 60.4304 W	160	MW444313	MW444494	MW453643
Tritonia vorax	SIO-BIC M13399	Antarctica: Shag Rocks	19-apr.-13	Blake trawl	53.54945 S 41.65085 W	128	MW444314	MW444495	MW453644
Tritonia vorax	SIO-BIC M13404	Antarctica: Shag Rocks	19-apr.-13	Blake trawl	53.5585 S 41.654417 W	130	MW444315	MW444496	MW453645
Tritonia vorax	SIO-BIC M13414	Antarctica: South Georgia	16-apr.-13	Blake trawl	53.5876 S 37.299983 W	444	MW444316	MW444497	MW453646
Tritonia vorax	SIO-BIC M13420	Falkland Islands: East	28-apr.-13	Blake trawl	51.637767 S 57.4938 W	124	MW444317	MW444498	MW453647
Tritonia vorax	SIO-BIC M13453	Falkland Islands: South	3-may.-13	Blake trawl	52.4799 S 60.4147 W	154	MW444318	MW444499	MW453648
Tritonia vorax	SIO-BIC M13507	Falkland Islands: Burdwood Bank East	24-apr.-13	Blake trawl	54.559817 S 56.8297 W	90	MW444319	MW444500	MW453649
Tritonia vorax	SIO-BIC M13577	Falkland Islands: East	28-apr.-13	Blake trawl	51.6377 S 57.4938 W	123	MW444320	MW444501	MW453650
Tritonia vorax	SIO-BIC M13805	Falkland Islands: Burdwood Bank South	31-oct.-11	Blake trawl	54.6715 S 61.2337 W	341	MW444321	MW444502	MW453651
Tritonia vorax	ZSM20020848	Falkland Islands: Burdwood Bank South	15-jul.-02	Bottom trawl	54.520332 S 56.148834 W	296	MW444322	MW444503	MW453652
Tritonia vorax	ZSM20020878	Falkland Islands: Burdwood Bank South	16-jul.-02	Agassiz trawl	54.503666 S 56.136665 W	292	MW444323	MW444504	MW453653
Tritonia vorax	ZSM20020887	Falkland Islands: Burdwood Bank South	17-jul.-02	Bottom trawl	54.520332 S 56.148834 W	296	MW444324	MW444505	MW453654
Tritonia vorax	ZSM20020931	Falkland Islands: Burdwood Bank South	22-jul.-02	Bottom trawl	54.520332 S 56.148834 W	296	MW444325	MW444506	MW453655
Tritonia vorax	ZSM20021040	Falkland Islands: Burdwood Bank South	1-aug.-02	Agassiz trawl	54.503666 S 56.136665 W	292	MW444326	MW444507	MW453656
Tritonia vorax	ZSM20021245	Falkland Islands: Burdwood Bank South	5-sep.-02	Bottom trawl	54.520332 S 56.148834 W	296	MW444327	MW444508	MW453657
Tritonia vorax	ZSM20021246	Falkland Islands: Burdwood Bank South	5-sep.-02	Bottom trawl	54.520332 S 56.148834 W	296	MW444328	MW444509	MW453658
Tritonia vorax	ZSM20021247	Falkland Islands: Burdwood Bank South	5-sep.-02	Bottom trawl	54.520332 S 56.148834 W	296	MW444329	MW444510	MW453659
Tritonia vorax	ZSM20070357	Chile: Magallanes, Channel Passage	9-mar.-06	SCUBA	50.463612 S 75.128334 W	23	MW444330	MW444511	MW453660

(*continued on next page*)

Table 1 (*continued*)

Species	Voucher number	Locality	Collection date	Collecting gear	Lat_Lon	Depth (m)	COI	16S	H3
Tritonia vorax	ZSM20090520	Chile: Magallanes, Channel Passage	29-mar.-07	SCUBA	50.463612 S 75.128334 W	20	MW444331	MW444512	MW453661
Tritonia vorax	ZSM20090524	Chile: Aysén, Messier Channel	15-mar.-06	SCUBA	47.979168 S 74.679726 W	20	MW444332	MW444513	MW453662
Tritonia vorax	ZSM20110266	Argentina: Tierra del Fuego, Beagle Channel, Islotes Gemelos	17-jun.-11	SCUBA	54.919417 S 67.363083 W	14	MW444333	MW444514	MW453663
Tritonia vorax	ZSM20110271	Argentina: Tierra del Fuego, Beagle Channel, Islotes Gemelos	17-jun.-11	SCUBA	54.919417 S 67.363083 W	13	MW444334	MW444515	MW453664
Tritonia vorax	ZSM20130484	Chile: Cueva	24-jan.-14	SCUBA	unknown	15	MW444335	MW444516	MW453665
Tritonia vorax	ZSM20130783	Chile: Isla Fitz Roy, Huinay Fiorden 21	25-jan.-14	SCUBA	45.83927 S 74.039811 W	20	MW444336	MW444517	MW453666
Tritoniella belli	ZSM20012172	Antarctica: Weddell Sea, Kapp Norvegia	2-apr.-00	Bottom trawl	71.188333 S 12.256667 W	318	MW444337	MW444518	MW453667
Tritoniella belli	ZSM20012249	Antarctica: East Weddell Sea, Austasen	7-apr.-00	Bottom trawl	70.84 S 10.586667 W	266	MW444338	MW444519	MW453668
Tritoniella belli	ZSM20012253	Antarctica: North of Kapp Norvegia	6-apr.-00	Bottom trawl	71.125167 S 11.451667 W	137	MW444339	MW444520	MW453669
Tritoniella belli	ZSM20012255	Antarctica: Weddell Sea, Kapp Norvegia	9-apr.-00	Bottom trawl	71.124167 S 11.460667 W	80	MW444340	MW444521	MW453670
Tritoniella belli	ZSM20090521	Antarctica: Weddell Sea, Kapp Norvegia	9-apr.-00	Bottom trawl	71.124167 S 11.460667 W	80	MW444341	MW444522	MW453671
Tritoniella belli	ZSM20090522	Antarctica: Weddell Sea, Kapp Norvegia	9-apr.-00	Bottom trawl	71.124167 S 11.460667 W	80	MW444342	MW444523	MW453672
Tritoniella sp. 1	SIO-BIC M13502	Falkland Islands: Burdwood Bank East, S Falkland Islands	26-apr.-13	Blake trawl	54.466733 S 55.68085 W	572	MW444343	MW444524	MW453673
Tritoniella sp. 2	ZSM20141002	Antarctica: South Shetland Islands, King George Island	19-feb.-10	SCUBA	62.210306 S 58.913868 W	40	MW444344	MW444525	MW453674
Tritoniella sp. 3	SIO-BIC M12606	Antarctica: South Georgia	26-sep.-11	Blake trawl	53.8069 S 37.217003 W	144	MW444345	MW444526	MW453675
Tritoniella sp. 3	SIO-BIC M12609	Antarctica: Shag Rocks	23-sep.-11	Blake trawl	53.45317 S 42.057903 W	186	MW444346	MW444527	MW453676
Tritoniella sp. 3	SIO-BIC M12618B	Antarctica: Shag Rocks	22-sep.-11	Blake trawl	53.42398 S 42.017738 W	160	MW444347	MW444528	MW453677
Tritoniella sp. 3	SIO-BIC M12648	Antarctica: South Georgia	26-sep.-11	Blake trawl	53.8069 S 37.217003 W	144	MW444348	MW444529	MW453678
Tritoniella sp. 3	SIO-BIC M12939	Antarctica: South Georgia	29-sep.-11	Blake trawl	55.051528 S 35.395497 W	119	MW444349	MW444530	MW453679
Tritoniella sp. 3	SIO-BIC M13488	Antarctica: Shag Rocks	24-sep.-11	Blake trawl	53.7211 S 41.462667 W	180	MW444350	MW444531	MW453680
Tritoniella sp. 3	SIO-BIC M13489	Antarctica: Shag Rocks	24-sep.-11	Blake trawl	53.534252 S 41.633907 W	133	MW444351	MW444532	MW453681
Tritoniella sp. 3	SIO-BIC M13492	Antarctica: Discovery Bank	15-oct.-11	Blake trawl	60.145708 S 34.919935 W	751	MW444352	MW444533	MW453682
Tritoniella sp. 4	SIO-BIC M12599	Antarctica: South Orkney	20-oct.-11	Blake trawl	60.550858 S 45.176113 W	278	MW444353	MW444534	MW453683
Tritoniella sp. 4	ZSM20110202-02	Antarctica: South Shetland Islands, King George Island	19-feb.-10	SCUBA	62.210306 S 58.913868 W	40	MW444354	MW444535	MW453684
Tritoniella sp. 5	SIO-BIC M12581B	Antarctica: Antarctic Peninsula, Bransfield Strait	25-oct.-11	Blake trawl	63.322833 S 59.851052 W	199	MW444355	MW444536	MW453685
Tritoniella sp. 5	SIO-BIC M12593	Antarctica: Antarctic Peninsula, Bransfield Strait	24-oct.-11	Blake trawl	62.86951 S 57.21682 W	247	MW444356	MW444537	MW453686
Tritoniella sp. 5	SIO-BIC M12608	Antarctica: South Sandwich Islands	6-oct.-11	Blake trawl	58.384513 S 26.281305 W	262	MW444357	MW444538	MW453687
Tritoniella sp. 5	SIO-BIC M12615	Antarctica: Antarctic Peninsula, Bransfield Strait	24-sep.-11	Blake trawl	62.752845 S 57.321675 W	292	MW444358	MW444539	MW453688
Tritoniella sp. 5	SIO-BIC M12617A	Antarctica: N South Sandwich Islands	25-oct.-11	Blake trawl	58.3773 S 26.2824 W	420	MW444359	MW444540	MW453689
Tritoniella sp. 5	SIO-BIC M12617B	Antarctica: N South Sandwich Islands	25-oct.-11	Blake trawl	58.3773 S 26.2824 W	420	MW444360	MW444541	MW453690
Tritoniella sp. 5	SIO-BIC M12617C	Antarctica: N South Sandwich Islands	25-oct.-11	Blake trawl	58.3773 S 26.2824 W	420	MW444361	MW444542	MW453691
Tritoniella sp. 5	SIO-BIC M12965	Antarctica: South Sandwich Islands	7-oct.-11	Blake trawl	59.393638 S 27.322693 W	188	MW444362	MW444543	MW453692
Tritoniella sp. 5	SIO-BIC M13011	Antarctica: South Sandwich Islands	6-oct.-11	Blake trawl	58.378473 S 26.283405 W	260	MW444363	MW444544	MW453693
Tritoniella sp. 5	SIO-BIC (NW31)	unknown	unknown	unknown	unknown	unknown	–	MW546278	MW544058

(*continued on next page*)

Table 1 (*continued*)

Species	Voucher number	Locality	Collection date	Collecting gear	Lat_Lon	Depth (m)	COI	16S	H3
Tritoniella sp. 5	ZSM20110202-01	Antarctica: South Shetland Islands, King George Island	19-feb.-10	SCUBA	62.210306 S 58.913868 W	40	MW444364	MW444545	MW453694
Tritoniella sp. 5	ZSM20110221-06	Antarctica: South Shetland Islands, King George Island	19-feb.-10	SCUBA	62.210306 S 58.913868 W	40	MW444365	MW444546	MW453695
Tritoniella sp. 5	ZSM20141003	Antarctica: South Shetland Islands, King George Island	19-feb.-10	SCUBA	62.210306 S 58.913868 W	40	MW444366	MW444547	MW453696
Tritoniella sp. 5	ZSM20141004	Antarctica: South Shetland Islands, King George Island	19-feb.-10	SCUBA	62.210306 S 58.913868 W	40	MW444367	MW444548	MW453697
Tritoniella sp. 5	ZSM20141005	Antarctica: South Shetland Islands, King George Island	19-feb.-10	SCUBA	62.210306 S 58.913868 W	40	MW444368	MW444549	MW453698

Tritonidoxa Bergh, 1908, molecular data is still missing. Taxonomically speaking, the family has undergone important restructuring, with many genera having been synonymized. For instance, *Duvaucelia* Risso, 1826 has been resurrected and *Tritonicula* Korshunova & Martynov, 2020 newly erected in a recent phylogenetic analysis, thus dividing the genus *Tritonia* into three genera based on morphological and molecular evidence (Korshunova and Martynov, 2020). Still, much controversy exists especially regarding many of the abovementioned genera.

Southern Ocean tritoniids are large and commonly sampled, but the incomplete morphoanatomical descriptions (Eliot, 1907a; von Martens and Pfeffer, 1886), unknown ranges of morphological variation, apparently wide distributional ranges of currently valid species, and the lack of molecular data contribute to taxonomic uncertainty (Rossi et al., 2021; Schrödl, 2009; Wägele, 1995). Two tritoniid genera are currently reported from Antarctica. *Tritoniella* is limited to the Southern Ocean, with the northernmost record from Shag Rocks (Wägele, 1989a), not extending north of the Antarctic convergence (Schrödl, 1999). *Tritoniella belli* Eliot, 1907 was originally described from the Ross Sea and synonymized to the sympatric *T. sinuata* Eliot, 1907 (Wägele, 1989a). This species is a widespread, chemically-protected, and facultative corallivore with direct development (Bryan et al., 1998; Wägele, 1989b). The second common genus in the Southern Ocean is *Tritonia*. In a careful morphological revision, Wägele (1995) showed all *Tritonia* specimens known from Antarctic waters belong to only two species. The widespread, high-Antarctic *T. antarctica* Pfeffer in von Martens and Pfeffer, 1886, and the Sub-Antarctic *T. vorax* (Odhner, 1926). The latter species is only known from deep waters (100–360 m) of the Beagle Channel, the Burdwood Bank, and South Georgia, and so far has never been reported from locations further south of the Antarctic convergence (Schrödl, 1999). Large egg sizes and larval morphology of Antarctic specimens from the Ross Sea suggest *T. antarctica* produces crawl-away juveniles or larvae with a short dispersive period (Woods and Moran, 2008), a common feature for Antarctic nudibranchs (e.g., Moles et al., 2017a). Later, *Tritonia dantarti* Ballesteros & Avila, 2006 was described from Bouvet Island (Ballesteros and Avila, 2006) and its distinction from *T. antarctica* was confirmed in a recent molecular study (Rossi et al., 2021). Nonetheless, as suggested by Wägele (1995), Sub-Antarctic tritoniids are not identifiable without an exhaustive molecular and morphological revision of Tritoniidae of the temperate waters of South America.

From the Magellanic faunal Province (see Brattström and Johanssen, 1983), which includes coastal waters of South America south to 41°S (the wide Patagonian shelf and the Falkland Islands), a considerable number of tritoniid specimens have been collected during several international expeditions (Bergh, 1898, 1884; Eliot, 1907a; Marcus, 1959; Marcus and Marcus, 1969; Odhner, 1926). These were assigned to five nominal species of the genus *Tritonia*, including species from the three then synonymised genera *Duvaucelia*, *Candiella* Gray, 1850, and the dubious *Microlophus* Rochebrune & Mabille, 1891. *Tritonia challengeriana* Bergh, 1884 was regarded to be conspecific with *T. antarctica* by Odhner (1926) and Marcus (1959). More recently, the status of both species has been questioned, and still to be solved with the aids of molecular data (Rossi et al., 2021; Schrödl, 1996; Wägele, 1995). Also, specimens from the Falklands described as *T. challengeriana* by Eliot (1907b) appeared aberrant in shape (see Wägele, 1995). *Tritonia australis* (Bergh, 1898) seemed to be the most widespread Magellanic species with records from the Pacific Juan Fernández Islands (Bergh, 1898) and central Chile (Schrödl, 1996) over the Magellan Strait area (Marcus, 1959) to the northern Argentinian coast (Marcus and Marcus, 1969). It is, however, also the most puzzling species since it cannot be unambiguously characterized by the various descriptions. For instance, there are no clear differences to *T. challengeriana* besides the larger jaws and thus, it has been considered a junior synonym (Schrödl, 2003). Similarly, from the warm-temperate Atlantic, some poorly known tritoniids were reported by Odhner (1926) and Eveline Marcus (1983). Marcus described two new species, *Tritonia eriosi* and *Marionia tedi*, and redescribed *Marionia cucullata* (Couthouy in Gould, 1952). The Uruguayan *T. eriosi* Marcus, 1983 is similar to *T. odhneri* Marcus, 1959 known from southern and central Chile (Marcus, 1959; Schrödl, 1996). Both areas are north of the widely accepted 41°S border of the Magellanic province and are characterized by a considerable faunal overlap of cold and warm temperate species (Schrödl, 2003, 1999, 1996).

All the species mentioned are in a state of extreme taxonomic confusion (see Schrödl, 2003, 1996; Wägele, 1995), which arose from (1) incomplete descriptions of (2) a few, preserved specimens, and (3) the apparent loss of type material. The inability to link names with living specimens and thus interpret intraspecific variation is a considerable obstacle, since Antarctic and Magellanic tritoniids are very similar to each other, especially when preserved (Wägele, 1995). During the last years, numerous tritoniid specimens have been observed, collected, and photographed alive, from the Chilean and Argentinian coasts, from Sub-Antarctic Islands, the Weddell Sea, and the Antarctic Peninsula. The present study aims are twofold: (1) to provide molecular data for a great number of southern tritoniids from this broad geographical range and (2) to ascertain the status of the current valid species and further delve into the potential of pseudocryptic speciation within the genera *Tritonia* and *Tritoniella*. The new data is included in a wider phylogenetic context of the family to provide some clarification at both the genus and species levels.

2. Material & methods

2.1. Taxa collection

Animals were collected during multiple campaigns covering a wide geographical range in the Atlantic sector of the Southern Ocean and the Scotia Sea either by dredging or trawling on board the RV Polarstern (AWI, Germany) and the RV Nathaniel B. Palmer (NSF, USA), by SCUBA diving in the Southern Chilean coast on board of the Yepayek (CONAF) and by other independent dive expeditions (Table 1). Distribution maps colour-coded by species for both the Southern Hemisphere (SH) *Tritoniella* (Fig. 1) and *Tritonia* (Fig. 2) were designed in Arc-GIS 10.3 (Esri, Redlands, CA). When possible, specimens were photographed alive and preserved in either 75% or 96% EtOH for molecular purposes. Additionally, material of *Marionia cucullata* (Couthouy, 1852) from the type-locality, southeastern Brazil, and three specimens of *Tritonia* cf. *festiva* from the Peruvian coast were included to provide phylogenetic context. Voucher specimens were deposited at the Bavarian State Collection of Zoology (ZSM, Munich), the Benthic Invertebrate Collection at Scripps Institution of Oceanography, University of California, San Diego (SIO-BIC, La Jolla, CA, USA), and the Colección de Zoologia Acuática (CZA) of the Universidad Peruana Cayetano Heredia, Peru.

2.2. Molecular data collection

DNA was extracted from a piece of the foot using a combination of CTAB extraction and NucleoSpin Tissue Kit (Macherey-Nagel, Germany). For tissue lysis, samples in a CTAB-buffer/mercaptoethanol solution were kept at 56 °C overnight. For extraction, samples went through the following steps: (1) incubation at 37 °C with RNase A for 30 min, (2) wash up step with 400 μl Chloroform-Isoamyl alcohol solution twice, (3) addition of Sodium Acetate/96% EtOH and centrifuge in a NucleoSpin column, (4) final wash with 96% EtOH. Further process followed the NucleoSpin Tissue Kit manufacturer's protocol. The three standard molecular markers amplified were the mitochondrial Cytochrome *c* Oxidase subunit I (COI; *ca.* 658 bp), using the LCO1490 and HCO2198 primer pair (Folmer et al., 1994), and 16S rRNA (16S; *ca.* 485 bp), using the 16S-H and 16S-R primers (Simon et al., 1994), and the nuclear gene histone-3 (H3; *ca.* 330 bp), using H3AD5′3′ and H3BD5′3′ (Colgan et al., 1998). PCR protocols are listed in Jörger et al. (2010). Successful PCR products were cleaned up with DNA Clean & Concentrator TM (Zymo Research, CA, USA). Sanger sequencing was performed

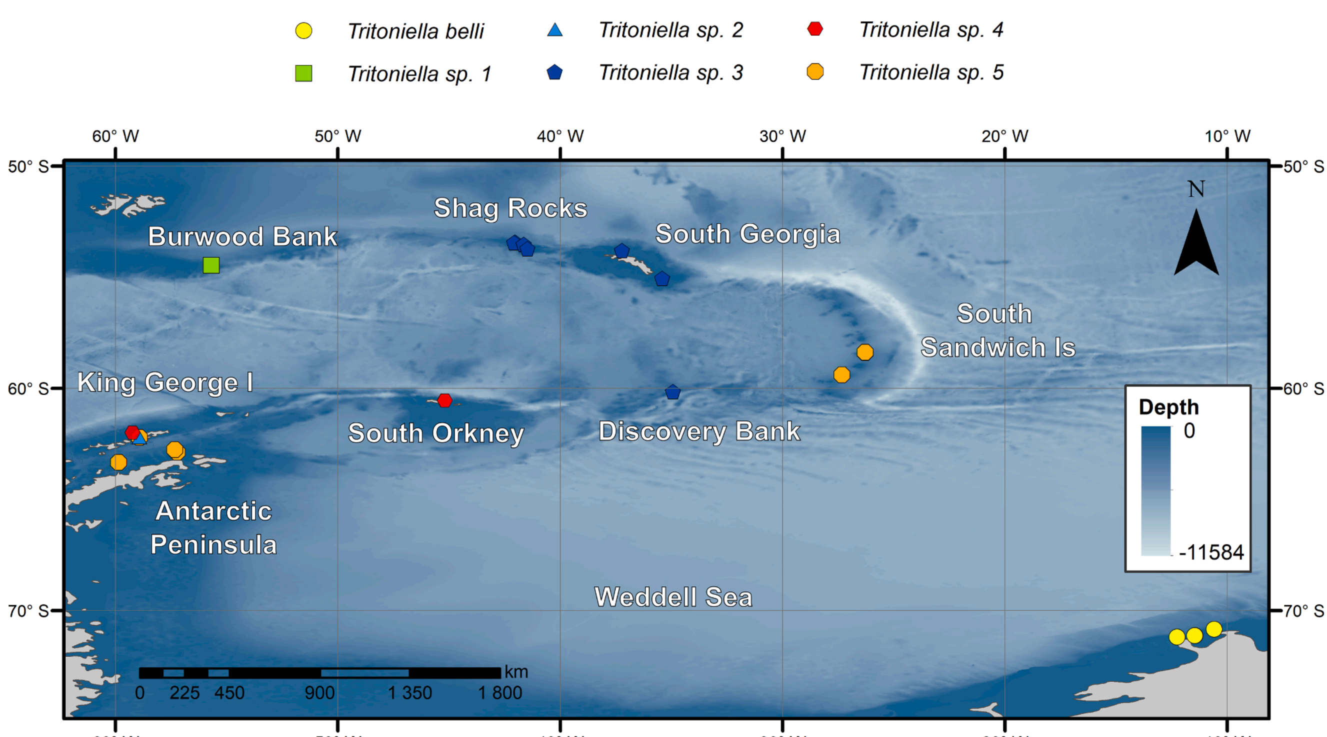

Fig. 1. Distribution map from the Scotia Sea and the Weddell Sea of all *Tritoniella* species sequenced in this study colour-coded by species. Yellow circles – *Tritoniella belli* from the Weddell Sea (Ross Sea sequence data from GenBank not depicted); green square – *Tritoniella* sp. 1 from Burdwood Bank; light blue triangle – *Tritoniella* sp. 2 from King George Island; dark blue pentagons – *Tritoniella* sp. 3 from Shag Rocks, South Georgia, and Discovery Bank; red hexagons – *Tritoniella* sp. 4 from South Orkney and King George Island; orange octagons – *Tritoniella* sp. 5 from South Sandwich Islands, King George Island, and Bransfield Strait. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

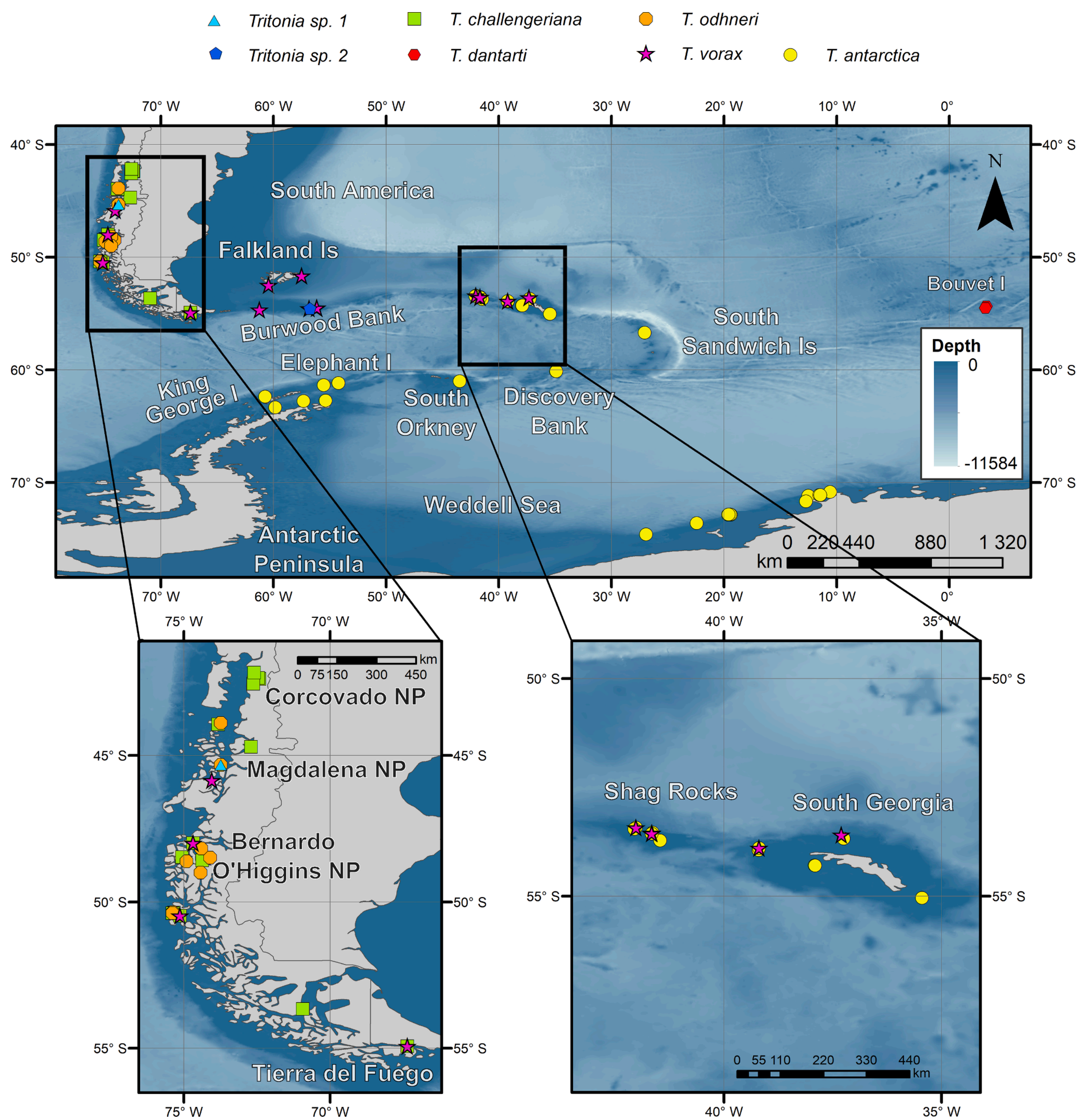

Fig. 2. Distribution map from South America, the Scotia Sea, and the Weddell Sea of the Southern Hemisphere *Tritonia* species sequenced in this study colour-coded by species. Light blue triangle – *Tritonia* sp. 1 from Isla Castillo in Chile; dark blue pentagons – *Tritonia* sp. 2 from Burdwood Bank; green square – *Tritonia challengeriana* from Chile and Tierra del Fuego; red hexagons – *Tritonia dantarti* from Bouvet Island; orange octagons – *Tritonia odhneri* from Chile; pink stars – *Tritonia vorax* from Chile, Tierra del Fuego, Falkland Islands, Burdwood Bank, Shag Rocks, and South Georgia; yellow circles – *Tritonia antarctica* from Shag Rocks, South Georgia, South Sandwich, South Orkney, South Shetland, Antarctic Peninsula, and the Weddell Sea (Ross Sea sequence data from GenBank not depicted). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

by the Sequencing Service of the Department of Biology Genomic Service Unit (GSU) of the Ludwig-Maximilians-University Munich. DNA aliquots are stored and publicly available through the DNA bank network (http://www.dnabank-network.org) or from the collection that houses the specimen. All sequences were deposited to GenBank at NCBI (see Table 1 for accession numbers). A total of 109 additional sequences from all available Tritoniidae (94) and additional outgroup taxa belonging to Cladobranchia and Doridina were downloaded from GenBank (see Table S1).

2.3. Phylogenetic analyses

Sequences were visualized, edited, and assembled in Geneious Prime® 2019.2.3 (Kearse et al., 2012) and contamination was assessed at NCBI through the BLAST algorithm (Altschul et al., 1997). Alignments were built in MAFFT v 7.450 (Katoh and Standley, 2013) using the G-INS-i (COI and H3) and L-INS-i (16S) algorithms. Each alignment was trimmed of primer regions and inspected visually for possible alignment errors. Additionally, poorly aligned positions and divergent regions

Table 2
Jukes-Cantor (JC69) intra- and interspecific genetic *p*-distances based on COI for *Tritoniella* species.

		1	2	3	4	5	6
1	***Tritoniella belli***	0–2.2					
2	***Tritoniella* sp. 1**	15.1–15.7	–				
3	***Tritoniella* sp. 2**	11.8–13.9	17.4	–			
4	***Tritoniella* sp. 3**	7.3–9.1	16–16.7	12.1–13.7	0–2.7		
5	***Tritoniella* sp. 4**	4.8–6.3	15.5–17.2	12.1–12.4	8.2–10.9	3.3	
6	***Tritoniella* sp. 5**	4.6–5.7	15.3–16.5	10.6–11.9	8.4–9.6	4.3–5	0–0.8

were trimmed in the 16S alignment using Gblocks v0.9b (Talavera and Castresana, 2007) under all relaxed parameters (retaining 91% of the original alignment). Phylogenetic analyses for each alignment as well as for the concatenated dataset were carried out online in CIPRES Science Gateway (Miller et al., 2015). A Maximum Likelihood (ML) approach implemented in IQ-TREE v 1.6.10 (Nguyen et al., 2015) used ModelFinder (Kalyaanamoorthy et al., 2017) to automatically select the best-fit partitioning scheme by merging partitions to reduce over-parameterization and increase model fit (TESTMERGE), also accounting for codon position for the protein-coding genes. Bootstrap support values (BS) were estimated via the ultrafast bootstrap algorithm with 1500 replicates (Minh et al., 2013). A Bayesian Inference (BI) approach was assessed with the partitioned concatenated alignment in MrBayes v 3.2.7 (Ronquist et al., 2011) under a GTRGAMMA model of evolution. We ran four parallel runs of four coupled Markov chain Monte Carlo (MCMC) chains for 20 million generations each and sampled every 1000 generations. The first 25% of the trees were discarded as burn-in for each MCMC run before convergence. Convergence was achieved when the average standard deviation of split frequencies (ASDSF) reached < 0.02% for all parameters. Topological robustness was assessed using posterior probabilities (PP). Trees were visualized in FigTree v. 1.4.4 (Rambaut, 2014) and edited in Inkscape v. 1 (Inkscape Project, 2020).

2.4. Species delimitation tests (SDT)

A preliminary assessment of species diversity was evaluated through the Automatic Barcode Discovery (ABGD; Puillandre et al., 2012) for the individual Southern Hemisphere (SH) *Tritonia* and *Tritoniella* COI alignments, *p*-distance matrices are depicted in Table 2 and 3, respectively. ABGD analysis was run using the Jukes-Cantor (JC69) TS/TV distance matrix with default parameters ($P_{min} = 0.001$, $P_{max} = 0.1$, 10 steps, 20 Nb bins, and relative gap width = 1.5). Species delimitation tests (SDT) based on ML trees for SH *Tritonia* and *Tritoniella* were performed via the webserver by both a Bayesian implementation of the Poisson tree processes model (bPTP; Zhang et al., 2013) at https://species.h-its.org/ and the multi-rate Poisson tree processes under ML and MCMC (mPTP; Kapli et al., 2017) at https://mptp.h-its.org/#/tree. We also used the Generalized Mixed Yule Coalescent approach (GMYC; Fujisawa and Barraclough, 2013) on ultrametric trees of the individual alignments generated in BEAST v1.10.4 (Drummond and Rambaut, 2007) under the best-fit evolutionary model obtained in ModelFinder and with a relaxed lognormal clock and a rate of 1.0 during 10 million generations. TreeAnnotator was used to discard the initial 25% of trees as burn-in. To further corroborate species hypothesis all SDTs were likewise performed on the 16S alignment, these will be discussed when differing from the COI-based SDT. Haplotype networks were generated for COI sequences of Southern Ocean *Tritoniella* and *Tritonia* species using a statistical parsimony network algorithm (Clement et al., 2000) implemented in PopART (Leigh and Bryant, 2015).

3. Results

3.1. Phylogenetic relationships

A total of 167 samples of *Tritonia* (n = 133), *Tritoniella* (n = 33), and one of *Marionia cucullata* were successfully sequenced and included in the final dataset of 276 specimens. The best-fit model of evolution according to BIC for individual alignments was TVM + F + I + G4 for COI and 16S, and TIM + F + I + G4 for H3 (accounting for codon positions in protein-encoding genes). The final concatenated alignment contained 1473 bp and the best-merged model of evolution was GTR + F + I + G4. Gene trees overall recovered topologies compatible with the concatenated one, thus we only refer to the final concatenated analyses. Only the BI tree recovered the monophyly of Tritoniidae with high support (Fig. 3; BS = 44, PP = 0.95). The monophyly of most of the Tritoniidae genera was recovered in ML and BI (see Figures S1 and S2), namely *Duvaucelia* (BS = 100, PP = 1), *Marionia* (BS = 100, but not in BI), *Tritoniopsis* (BS = 100, PP = 1), *Tochuina* (BS = 100, PP = 1), *Tritonia* (BS = 98, PP = 0.96), *Tritonicula* (BS = 100, but not in BI), *Tritoniella* (BS = 99, PP = 1), and the relationship of the monotypic *Marianina* as sister taxon to *Tritoniopsis* (BS = 97, PP = 0.96). Type species of all genera are represented here except for *Tritoniopsis*. These include (type locality; sampling collection GenBank) *Duvaucelia manicata* (Deshayes, 1853) (both from the Western Mediterranean), *Marianina rosea* (Pruvot-Fol, 1930) (New Caledonia; Malaysia), *Marionia blainvillea* (Risso, 1818) (Marseille; Italian and Spanish coasts), *Tochuina gigantea* (Bergh, 1904) (Alaska; Washington State), *Tritonicula hamnerorum* (Gosliner and Ghiselin, 1987) (Gulf of Mexico; Bermuda), *Tritoniella belli* Eliot, 1907 (both from the Ross Sea). Within Tritoniidae, only the monotypic and elusive genera *Paratritonia* and *Tritonidoxa* were not included in our dataset. *Marionia* is monophyletic only in the ML and includes our newly sequenced *M. cucullata*, the type and additional species, and many unidentified ones available in GenBank. Also, we corroborate the recent results clustering *Tochuina nigritigris* (Valdés, Lundsten & Wilson, 2018) with *T. gigantea* (Korshunova and Martynov, 2020; Valdés et al., 2018). This is based on molecular data and shared morphological characters, i.e., absence of velar processes and presence of hundreds of tiny gills on the notal margins, characteristic features of the genus *Tochuina* (Marcus,

Table 3
Jukes-Cantor (JC69) intra- and interspecific genetic *p*-distances based on COI for *Tritonia* species from the Southern Hemisphere.

		1	2	3	4	5	6	7	8
1	***Tritonia* sp. 3**	0–0.3							
2	***Tritonia odhneri* ZSM**	3.6–4.6	0–0.6						
3	***Tritonia 'challengeriana'* sp. 1**	18–18.8	18–19.1	–					
4	***Tritonia 'challengeriana'* sp. 2**	20.1–20.9	19.3–21.5	14	–				
5	***Tritonia challengeriana***	20.1–22.1	18.9–21.5	11.7–13.1	4.5–5.3	0–1.2			
6	***Tritonia dantarti***	16–21.8	17.5–24	15.9–17.2	14.3–15.5	12.5–17	0.3–1.2		
7	***Tritonia vorax***	16.4–18.8	17.4–21.1	13.6–18	14.8–16.3	14.2–17.5	2.5–7.4	0–4	
8	***Tritonia antarctica***	18.4–22.1	19–23.8	15.4–17.6	14.6–16.6	14.4–20.9	12.3–16.5	11.4–25.4	0–9.5

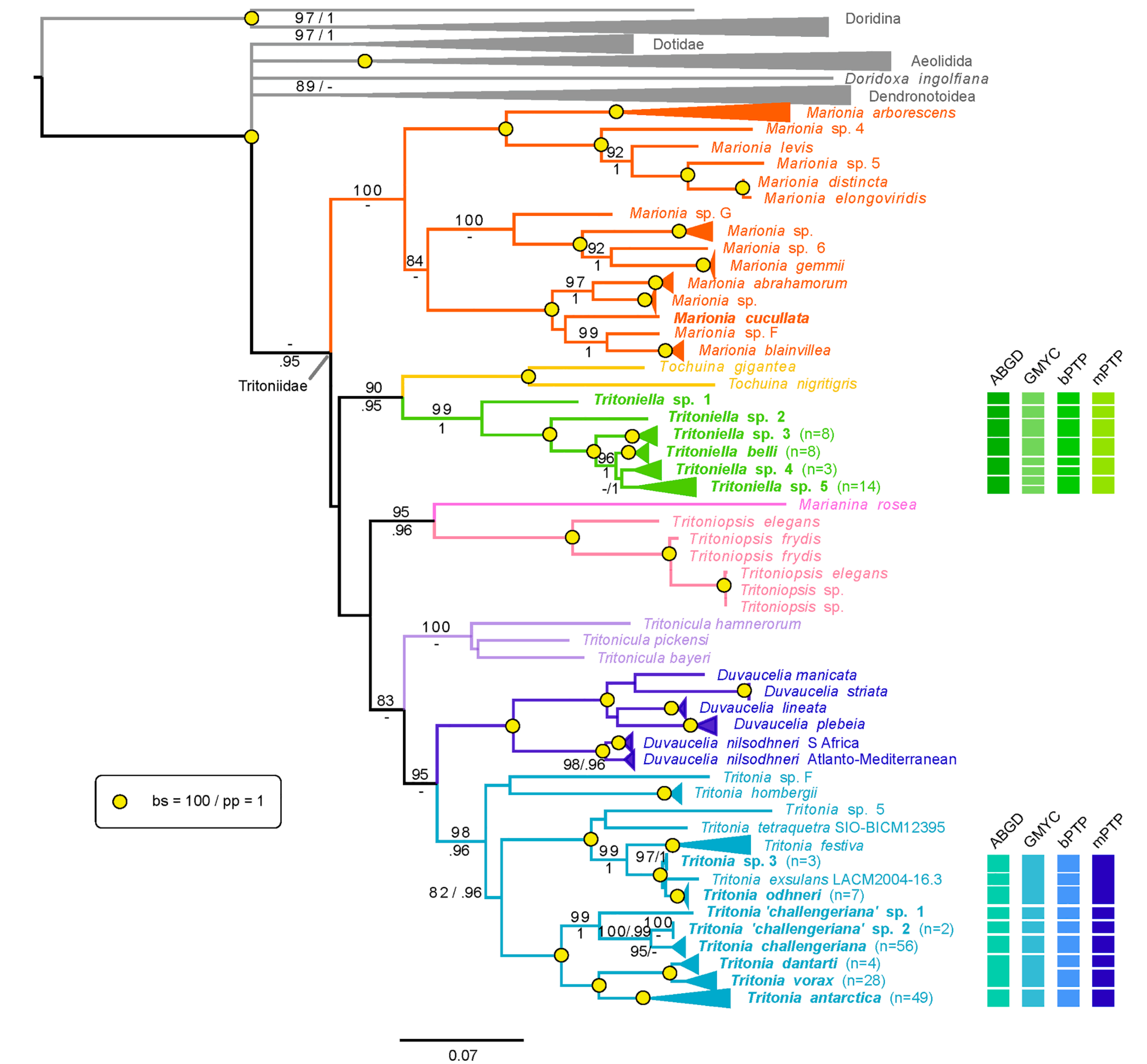

Fig. 3. Phylogeny of Tritoniidae based on the concatenated ML analysis rooted with species of Doridina depicted in grey. Additional outgroup taxa include Aeolidida, Dendronotoidea, and *Doridoxa* are also depicted in grey. Tritoniidae genera are independently coloured, branches with several replicates for each species hypothesis were collapsed for clarity, our sequenced species are highlighted in bold with the number of sequenced specimens shown in brackets. Node support values include bootstrap support (BS) and posterior probabilities (PP) values on the left/top and right/bottom sides, respectively. Maximum supported nodes for both analyses are depicted with a yellow circle. Species delimitation tests (ABGD, GMYC, bPTP, mPTP) for the Southern Hemisphere species of *Tritonia* and *Tritoniella* species depicted in bars on the right side. Scale bar indicates substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1983).

3.2. Species discovery

A wide taxon sampling of specimens belonging to *Tritoniella,* from its entire distributional range, was available and demonstrated several undescribed new species, all restricted to Antarctic and Sub-Antarctic regions. Almost all SDT based on COI split the species complex of *Tritoniella belli* into six species hypotheses according to the following geographic regions (Fig. 3): (A) *Tritoniella* sp. 1 – a singleton found on Burdwood Bank East (south from the Falkland Islands) at 560 m depth, (B) *Tritoniella* sp. 2 – a singleton from King George Island at 30 m depth, (C) *Tritoniella* sp. 3 – Shag Rocks, South Georgia, and Discovery Bank (north and east of Scotia Sea) from 119 to 751 m depth, (D) *Tritoniella belli* – Ross and Weddell seas from 80 to 318 m depth, (E) *Tritoniella* sp. 4 – the South Orkney Islands and King George Island, 20–278 m depth, and (F) *Tritoniella* sp. 5 – South Sandwich Islands, Bransfield Strait, and King George Island, 20–420 m depth. The SDT results based on 16S mainly recover four species and clustered the specimens distributed in higher latitudes belonging to *T. belli* with the species hypotheses sp. 4 and sp. 5.

Species previously ascribed to *Tritonia* now split into three genera. First, a small clade composed of the newly erected genus *Tritonicula* is comprised of *T. hamnerorum* and *T. bayeri* from the Caribbean Sea

(Goodheart et al., 2016), and *T. pickensi*, from Baja California (E.B.-R. Marcus and Marcus, 1967). This genus is the sister taxon to the remaining clades. A second clade is composed of species from the Mediterranean, Eastern Atlantic, and South Africa now under the genus *Duvaucelia* (*D. manicata, D. striata, D. lineata, D. plebeia*, and *D. nilsodhneri*), which is sister to a larger clade including Eastern Pacific and Southern Hemisphere species. Finally, the Eastern Pacific clade includes the currently recognized *Tritonia* species *T. exsulans*, *T. festiva*, *T. tetraquetra*, an undescribed Peruvian *Tritonia* sp. 3, and the Chilean-Argentinian *T. odhneri*. In the Southern Hemisphere clade, the South American *T. challengeriana* is a complex of three species sister to a clade formed by the Sub-Antarctic species *T. dantarti* plus *T. vorax* (BS = 100, PP = 1) and the Antarctic specimens of '*T. challengeriana*' (BS = 100, PP = 1), which were once recognized as the distinctive species *T. antarctica* (Wägele, 1995), which is henceforth resurrected. Among these above-mentioned species, all SDT for both COI and 16S agree on the species composition of *T. odhneri* from Aysén and Magallanes channels in Southern Chile, 5–35 m depth, and the Peruvian *Tritonia* sp. 3 from 5 to 20 m depth. The same agreement occurs for *T. antarctica* from the Scotia Sea, Antarctic Peninsula, Ross, and Weddell Seas, 62–789 m depth (Rossi et al., 2021). We found evidence of the presence of at least three species within the complex *T. challengeriana*, (A) *Tritonia* sp. 1 – a singleton from Isla Castillo in Chile at 28 m depth, (B) *Tritonia* sp. 2 – two specimens from Burdwood Bank East at 109 m depth, and (C) *T. challengeriana* – numerous specimens along the Southern Chilean coast to Tierra del Fuego, 1–30 m depth. Incongruence among SDT based on COI or 16S was only found in the identity of *T. dantarti* (from Bouvet Island at 130 m depth), recognized as *T. vorax* in the 16S dataset present in Tierra del Fuego, Falkland Islands, Burdwood Bank, and South Georgia at 3–444 m depth.

4. Discussion

This study benefited from an unprecedented taxon representation of Tritoniidae species from remote areas in the Southern Ocean and nearby regions, including material from Sub-Antarctic Islands and South America. Overall, we recovered the monophyly of Tritoniidae only in the BI analysis and the monophyly of the genera represented within. The genus *Tritonia sensu lato* is rendered monophyletic once the latest taxonomic restructuring was applied (Korshunova and Martynov, 2020), i.e. the restatement of *Duvaucelia* and the newly erected genus *Tritonicula*, as well as the inclusion of *Tritonia nigritigris* into the genus *Tochuina*. *Tritonia sensu lato* included a well-supported clade with representatives from the Atlantic, Pacific, and Southern oceans and a clade mainly formed by both Central Eastern Pacific and Western Atlantic oceans (composed by *T. bayeri*, *T. hamnerorum*, *T. pickensi*). The latter group, together with *T. wellsi* Er. Marcus, 1961 and *T. khaleesi* Silva, Azevedo & Matthews-Cascon, 2014, is characterized by a rather slender body, a white diffuse net of white pigmentation on the notum, a narrow radula with few lateral teeth, and a fleshy extension on the rhinophoral sheath (Silva et al., 2014; Gosliner and Ghiselin, 1987; E. Marcus and Marcus, 1967). Previous work has also recovered the genus *Tritonia* as polyphyletic in phylogenies generated by different morphological data (Bertsch et al., 2009), Sanger sequencing (Hulett et al., 2015; Korshunova and Martynov, 2020; Valdés et al., 2018), and transcriptomes (Goodheart, 2017), when these species were included. Since the type of *Tritonia* belongs to the Pacific + Southern Ocean clade, the Central Western Atlantic and Eastern Pacific clade may be better characterized as a different genus, for which *Tritonicula* was recently erected (Korshunova and Martynov, 2020). Therefore, we propose *Tritonicula khaleesi* comb. nov. to reflect the current taxonomy and recommend including it in future molecular assessments (as also mentioned in Korshunova and Martynov, 2020). Likewise, our analyses also support the recent restatement of *Duvaucelia* to comprise the species present in a clade formed by Mediterranean and Eastern Atlantic representatives. The latter genus is defined by relatively small size, a non-bilobed, small oral veil and a moderate number of lateral teeth (as also commented in Korshunova and Martynov, 2020). An interesting model system of speciation to study nudibranch evolution and distributional history within *Tritonia* is the complex of species composed of the orange *Tritonia* (*T. tetraquetra*, *T. odhneri*, *T. exsulans*). Chilean and Argentinian populations of the warm-temperate adapted *T. odhneri* may be geographically separated but still appear morphologically identical (Schrödl, 2009). *Tritonia odhneri* is considered here a single species with (probably unidirectional) gene flow around Cape Horn, or undergoing speciation, together with its northern Pacific twin species *T. tetraquetra* and *T. exsulans* and our undescribed *Tritonia* sp. 3 from Peru (which externally mostly resembles *T. festiva*). The similarly coloured species *Marionia cucullata* distributed from Florida to Brazil (Muniain, 1998) presents a characteristic notal reticulation and it was previously confused with *T. odhneri*, found in northern Argentina. The species was sequenced here and its distinct identity confirmed as not belonging to *Tritonia*, thus resulting in its absence from Magellanic waters. Still, a comparative revision of southern Atlantic *Marionia* species, especially *M. tedi*, is required. Remarkably, *Marionia* formed two sister groups, concordant with other studies (Almón et al., 2018; Silva et al., 2019), thus including the molecular data for the type species of *Marioniopsis* Odhner, 1934 and *Paratritonia* in a comparative morpho-anatomical analysis is urged.

Regarding the southern tritoniids, we provide evidence for 13 + species compared to five traditionally recognised ones. Analyses with broader taxon sampling reject the monophyly of *Tritoniella belli* and *Tritonia challengeriana,* demonstrating pseudocryptic speciation that has misled morphology-based taxonomy (Schrödl, 2009; Wägele, 1995, 1989a). Such radiations of Antarctic *Tritoniella* and Southern Ocean *Tritonia* species support the hypothesis of the Antarctic biodiversity pump (*sensu* Rogers, 2007), and sustains habitat fragmentation during glacial maxima as the driving force towards allopatric speciation. This pattern is particularly evidenced in species with intracapsular development (Bryan et al., 1998; Woods and Moran, 2008), as for the ubiquitous and sympatric nudibranch *Doris kerguelenensis sensu lato* (Bergh, 1884) (Moles et al., 2017a; Wilson et al., 2013, 2009) and other heterobranch relatives (Moles et al., 2021, 2019). Our phylogeny supports two independent invasions of tritoniids into cold Antarctic waters by the genera *Tritoniella* and *Tritonia*. *Tritoniella* is confirmed as a valid genus characterized by a conspicuous dorsal ridge and the presence of lateral flaps in the dorsum (instead of ramified gills as in *Tritonia*; Wägele, 1989a), for which we provide molecular evidence for many species hypotheses. These are also supported by our preliminary morpho-anatomical descriptions, although formal species descriptions will be presented elsewhere.

Interestingly, the sister taxon to *Tritoniella* is the deep-sea genus *Tochuina,* with three species now known from the North Pacific Ocean (Behrens and Hermosillo, 2005; Korshunova and Martynov, 2020; Valdés et al., 2018). Bipolar distributions have been described in many lineages of molluscs (Crame, 1993), including nudibranchs (Moles et al., 2017b). In cooler times through Earth's history, a prior cosmopolitan species may have undergone vicariant isolation in high latitudes during interglacial periods (Allcock and Griffiths, 2015). Each of the resulting species may have ultimately become extinct or persisted and found refuge in deep waters at lower latitudes (Stepanjants et al., 2006). *Tochuina* + *Tritoniella* illustrate this pattern of distribution with lower latitude species commonly found in deeper waters than the high latitude ones, i.e. *Tochuina nigritigris* found at 1733 m depth 37°N of the Pacific Ocean (Valdés et al., 2018) and *Tritoniella* sp. 1 found at 570 m depth 54.5°S on Burdwood Bank herein. *Tritoniella belli* seems restricted to the Weddell and Ross Seas, a distribution probably explained due to the Weddell-Ross seaway during the Miocene (Linse et al., 2006), as described for Antarctophilinidae heterobranchs (see Moles et al., 2021). The other *Tritoniella* species seem to be endemic to distant Sub-Antarctic Islands, although the abundance of singletons found in this study highlights the difficulties collecting in these remote localities. For

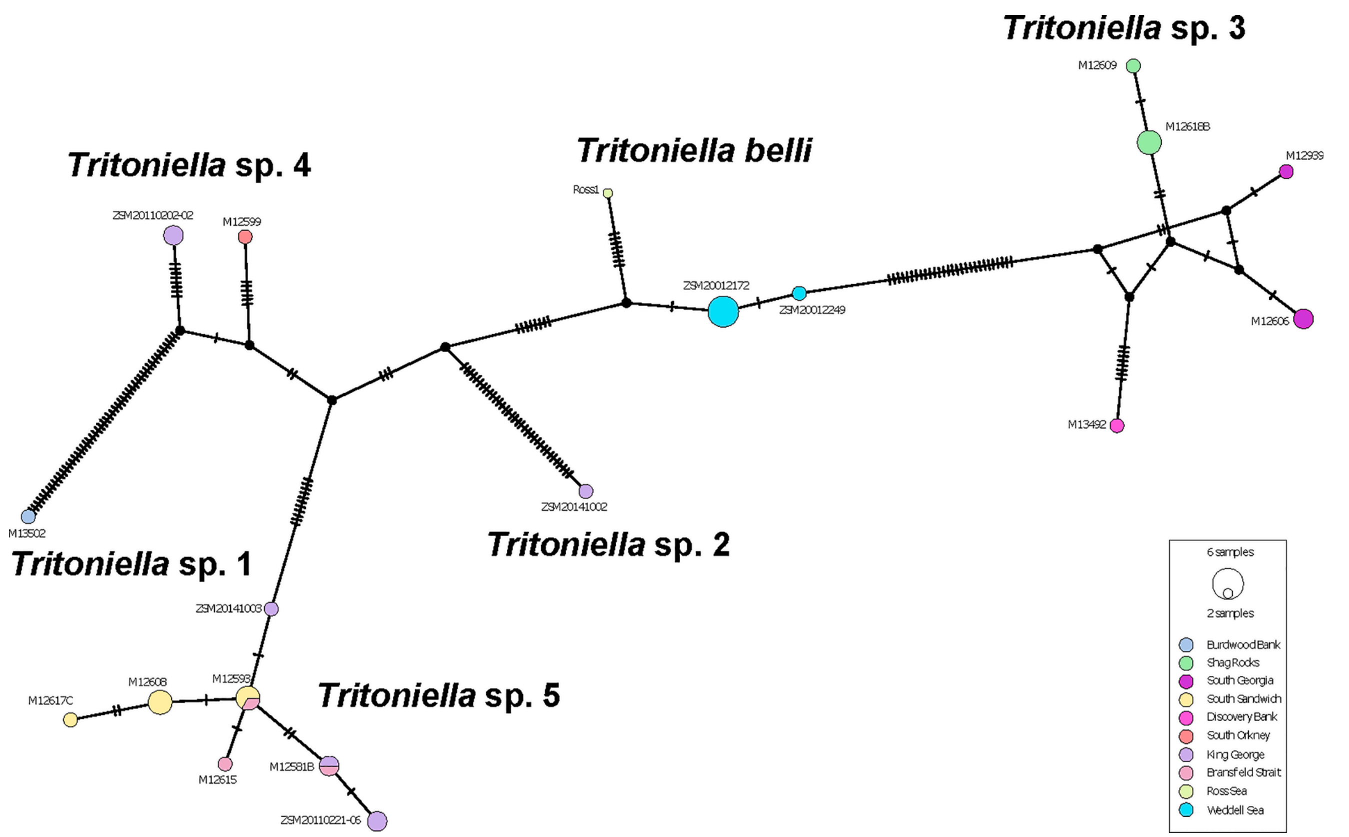

Fig. 4. Haplotype network reconstructed with PopART for the COI marker of species of *Tritoniella* from the Southern Hemisphere. Codes refer to voucher numbers from this study and two specimens of *T. belli* from the Ross Sea obtained from GenBank (Heimeier et al., 2010; Shields et al., 2009). Crossing lines between haplotypes indicate mutational steps.

instance, a well-sampled station in King George Island unravels three species living in sympatry in shallow waters. Some species are more widespread, e.g. *Tritoniella* sp. 5, which shows shared haplotypes between the Bransfield Strait and the South Sandwich Islands (see Fig. 4), a dispersal pattern probably favoured by the clockwise Weddell Gyre (Orsi et al., 1993). Overall, the species distribution and patchiness found here support the hypothesis of historic isolation and local radiation leading to speciation processes in *Tritoniella*.

Complex distribution patterns within Southern Ocean *Tritonia* species suggest a single invasion event of Antarctica from Sub-Antarctic regions with isolation leading to speciation and well-defined population structure (see Fig. 5). During repeated glaciation cycles, molluscs from Magellanic and Antarctic waters have been subjected to alternating periods of population decrease, fragmentation or extinction, and subsequent range expansion (González-Wevar et al., 2013; Strugnell et al., 2012; Wilson et al., 2013). Among those, *T. challengeriana,* which was once considered widespread, is now understood to be restricted to Chilean and Argentinian waters, with its southernmost record found in the Beagle Channel (Tierra del Fuego). Its closest relatives, which include *Tritonia* sp. 2, found in shallow waters in the Chilean locality of Aysén and *Tritonia* sp. 1, found at 110 m depth in the Burdwood Bank; both species are in the process of being described elsewhere. Whether the morphologically identical conspecifics *T. australis* or *T.* (*Microlophus*) *poirieri* are valid species will require additional taxonomical investigation, although are presently regarded as junior synonyms to *T. challengeriana* (Schrödl, 2003). The sister clade to the *T. challengeriana* species complex is composed of three species found in higher latitudes. *Tritonia vorax* is now found for the first time at shallow water depths in the Chilean fiords down to deeper waters on the Burdwood Bank and South Georgia thus importantly, crossing the Antarctic Convergence (Schrödl, 1999). This species is one of the easier species to discern due to the extremely large pharynx and jaw size, and externally by the typical colour pattern (Schrödl, 2009; Wägele, 1995). The closest relative to *T. vorax* is *T. dantarti,* which seems restricted to remote Bouvet Island and also presents a heterogeneously coloured notum (Ballesteros and Avila, 2006). Nevertheless, colouration from translucent white to bright orange may be artefactual due to dietary pigments acquired by the southern *Tritonia* slugs (Rossi et al., 2021). Even if not all SDT clearly distinguish among both species, we consider both species valid due to most molecular results, morphological distinctiveness (especially foregut anatomy Rossi et al., 2021), and biogeography. Finally, *T. antarctica* is now considered a valid species with an apparent circumpolar distribution, but still encompassing much population structure. Although the Weddell and Ross Seas populations seem genetically more closely related to each other, additional structured populations are found across the Antarctic Peninsula and Scotia Arc. Ultimately, intraspecific variability described in morphological descriptions (Schrödl, 2003; Wägele, 1995) will now have to be reconsidered in light of the genetic structure here described. Among these characters, the living colouration, body size, notal structure, jaw size and structure, radula formula, the cuticular lining of the stomach, and the shape of the penial papilla will have to be taken into consideration.

In this study, we evaluated the evolutionary history of Southern Ocean and South American Tritoniidae with increased taxon sampling encompassing species of *Tritonia* and *Tritoniella*. Our phylogeny supports the current systematic knowledge on Tritoniidae by providing ample data from remote areas of the Southern Hemisphere. Phylogenetic analyses demonstrated the evolution of southern tritoniids is more complex than expected with 13 species discovered from the five traditional taxa. New species of *Tritonia* and *Tritoniella* are in process of being described elsewhere with a thorough taxonomical reassessment of type taxa and literature. Biodiversity and biogeographical patterns show a

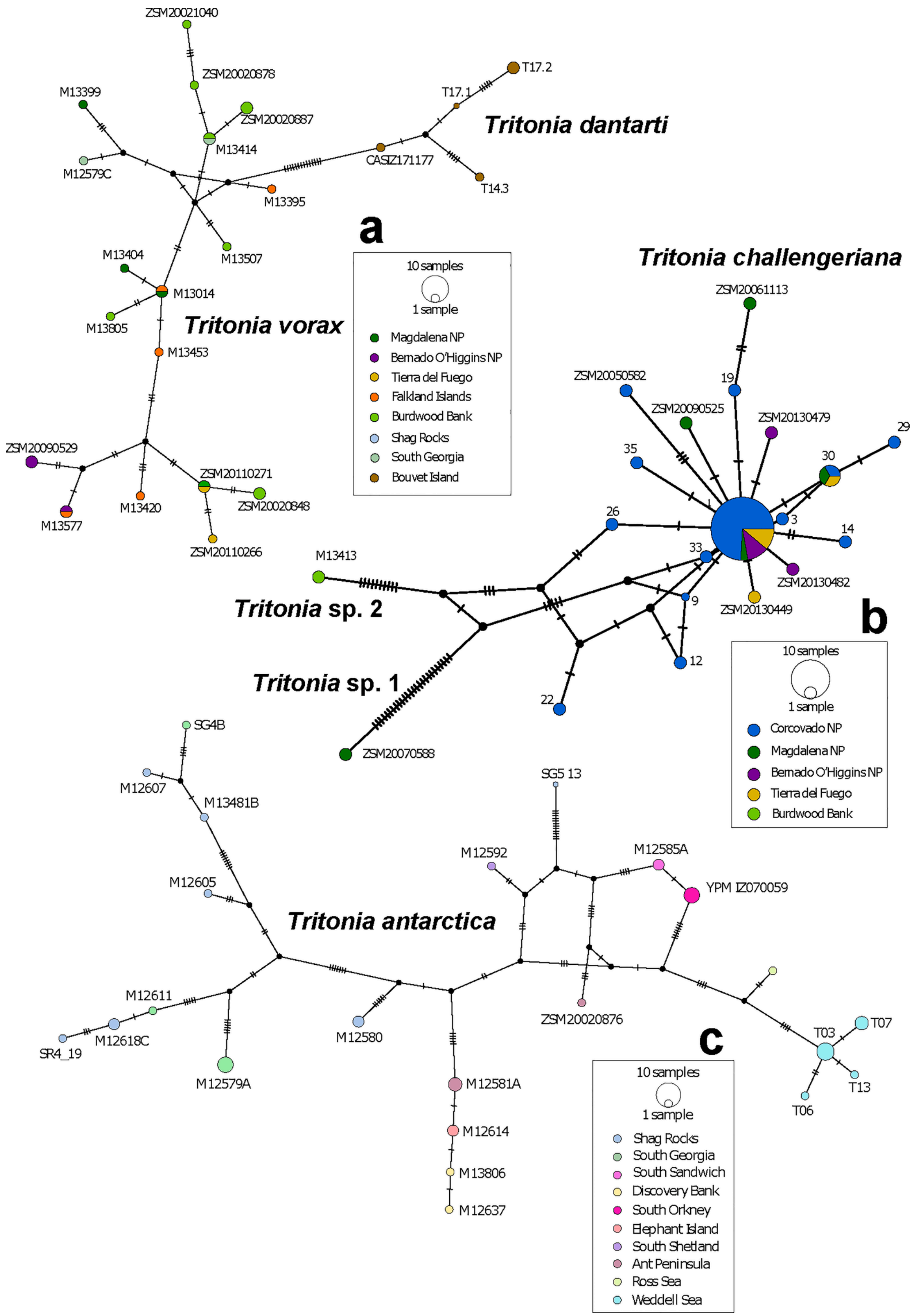

Fig. 5. Haplotype network reconstructed with PopART for the COI marker of species of *Tritonia* from the Southern Hemisphere. Codes refer to voucher numbers from this study and specimens of *T. dantarti* from Bouvet Island and *T. antarctica* from the Weddell and Ross Seas obtained from GenBank (Pola and Gosliner, 2010; Rossi et al., 2021; Shields et al., 2009). Crossing lines between haplotypes indicate mutational steps.

more restricted distribution and endemicity likely driven by specific historical and ecological processes. Our contribution highlights the necessity of inventory work, essential to monitor and evaluate the consequences of the rapid environmental change on sensitive Southern Ocean taxa. In light of this study, future works assessing the systematics, taxonomy, and ecology of these species are encouraged to better comprehend this lineage of sea slugs with many interesting lines of research such as chemical ecology, neurophysiology, and behaviour.

CRediT authorship contribution statement

J.M. and M.S. work in the conceptualization. Y.H., V.P., M.S., and N.G.W. collected specimens. M.I.B. and V.P. performed lab work. J.M. and N.G.W. performed analyses. M.S. contributed to resources. J.M. wrote the original draft. J.M., V.P., N.G.W., and M.S. reviewed and edited the final version of the manuscript.

Acknowledgements

We are indebted to the crew and participants of the RV Polarstern (AWI, Germany) during the ANT XVII/3, ANT XIX/5, and ANT-XXVIII/4, the RV Nathaniel B. Palmer (NSF, USA) during the NBP11-05 and NBP13-03 (National Science Foundation; ANT-1043749), and the Yepayek (CONAF, Chile) during the South Chile Fjord Expedition of 2006. For kind help in the field, we would like to thank the following friends: Roland Anton, Liz Atwood, Günter Försterra, Sebastian Gigglinger, Vreni Häussermann, Katrin Linse (BAS), Roland Melzer (ZSM), Sandra Millen, Martin Rauschert, Nicola Reiff, Greg Rouse, Klaus Salger, Xavier Sanchez, Dirk Schories, and Philippe Willenz. The Victor Hensen Magellan Campaign 1994 coordinated by Wolf Arntz (AWI) provided the material from the Beagle Channel. Special thanks go to Philippe Bouchet (BNHN, France), Matthias Glaubrecht (ZMB, Germany), David Reid (BMNH, UK), Anders Warén (SMNH, Sweden), Alejandro Tablado (MACN, Argentina), Fabián Tricárico (MACN), Greg Rouse, and Charlotte Seid (BIC-SIO, USA). We appreciate Dirk Schories for kindly providing photographs of live specimens and Joan Giménez for helping with the map design. Software and literature were supported by the Faculty of Arts and Sciences, Harvard University, and Gonzalo Giribet. Three anonymous reviewers provided comments that helped improve this paper. This project was supported by the GeoBio-Center and funded by the DFG-SPP1158 to MS (SCHR667/15-1). This study was part of the PhD of VP funded by CNPq-Brazil and DAAD-Germany. JM postdoctoral fellowship was supported by the Alexander von Humboldt Foundation (Germany).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2021.107209.

References

Allcock, A.L., Griffiths, H.J., 2015. Bipolarity, in: Biogeographic Atlas of the Southern Ocean. pp. 431–436.

Almón, B., Pérez, J., Caballer, M., 2018. Expect the unexpected: A new large species of *Marionia* (Heterobranchia: Nudibranchia: Tritoniidae) from western Europe. Invertebr. Syst. 32, 890–906. https://doi.org/10.1071/IS17073.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipmann, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Ballesteros, M., Avila, C., 2006. A new tritoniid species (Mollusca: Opisthobranchia) from Bouvet Island. Polar Biol. 29, 128–136. https://doi.org/10.1007/s00300-005-0059-4.

Behrens, D.W., Hermosillo, A., 2005. Eastern Pacific Nudibranchs: A guide to the Opisthobranchs from Alaska to Central America. Sea Challengers, Monterey, CA.

Bergh, L.S.R., 1904. Malacologische Untersuchungen. Reis. im Arch. der Philipp. von Dr. Carl Gottfried Semper. Zweiter Theil. Wissenschaftliche Result. 9, 26–28.

Bergh, L.S.R., 1898. Die Opisthobranchier der Sammlung Plate. Zool. Jahrbücher Suppl. 4, 481–582.

Bergh, R., 1884. Report on the Nudibranchiata collected by H.M.S. Challenger, during the years 1873–1876, in: Thompson, C.V., Murray, J. (Eds.), Report on the Scientific Results of the Voyage of H.M.S. Challenger during the Years 1873–76 under the Command of Captain George S. Nares, R.N., F.R.S., and Captain Frank Tourle Thomson, R.N. Zoology. Vol. 10. Stationery Office, London, p. 154.

Bertsch, H., Gosliner, T., Valdés, Á., 2009. A new species of tritoniid nudibranch, the first found feeding on a zoanthid anthozoan, with a preliminary phylogeny of the Tritoniidae. Proc. Calif. Acad. Sci. 60, 431–446.

Brattström, H., Johanssen, A., 1983. Ecological and regional zoogeography of the marine benthic fauna of Chile. Report no. 49 of the Lund University Chile Expedition 1948–49. Sarsia 68, 289–339.

Bryan, P.J., McClintock, J.B., Baker, B.J., 1998. Population biology and antipredator defenses of the shallow-water Antarctic nudibranch *Tritoniella belli*. Mar. Biol. 132, 259–265. https://doi.org/10.1007/s002270050391.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1659.

Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G., Gray, M.R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Aust. J. Zool. 46, 419–437. https://doi.org/10.1071/ZO98048.

Crame, J.A., 1993. Bipolar molluscs and their evolutionary implications. J. Biogeogr. 20, 145–161.

Deshayes, G.P., 1853. Traité élémentaire de conchyliologie: avec les applications de cette science à la géologie.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214. https://doi.org/10.1186/1471-2148-7-214.

Eliot, C., 1907a. Mollusca IV: Nudibranchiata. National Antarctic Expedition 1901–1904. Nat. Hist. 2, 1–28.

Eliot, C., 1907b. Nudibranchs from New Zealand and the Falkland Islands. J. Molluscan Stud. 7, 327–361.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299. https://doi.org/10.1371/journal.pone.0013102.

Fujisawa, T., Barraclough, T.G., 2013. Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent Approach: A revised method and evaluation on simulated data sets. Syst. Biol. 62, 707–724. https://doi.org/10.1093/sysbio/syt033.

González-Wevar, C.A., Saucède, T., Morley, S.A., Chown, S.L., Poulin, E., 2013. Extinction and recolonization of maritime Antarctica in the limpet *Nacella concinna* (Strebel, 1908) during the last glacial cycle: toward a model of Quaternary biogeography in shallow Antarctic invertebrates. Mol. Ecol. 22, 5221–5236. https://doi.org/10.1111/mec.12465.

Goodheart, J.A., 2017. Insights into the systematics, phylogeny, and evolution of Cladobranchia (Gastropoda: Heterobranchia). Am. Malacol. Bull. 35, 73–81.

Goodheart, J.A., Ellingson, R.A., Vital, X.G., Filho, H.C.G., Mccarthy, J.B., Medrano, S.M., Bhave, V.J., García-Méndez, K., Jiménez, L.M., López, G., Hoover, C.A., Awbrey, J.D., Jesus, J.M.D., Gowacki, W., Krug, P.J., Valdés, Á., 2016. Identification guide to the heterobranch sea slugs (Mollusca: Gastropoda) from Bocas del Toro, Panama. Mar. Biodivers. Rec. 9, 56. https://doi.org/10.1186/s41200-016-0048-z.

Gosliner, T., Ghiselin, M., 1987. A new species of *Tritonia* (Opisthobranchia: Gastropoda) from the Caribbean Sea. Bull. Mar. Sci. 40, 428–436.

Gosliner, T.M., Valdés, Á., Behrens, D.W., 2018. Nudibranch and Sea Slug Identification Indo-Pacific, 2nd ed. New World Publications Inc, Jacksonville, Florida.

Heimeier, D., Lavery, S., Sewell, M.A., 2010. Using DNA barcoding and phylogenetics to identify Antarctic invertebrate larvae: Lessons from a large scale study. Mar. Genomics 3, 165–177.

Hulett, R.E., Mahguib, J., Gosliner, T.M., Valdés, Á., 2015. Molecular evaluation of the phylogenetic position of the enigmatic species *Trivettea papalotla* (Bertsch) (Mollusca: Nudibranchia). Invertebr. Syst. 29, 215–222.

Inkscape Project, 2020. Inkscape [WWW Document]. https://inkscape.org. URL https://inkscape.org.

Jörger, K.M., Stöger, I., Kano, Y., Fukuda, H., Knebelsberger, T., Schrödl, M., 2010. On the origin of Acochlidia and other enigmatic euthyneuran gastropods, with implications for the systematics of Heterobranchia. BMC Evol. Biol. 10, 323. https://doi.org/10.1186/1471-2148-10-323.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589. https://doi.org/10.1038/nmeth.4285.

Kapli, P., Lutteropp, S., Zhang, J., Kobert, K., Pavlidis, P., Stamatakis, A., Flouri, T., 2017. Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. Bioinformatics 33, 1630–1638. https://doi.org/10.1093/bioinformatics/btx025.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A.S., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.

Korshunova, T., Martynov, A.V., 2020. Consolidated data on the phylogeny and evolution of the family Tritoniidae (Gastropoda: Nudibranchia) contribute to genera reassessment and clarify the taxonomic status of the neuroscience models. PLoS ONE 15, e0242103. https://doi.org/10.1371/journal.pone.0242103.

Leigh, J.W., Bryant, D., 2015. POPART: Full-feature software for haplotype network construction. Methods Ecol. Evol. 6, 1110–1116. https://doi.org/10.1111/2041-210X.12410.

Linse, K., Griffiths, H.J., Barnes, D.K.A.A., Clarke, A., 2006. Biodiversity and biogeography of Antarctic and sub-Antarctic mollusca. Deep. Res. Part II Top. Stud. Oceanogr. 53, 985–1008. https://doi.org/10.1016/j.dsr2.2006.05.003.

Marcus, E.-B.-R., 1983. The Western Atlantic Tritoniidae. Bol. Zool. Univ. São Paulo 6, 177–214.

Marcus, E.-B.-R., Marcus, E., 1967a. Some opisthobranchs from Sapelo Island, Georgia, U.S.A. Malacologia 6, 199–222.

Marcus, E.R., 1959. Lamellariacea und Opisthobranchia, in: Reports of the Lund University Chile Expedition 1948-1949. The Lunds Universitets. Arsskrift. N. F., pp. 69–71.

Marcus, E.v., Marcus, E.r., 1969. Opisthobranchian and Lamellarian gastropods collected by the "Vema". Am. Museum Novit. 2368, 1–34.

Marcus, E., Marcus, Er., 1967b. American opisthobranch mollusks. Stud. Trop. Oceanogr. 6, 1–256.

Martens, E. von, Pfeffer, G., 1886. Die Mollusken von Sud-Georgien nach der Ausbeute der Deutschen Station, in: Jahrbuch Der Hamburgischen Wissenschaftlichen Anstalten. Hamburg, pp. 65–135.

Miller, M.A., Schwartz, T., Pickett, B.E., He, S., Klem, E.B., Scheuermann, R.H., Passarotti, M., Kaufman, S., O'Leary, M.A., 2015. A RESTful API for access to phylogenetic tools via the CIPRES science gateway. Evol. Bioinforma. 11, 43–48. https://doi.org/10.4137/EBO.S21501.

Minh, B.Q., Nguyen, M.A.T., von Haeseler, A., 2013. Ultrafast approximation for phylogenetic bootstrap. Mol. Biol. Evol. 30, 1188–1195. https://doi.org/10.1093/molbev/mst024.

Moles, J., Avila, C., Malaquias, M.A.E., 2019. Unmasking Antarctic mollusc lineages: novel evidence from philinoid snails (Gastropoda: Cephalaspidea). Cladistics 35, 487–513. https://doi.org/10.1111/cla.12364.

Moles, J., Derkarabetian, S., Schiaparelli, S., Schrödl, M., Troncoso, J.S., Wilson, N.G., Giribet, G., 2021. An approach using ddRADseq and machine learning for understanding speciation in Antarctic Antarctophilinidae gastropods. Sci. Rep. 11, 8473. https://doi.org/10.1038/s41598-021-87244-5.

Moles, J., Wägele, H., Cutignano, A., Fontana, A., Ballesteros, M., Avila, C., 2017a. Giant embryos and hatchlings of Antarctic nudibranchs (Mollusca: Gastropoda: Heterobranchia). Mar. Biol. 164, 114. https://doi.org/10.1007/s00227-017-3143-8.

Moles, J., Wägele, H., Uhl, G., Avila, C., 2017b. Bipolarity in sea slugs: a new species of *Doridunculus* (Mollusca: Nudibranchia: Onchidoridoidea) from Antarctica. Org. Divers. Evol. 17, 101–109. https://doi.org/10.1007/s13127-016-0309-z.

Muniain, C., 1998. Moluscos opistobranquios de Argentina: revisión taxonómica y relación de ecología química en algunas especies patagónicas. Universidad de Oviedo, Spain.

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274. https://doi.org/10.1093/molbev/msu300.

Odhner, N.H.J., 1926. Die Opisthobranchien. Furth. Zool. Results Swedish Antarct. Exped. 1901–1903 (2), 1–100.

Orsi, A.H., Nowlin Jr, W.D., Whitworth III, T., 1993. On the circulation and stratification of the Weddell Gyre. Deep Sea Res. Part I Oceanogr. Res. Pap. 40, 169–203.

Pola, M., Gosliner, T.M., 2010. The first molecular phylogeny of cladobranchian opisthobranchs (Mollusca, Gastropoda, Nudibranchia). Mol. Phylogenet. Evol. 56, 931–941. https://doi.org/10.1016/j.ympev.2010.05.003.

Pruvot-Fol, A., 1930. Diagnoses provisoires (incomplètes) des espèces nouvelles et liste provisoire des mollusques nudibranches recueillis par Mme A. Pruvot-Fol en Nouvelle Cadédonie (Ile de Pins). Bull. du Muséum Natl. d'Histoire Nat. ser. 2 2, 229–233.

Puillandre, N., Lambert, A., Brouillet, S., Achaz, G., 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Mol. Ecol. 21, 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x.

Rambaut, A., 2014. Figtree, a graphical viewer of phylogenetic trees.

Risso, A., 1818. Mémoire sur quelques Gastropodes nouveaux, Nudibranches et Tectibranches observés dans la mer de Nice. J. Phys. Chim. d'Histoire Nat. des Arts 87, 368–377.

Rogers, A.D., 2007. Evolution and biodiversity of Antarctic organisms: a molecular perspective. Philos. Trans. R. Soc. B 362, 2191–2214. https://doi.org/10.1098/rstb.2006.1948.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M., Huelsenbeck, J., 2011. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Rossi, M.E., Avila, C., Moles, J., 2021. Orange is the new white: taxonomic revision of *Tritonia* species (Gastropoda: Nudibranchia) from the Weddell Sea and Bouvet Island. Polar Biol. 44, 559–573. https://doi.org/10.1007/s00300-021-02813-8.

Schrödl, M., 2009. Opisthobranchia - Sea slugs, in: Marine Benthic Fauna of Chilean Patagonia. pp. 505–542.

Schrödl, M., 2003. Sea slugs of Southern South America. ConchBooks, Hackenheim.

Schrödl, M., 1999. Zoogeographic relationships of Magellan Nudibranchia (Mollusca: Opisthobranchia) with particular reference to species from adjacent regions. Sci. Mar. 63, 409–416. https://doi.org/10.3989/scimar.1999.63s1409.

Schrödl, M., 1996. Nudibranchia and Sacoglossa of Chile: External morphology and distribution. Gayana Zool. 60, 17–62.

Shields, C.C., Marko, P.B., Woods, H.A., Moran, A.L., 2009. Nudibranchs in the Ross Sea, Antarctica: Lineage Diversity and divergence estimated using methods of molecular phylogenetics and sequence divergence. Clemson University, Clemson, South Carolina.

Silva, F.D.V., Herrero-Barrencua, A., Pola, M., Cervera, J.L., 2019. Description of a new species of *Marionia* (Gastropoda: Heterobranchia: Tritoniidae) from the Gulf of Guinea (eastern Atlantic Ocean). Bull. Mar. Sci. 95, 431–447. https://doi.org/10.5343/bms.2018.0096.

Silva, F.V., Azevedo, V.M., Matthews-Cascon, H., 2014. A new species of *Tritonia* (Opisthobranchia: Nudibranchia: Tritoniidae) from the tropical South Atlantic Ocean. J. Mar. Biol. Assoc. United Kingdom 94, 579–585. https://doi.org/10.1017/S0025315413001586.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651–670.

Stepanjants, S.D., Cortese, G., Kruglikova, S.B., Bjørklund, K.R., 2006. A review of bipolarity concepts: History and examples from Radiolaria and Medusozoa (Cnidaria). Mar. Biol. Res. 2, 200–241. https://doi.org/10.1080/17451000600781767.

Strugnell, J.M., Watts, P.C., Smith, P.J., Allcock, A.L., 2012. Persistent genetic signatures of historic climatic events in an Antarctic octopus. Mol. Ecol. 21, 2775–2787. https://doi.org/10.1111/j.1365-294X.2012.05572.x.

Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56, 564–577.

Valdés, Á., Lundsten, L., Wilson, N.G., 2018. Five new deep-sea species of nudibranchs (Gastropoda: Heterobranchia: Cladobranchia) from the Northeast Pacific. Zootaxa 4526, 401–443. https://doi.org/10.11646/zootaxa.4526.4.1.

Wägele, H., 1995. The morphology and taxonomy of the Antarctic species of *Tritonia* Cuvier, 1797 (Nudibranchia: Dendronotoidea). Zool. J. Linn. Soc. 113, 21–46.

Wägele, H., 1989a. On the anatomy and zoogeography of *Tritoniella belli* Eliot, 1907 (Opisthobranchia, Nudibranchia) and the synonymy of *Tritoniella sinuata* Eliot, 1907. Polar Biol. 9, 235–243.

Wägele, H., 1989b. Diet of some Antarctic nudibranchs (Gastropoda, Opisthobranchia, Nudibranchia). Mar. Biol. 100, 439–441. https://doi.org/10.1007/BF00394819.

Willows, A.O.D., 1976. Trigger neurons in the mollusk *Tritonia*. In: Neural Control of Locomotion. Springer, Boston, MA, pp. 327–349.

Wilson, N.G., Maschek, J.A., Baker, B.J., 2013. A species flock driven by predation? Secondary metabolites support diversification of slugs in Antarctica. PLoS ONE 8, e80277. https://doi.org/10.1371/journal.pone.0080277.

Wilson, N.G., Schrödl, M., Halanych, K.M., 2009. Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). Mol. Ecol. 18, 965–984. https://doi.org/10.1111/j.1365-294X.2008.04071.x.

Woods, H.A., Moran, A.L., 2008. Temperature-oxygen interactions in Antarctic nudibranch egg masses. J. Exp. Biol. 211, 798–804. https://doi.org/10.1242/jeb.014621.

WoRMS Editorial Board, 2020. World Register of Marine Species. Available from [WWW Document]. http//www.marinespecies.org VLIZ. https://doi.org/10.14284/170.

Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29, 2869–2876. https://doi.org/10.1093/bioinformatics/btt499.