Unveiling a new species of *Trapania* (Gastropoda: Nudibranchia: Goniodorididae) from the South-eastern Pacific using anatomical and molecular tools

Roberto A. Uribe[1] ⓘD, Alejandro Mendivil[2] ⓘD, Ruslan Pastor[3] ⓘD,

Dennis Atoche-Suclupe[1] ⓘD, Solange Aleman[4] ⓘD, Lorenzo Flores[1] ⓘD and

Fabiola Sepulveda[5] ⓘD

[1]Área de Macroalgas y Biodiversidad, Instituto del Mar del Perú – IMARPE, Av. La Ribera # 805, Huanchaco, La Libertad, Perú; [2]Laboratorio de Biología y Sistemática de Invertebrados Marinos (LaBSIM), Facultad de Ciencias Biológicas, Universidad Nacional Mayor de San Marcos, Lima, Perú; [3]Área Funcional de Investigación Marino Costera (AFIMC), Instituto del Mar del Perú – IMARPE, La Punta, Callao, Perú; [4]Laboratorio Costero de Tumbes, Instituto del Mar del Perú – IMARPE, Tumbes, Perú and [5]Laboratorio de Eco-Parasitología y Epidemiología Marina (LEPyEM), Instituto de Ciencias Naturales Alexander von Humboldt, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Antofagasta, Chile

Abstract

Morphological and molecular methods were used to describe a new species of *Trapania* Pruvot-Fol, 1931 from shallow water kelp forests on the north-central coast of Peru. The new species, *Trapania huarmeyana* sp. nov., is distinguished from other species along the Eastern Pacific by external morphological characters such as its translucent white body with brown stripes and small spots on the dorsum, blotches on the base of the extra-branchial processes, extra rhinophoral processes and gill branches. Internally, *T. huarmeyana* sp. nov. is distinguishable by several morphological characteristics of the radula, jaws and genital organs. Phylogenetic trees recovered using Bayesian Inference and Maximum Likelihood analysis of DNA sequences support its distinct status and clarify its relationship to other species from the Eastern Pacific. This new species constitutes the first record of *Trapania* from the Humboldt Current Ecosystem, contributing to our understanding of the distribution of the genus in the South-eastern Pacific.

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Introduction

The monophyletic genus *Trapania* Pruvot-Fol, 1931 is one of the 11 genera of the family Goniodorididae, and the largest in the subfamily Anculinae Pruvot-Fol, 1954 (Paz-Sedano *et al.*, 2024*a*). Members of this highly diverse genus are characterized by having an elongated body and a single pair of extra-rhinophoral and extra-branchial appendages, with a single lateral tooth on each side of the radula (Smirnoff *et al.*, 2022; Paz-Sedano *et al.*, 2024*a*). In recent years, many new species of *Trapania* have been described from different regions around the world, including the Indo-Pacific (Gosliner and Fahey, 2008; Smirnoff *et al.*, 2022), the Eastern Pacific (Gosliner and Fahey, 2008), the Caribbean Sea (Valdés, 2009), Western Africa (Edmunds, 2009), Argentina (Cetra and Roche, 2019) and Australia (Paz-Sedano *et al.*, 2024*b*). Recent studies have also led to the reassignment of certain species within the genus *Trapania*, particularly those found along the temperate coasts of the Eastern Atlantic and the Mediterranean Sea (Paz-Sedano *et al.*, 2022). These changes include the reclassification of *Trapania hispalensis* Cervera and García-Gómez, 1989, *Trapania lineata* Haefelfinger, 1960 and *Trapania pallida* Kress, 1968 as synonyms, and have resulted in the recognition of 55 valid *Trapania* species (MolluscaBase, 2024), highlighting the need for ongoing taxonomic revision within the genus.

Five species of *Trapania* have been previously described from the Eastern Pacific based on morphological data: *Trapania velox* (Cockerell, 1901) from California, *Trapania goslineri* Millen and Bertsch, 2000 from the Gulf of California, *Trapania goddardi* Hermosillo and Valdés, 2004 from Mexico, *Trapania inbiotica* Camacho-García and Ortea, 2000 from Costa Rica and *Trapania darwini* Gosliner and Fahey, 2008 from the Galapagos Islands. Externally, *T. darwini* most closely resembles *T. goslineri* and *T. velox* (Gosliner and Fahey, 2008), all three species sharing a white body with dark markings and yellow ornamentations, although they differ in the pattern and distribution of these pigments. The body of *T. goddardi* is covered by irregular small or large brown blotches with no yellow pigment (Hermosillo and Valdés, 2004), while *T. inbiotica* presents irregular red patches arranged over the entire dorsum, varying in shape and size (Camacho-García and Ortea, 2000). Despite significant differences in the external coloration between these species the diagnostic nature of the colour patterns should be

considered with caution because of intraspecific colour and morphological variability which direct the need to include molecular data to confirm taxonomic decisions (Padula et al., 2016; Paz-Sedano et al., 2017, 2022).

The coastal transition zone off northern Peru is characterized by the upwelling of nutrient-rich cool water, which dramatically increases biological diversity in benthic communities (Riascos et al., 2016). This zone includes habitat-forming species such as kelp forests, which are crucial for providing habitat complexity, food resources and shelter. Consequently, these forests support higher levels of biodiversity compared to adjacent ecosystems (Uribe et al., 2022). However, only 31 species of nudibranchs have been reported from the coast of Peru, indicating a relatively low species richness in this region compared to other areas of the continent (Grández et al., 2023). Notably, there are no recorded species of Trapania from Peruvian waters, and within the family Goniodorididae, only Okenia luna Millen, Schrödl, Vargas and Indacochea, 1994, has been reported from the central coast of Peru (Uribe et al., 2013). The available information on nudibranchs in the South-eastern Pacific suggests a lack of research effort in this area (Uribe and Pacheco, 2012), emphasizing the need for further taxonomic attention.

In the present study, we described a new species of Trapania from the north-central coast of Peru based on the combined use of molecular and morphological analyses, an integrative approach that has proven very useful for determining new species in little-explored areas such as the South-eastern Pacific (Ornelas-Gatdula et al., 2012; Hoover et al., 2017; Uribe et al., 2018; Valdés et al., 2018). We performed a detailed anatomical study in conjunction with phylogenetic and species delimitation analyses to verify the status of these specimens, including ecological information when available. Additionally, we used these results to perform a comparative analysis with other recorded Trapania from the Eastern Pacific.

Materials and methods

Sampling

Six specimens of an undescribed species of Trapania were collected in April 2023 from rocky subtidal sites in Rio Seco, Huarmey province, Ancash region, north-centre of Peru (−78.228080°, −9.807092°), at 8 m depth by SCUBA diving, during a monitoring of the biodiversity associated with the kelp forest Eisenia cokeri Howe, 1914. Samples were preserved in 95–99% ethanol and deposited at the Colección Científica del Instituto del Mar del Peru (IMARPE).

DNA extraction, amplification and sequencing

Three specimens were used for the molecular analysis. A small sample of foot tissue was cut for the DNA extraction. The DNA was isolated following a modified protocol based on Miller et al. (1988) involving treatment with sodium dodecyl sulphate, digestion with proteinase K, NaCl protein precipitation and subsequent ethanol precipitation. DNA was eluted in nuclease-free water and quantified in a spectrophotometer BioSpec-nano.

Partial sequences from two mitochondrial genes, 16S ribosomal RNA (16S) and cytochrome oxidase c subunit I (COI), and one nuclear gene, histone 3 (H3), were amplified by polymerase chain reaction (PCR). The primers used were 16Sar-L and 16Sbr-H for 16S (Palumbi, 1991), LCO1490 and HCO2198 for COI (Folmer et al., 1994) and HexAF and HexAR for H3 (Colgan et al., 1998) (Supplementary Table S1). Each PCR had a final volume of 35 μl including five standard units of GoTaq DNA polymerase (Promega, Madison, USA), 7 μl 5X PCR buffer,

5.6 μl MgCl$_2$ (25 mM), 2.1 μl BSA (10 mg ml^{-1}), 0.7 μl of deoxynucleotide triphosphate (dNTP) (10 mM), 10 pM of each primer and 3 μl template DNA. The optimized PCR conditions for 16S and H3 datasets started with an initial DNA denaturation at 94°C for 2 min; followed by 35–40 cycles of 30 s denaturation at 94°C, 30 s annealing at 50°C and 45 s–1 min extension at 72°C; with a final extension at 72°C for 10 min. For COI, we used an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation 94°C at 1 min, annealing 44°C at 30 s, extension 72°C at 1 min and final extension 72°C at 7 min. PCR amplifications were confirmed in a 1% agarose gel using GelRed™ fluorescent dye. PCR products were sent to Macrogen (http://www.macrogen.com) for purification and sequencing of both DNA forward and reverse strands. Complementary sequences were assembled and edited using ProSeq v2.9 (Filatov, 2002).

Sequence alignment

The sequences obtained in this study were aligned with the sequences of Trapania spp. retrieved from GenBank, along with seven other members of the Goniodorididae family and one member of each of the families Onchidorididae, Corambidae and Calycidordidae as outgroups (Table 1). The scientific names of all sequences used in this study were checked using MolluscaBase (2024). Each alignment was conducted using MAFFT v.7 (Katoh and Standley, 2013), using the L-INS-i iterative algorithm for 16S, and G-INS-I for COI and H3 following Paz-Sedano et al. (2024a). Ambiguous aligned regions (with internal gaps) of 16S were removed using relaxed parameters to allow half the gaps with the GBlock 0.91.1 program (Castresana, 2000; Talavera and Castresana, 2007; Lemoine et al., 2019). Finally, 16S, COI and H3 alignments were trimmed to 346, 594 and 328 base pairs, respectively. We concatenated the three aligned loci using Mesquite v.2.75 (Maddison and Maddison, 2011). Sampled specimens used, voucher numbers, GenBank accession numbers and specimen localities are listed in Table 1.

Phylogenetic analyses

Phylogenetic reconstruction was conducted for the concatenated dataset, using the Bayesian Inference (BI) performed in MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001) and the Maximum Likelihood (ML) performed in the webserver W-IQ-TREE v.2 (Trifinopoulos et al., 2016; http://iqtree.cibiv.univie.ac.at/). The evolutionary models were selected using jModelTest v.0.1.1 (Posada, 2008), based on the corrected Akaike information criterion (Akaike, 1974). The optimal models found by jModelTest were TPM2uf + I + G for 16S, GTR + I + G for COI and TrN + G for H3, but, because not all evolutionary models are available in MrBayes software, we replaced them with GTR + G + I (Ronquist and Huelsenbeck, 2003).

BI was conducted with the following parameters: nst = 6, rates = invgamma and run for 10,000,000 generations. Analyses included two runs of four chains, with sampling every 1000 generations. Support for nodes in the BI tree topology was obtained by posterior probability burn-in the initial 25% of samples. Results were visualized in TRACER v.1.7 (Drummond and Rambaut, 2007). For the ML analyses, we used the default options in the server W-IQ-TREE. The robustness of ML tree topology was assessed by bootstrap reiterations of the observed data 1000 times. The trees were visualized and edited in FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). Following Muff et al. (2022) we defined the following five categories for BI nodal support as: PP = 1: fully supported; PP = 0.99–0.90: strongly supported; PP = 0.89–0.80: moderated support; PP = 0.79–0.70: weakly supported; PP ≤ 0.69: not supported.

Table 1. Specimens used for molecular analysis, including locality data, GenBank accession numbers and museum voucher numbers

Species	Locality	Voucher	GenBank accession No.		
			16S	*COI*	*H3*
Calycidordidae					
Diaphorodoris mitsuii (Baba, 1938)	Philippines	CASIZ 185986	KP340310	KP340406	KP340424
Corambidae					
Corambe obscura (A. E. Verrill, 1870)	New Hampshire, USA	CASIZ 183942	KP340303	KP340399	KP340419
Onchidorididae					
Onchidoris bilamellata (Linnaeus, 1767)	California, USA	CASIZ 101555	KP340312	KP340408	–
Goniodorididae					
Trapania aurata Rudman, 1987	Philippines, Anilao	CASIZ 186205	MZ325731	MZ322636	MZ399609
Trapania cf. aurata	Australia	QM MO 86026	OR665615	OR665816	OR667639
Trapania brunnea Rudman, 1987	Australia	QM MO 86027	–	OR665815	–
Trapania circinata Gosliner and Fahey, 2008	Marshall Islands, Kwajalein Atoll	CASIZ 181785	–	MZ322629	–
Trapania cirrita Gosliner and Fahey, 2008	South Africa, False Bay South	CASIZ 176143	MZ325718	MZ322625	MZ399599
Trapania darvelli Rudman, 1987	The Philippines, Balayan Bay	CASIZ 222004	MZ325752	MZ322654	MZ399626
Trapania darvelli Rudman, 1987	The Philippines, Verde Island Passage	CASIZ 202112	MZ325740	MZ322644	MZ399614
Trapania darvelli Rudman, 1987	The Philippines, Verde Island Passage	CASIZ 197303	MZ325735	MZ322641	MZ399612
Trapania darvelli Rudman, 1987	The Philippines, Maricaban Island	CASIZ 186129	–	MZ322632	MZ399606
Trapania euryeia Gosliner and Fahey, 2008	The Philippines, Verde Island	CASIZ 208197	MZ325741	MZ322645	MZ399615
Trapania franae Paz-Sedano, Cobb, Gosliner and Pola, 2024b	Australia, Queensland	QM MO 86031	–	OR665820	OR667644
Trapania gibbera Gosliner and Fahey, 2008	The Philippines, Balayan Bay	CASIZ 224694	MZ325759	MZ322660	MZ399632
Trapania gibbera Gosliner and Fahey, 2008	The Philippines, Romblon Island	CASIZ 222092	MZ325755	MZ322657	MZ399629
Trapania gibbera Gosliner and Fahey, 2008	The Philippines, Romblon Island	CASIZ 222062	MZ325753	MZ322655	MZ399627
Trapania gibbera Gosliner and Fahey, 2008	The Philippines, Verde Island Passage	CASIZ 197300	MZ325734	MZ322640	–
Trapania gibbera Gosliner and Fahey, 2008	Malaysia, Pulau Tenggol	CASIZ 178334	MZ325721	MZ322627	MZ399601
Trapania goddardi Hermosillo and Valdés, 2004	Ecuador, Galapagos Islands	CASIZ 173620	MZ325717	MZ322624	MZ399598
Trapania huarmeyana	**Peru, Huarmey, Rio Seco**	**IMARPE 04-002373**	**PP693442**	**PP693141**	**–**
Trapania huarmeyana	**Peru, Huarmey, Rio Seco**	**IMARPE 04-002333**	**PP693443**	**PP693140**	**PP704681**
Trapania huarmeyana	**Peru, Huarmey, Rio Seco**	**IMARPE 04-002374**	**–**	**PP693142**	**PP704682**
Trapania japonica (Babba, 1935)	The Philippines, Anilao	CASIZ 182896	MZ325726	–	–
Trapania japonica (Babba, 1935)	The Philippines, Mabini	CASIZ 222116	MZ325757	–	–
Trapania kahel Smirnoff, Donohoo and Gosliner, 2022	Mabini, Balayan Bay	CASIZ 186133	MZ325729	MZ322634	–
Trapania kamagong Smirnoff, Donohoo and Gosliner, 2022	The Philippines, Puerto Galera	CASIZ 208585A	MZ325745	MZ322648	MZ399619
Trapania kamagong Smirnoff, Donohoo and Gosliner, 2022	The Philippines, Calumpan Peninsula	CASIZ 177526	MZ325719	MZ322626	MZ399600
Trapania Kanaloa Smirnoff, Donohoo and Gosliner, 2022	USA, Hawaii, Maui	CASIZ 189444	MZ325733	MZ322639	MZ399611
Trapania cf. Kanaloa Smirnoff, Donohoo and Gosliner, 2022	USA, Hawaii, Kauai	CASIZ 199250	MZ325736	MZ322642	MZ399613

(Continued)

Table 1. (*Continued.*)

Species	Locality	Voucher	GenBank accession No.		
			16S	*COI*	*H3*
Trapania lemnalioides Smirnoff, Donohoo and Gosliner, 2022	The Philippines, Romblon Island	CASIZ 222066	MZ325754	MZ322656	MZ399628
Trapania lemnalioides Smirnoff, Donohoo and Gosliner, 2022	The Philippines, Romblon Island	CASIZ 221979	MZ325750	MZ322653	MZ399624
Trapania lemnalioides Smirnoff, Donohoo and Gosliner, 2022	The Philippines, Puerto Galera	NMP 041335	MZ325744	MZ322647	MZ399618
Trapania lineata Haefelfinger, 1960	Ireland	MNCN 15.05/94372	OM237328	OM220107	–
Trapania lineata Haefelfinger, 1960	Ireland, Galway	MNCN 15.05/94371	OM237327	OM220106	–
Trapania lineata Haefelfinger, 1960	Spain	MNCN 15.05/94353	OM237326	OM220105	OM257122
Trapania lineata Haefelfinger, 1960	Spain	MNCN 15.05/94351	OM237325	OM220104	–
Trapania lineata Haefelfinger, 1960	Spain	MNCN 15.05/94352	OM237324	OM220103	OM257121
Trapania lineata Haefelfinger, 1960	Italy, Naples	MNCN 15.05/94345	OM237323	OM220102	OM257120
Trapania lineata Haefelfinger, 1960	Spain	MNCN 15.05/94368	OM237322	OM220101	OM257119
Trapania lineata Haefelfinger, 1960	France, Cap Ferret	MNCN 15.05/94370	OM237321	OM220100	OM257118
Trapania lineata Haefelfinger, 1960	Morocco, Jebha	MNCN 15.05/94366	OM237320	OM220099	OM257117
Trapania lineata Haefelfinger, 1960	Morocco	MNCN 15.05/94360	OM237319	OM220098	–
Trapania lineata Haefelfinger, 1960	Morocco	MNCN 15.05/94359	OM237318	OM220097	–
Trapania lineata Haefelfinger, 1960	Spain	MNCN 15.05/94349	OR665616	OR665817	OR667640
Trapania lineata Haefelfinger, 1960	Italy: Sardinia	RM3_698	OP965560	OQ001357	OQ096467
Trapania lineata Haefelfinger, 1960	Portugal: Aveiro	MNCN:15.05/55504	JX274048	JX274080	–
Trapania maculata Haefelfinger, 1960	Spain	MNCN 15.05/94374	OM237330	OM220109	OM257124
Trapania maculata Haefelfinger, 1960	Spain	MNCN 15.05/94373	OM237329	OM220108	OM257123
Trapania miltabrancha Gosliner and Fahey, 2008	Australia, Queensland	WAMS 72104	OR665619	OR665819	OR667643
Trapania naeva Gosliner and Fahey, 2008	Tanzania, Zanzibar	CASIZ 227553	MZ325760	MZ322661	MZ399633
Trapania orteai García-Gómez and Cervera, 1989	Spain	MNCN 15.05/94383	OM237335	OM220114	OM257129
Trapania orteai García-Gómez and Cervera, 1989	Spain	MNCN 15.05/94381	OM237334	OM220113	OM257128
Trapania orteai Gómez and Cervera, 1989	Morocco, Taghazout	MNCN 15.05/94389	OM237333	OM220112	OM257127
Trapania orteai Gómez and Cervera, 1989	Morocco, Capghir	MNCN 15.05/94384	OM237332	OM220110	OM257126
Trapania orteai Gómez and Cervera, 1989	Spain	MNCN 15.05/94382	OR665617	–	OR667641
Trapania palmula Gosliner and Fahey, 2008	Philippines, Siquijor Island	CASIZ 217428	MZ325748	MZ322651	MZ399622
Trapania palmula Gosliner and Fahey, 2008	Philippines, Romblon Island	CASIZ 221984	MZ325751	–	MZ399625
Trapania palmula Gosliner and Fahey, 2008	Philippines, Batangas Bay	CASIZ 182771	MZ325725	–	MZ399604
Trapania reticulata Rudman, 1987	Papua New Guinea, Tab Island.	CASIZ 191431	MF958303	MF958432	–
Trapania sanctipetrensis Cervera, García-Gómez and Megina, 2000	Spain	MNCN 15.05/94390	OM237316	–	OM257115
Trapania sanctipetrensis Cervera, García-Gómez and Megina, 2000	Spain	MNCN 15.05/94391	OM237317	–	OM257116
Trapania scurra Gosliner and Fahey, 2008	Philippines, Batangas Bay	CASIZ 182768	MZ325724	MZ322630	–
Trapania scurra Gosliner and Fahey, 2008	Australia, Queensland	QM MO 86029	OR665618	OR665818	OR667642
Trapania scurra Gosliner and Fahey, 2008	Philippines, Batangas Bay	CASIZ 201938A	MZ325739	–	–
Trapania sp. A	Philippines, Anilao	NMP 041329	MZ325727	MZ322631	MZ399605
Trapania stegodon Smirnoff, Donohoo and Gosliner, 2022	Philippines, Anilao	CASIZ 186206	–	MZ322637	MZ399610
Trapania tamaraw Smirnoff, Donohoo and Gosliner, 2022	Philippines, Puerto Galera	CASIZ 208391	MZ325742	MZ322646	MZ399616
Trapania tatsulok Smirnoff, Donohoo and Gosliner, 2022	Philippines, Verde Island Passage	CASIZ 200530	MZ325737	–	–

(*Continued*)

Table 1. (*Continued.*)

| Species | Locality | Voucher | GenBank accession No. | | |
			16S	COI	H3
Trapania tigger Smirnoff, Donohoo and Gosliner, 2022	Philippines, Mabini	CASIZ 180412	MZ325723	MZ322628	MZ399603
Trapania toddi Rudman, 1987	Australia, Queensland	QM MO 86030	–	OR665822	OR667646
Trapania toddi Rudman, 1987	Australia, Queensland	QM MO 86028	–	OR665821	OR667645
Trapania undulata Smirnoff, Donohoo and Gosliner, 2022	Philippines, Balayan Bay	NMP 041331	MZ325728	MZ322633	MZ399607
Trapania velox (Cockerell, 1901)	USA, California	CASIZ 209038	MZ325747	MZ322650	MZ399621
Trapania velox (Cockerell, 1901)	USA, California	CASIZ 208954	MZ325746	MZ322649	MZ399620
Trapania vitta Gosliner and Fahey, 2008	Philippines, Romblon Island	CASIZ 224693	MZ325758	MZ322659	MZ399631
Trapania vitta Gosliner and Fahey, 2008	Philippines, Romblon Island	CASIZ 222097	MZ325756	MZ322658	MZ399630
Trapania vitta Gosliner and Fahey, 2008	Palau: Babeldaob	CASIZ 220484	MZ325749	MZ322652	MZ399623
Trapania vitta Gosliner and Fahey, 2008	Philippines, Maricaban Strait	CASIZ 186204	MZ325730	MZ322635	MZ399608
Trapania vitta Gosliner and Fahey, 2008	Australia, Queensland	AM C270713	OR665620	OR665823	–
Trapania vitta Gosliner and Fahey, 2008	Malaysia, Pulau Tioman	CASIZ 178337	MZ325722	–	MZ399602
Trapania vitta Gosliner and Fahey, 2008	Philippines, Puerto Galera	CASIZ 208424	MZ325743	–	MZ399617
Ancula gibbosa (Risso, 1818)	Maine, USA	CASIZ 182028	KP340291	KP340388	KP340413
Pelagella joubini (Risbec, 1928)	Maui, Hawaii, USA	CASIZ 164920	MZ325716	MZ322623	MZ399597
Bermudella brunneomaculata (Gosliner, 2004)	Philippines	CASIZ 177712	MZ325720	KF744236	KF744242
Ceratodoris kendi (Gosliner, 2004)	Papua New Guinea	CASIZ 191574	OR665585	OR665789	OR667598
Pelagella albopunctata Paz-Sedano, Smirnoff, Gosliner and Pola, 2023	Marshall Islands	CASIZ 181619	ON426422	ON419299	ON419524
Pelagella rubrobranchiata Paz-Sedano, Smirnoff, Gosliner and Pola, 2023	Australia	WAM S98752	ON426443	ON419315	ON419531
Pelagella scottjohnsoni Paz-Sedano, Smirnoff, Gosliner and Pola, 2023	Marshall Islands	CASIZ 182225	ON426437	ON419311	ON419528

Species delimitation analyses

The COI dataset was further interrogated to test the genetic relatedness of the candidate species *T. huarmeyana* sp. nov. sequences to congeners. Species delimitation was examined using Automatic Barcode Gap Discovery (ABGD; Puillandre *et al.*, 2012) and Assemble Species by Automatic Partitioning (ASAP; Puillandre *et al.*, 2021). Sixty-six sequences of *Trapania* spp. were retrieved from GenBank and three were of the candidate species *T. huarmeyana* sp. nov. For the ABGD analysis, COI alignments were uploaded at https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html using the default settings: Pmin = 0.001, Pmax = 0.1, Steps = 10, X (relative gap width) = 1.0, Nb bins = 20 and with Kimura (K2P) distance. The ASAP was conducted using the web tool (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html), and under the Kimura (K80) model, with a default ts/tv rate of 2.0, and a 0.05 threshold distance. Additionally, to compare the genetic distance among specimens of *Trapania*, we calculated the pairwise *p*-distance (between individual sequences and in average by species) for 16S, COI and H3 using MEGA v.6 (Tamura *et al.*, 2013).

Morphological examination

The external morphology of all collected specimens was examined using a stereomicroscope. Additionally, the internal morphology of two specimens was examined, with a focus on the digestive and reproductive systems as the main internal traits for species identification and characterization. The reproductive organs were examined by removing them from the animal through a ventral incision and drawn under a Leica S APO dissecting microscope. The buccal mass of each examined specimen was removed and dissected to isolate the jaws and radula. Penises, jaws and radulae were rinsed in water, dried, mounted and sputter coated for examination under a FEI Inspect S50 variable pressure SEM at the Universidad Nacional Mayor de San Marcos (Lima, Peru).

Results

Phylogenetic analysis

A total of two partial sequences of 16S (of 453 bp), three of COI (of 631–666 bp) and two of H3 (of 339 bp) were obtained for *T. huarmeyana* sp. nov. The concatenated dataset contained 1268 bp, with 455 parsimony informative sites. The BI and ML analyses of the concatenated dataset, which included 32 of the 55 species of *Trapania*, produced phylogenetic trees with partial concordance of statistical support (BI posterior probabilities [pp] and ML bootstrap values [mlb]) (Figure 1). Both trees clustered the *Trapania* species in a monophyletic group (pp = 0.99; mlb = 100%), recovering two internal subclades with moderate-to-strong statistical support. The phylogenetic trees consistently grouped the Peruvian *T. huarmeyana* sp. nov. with Galapagos sequences
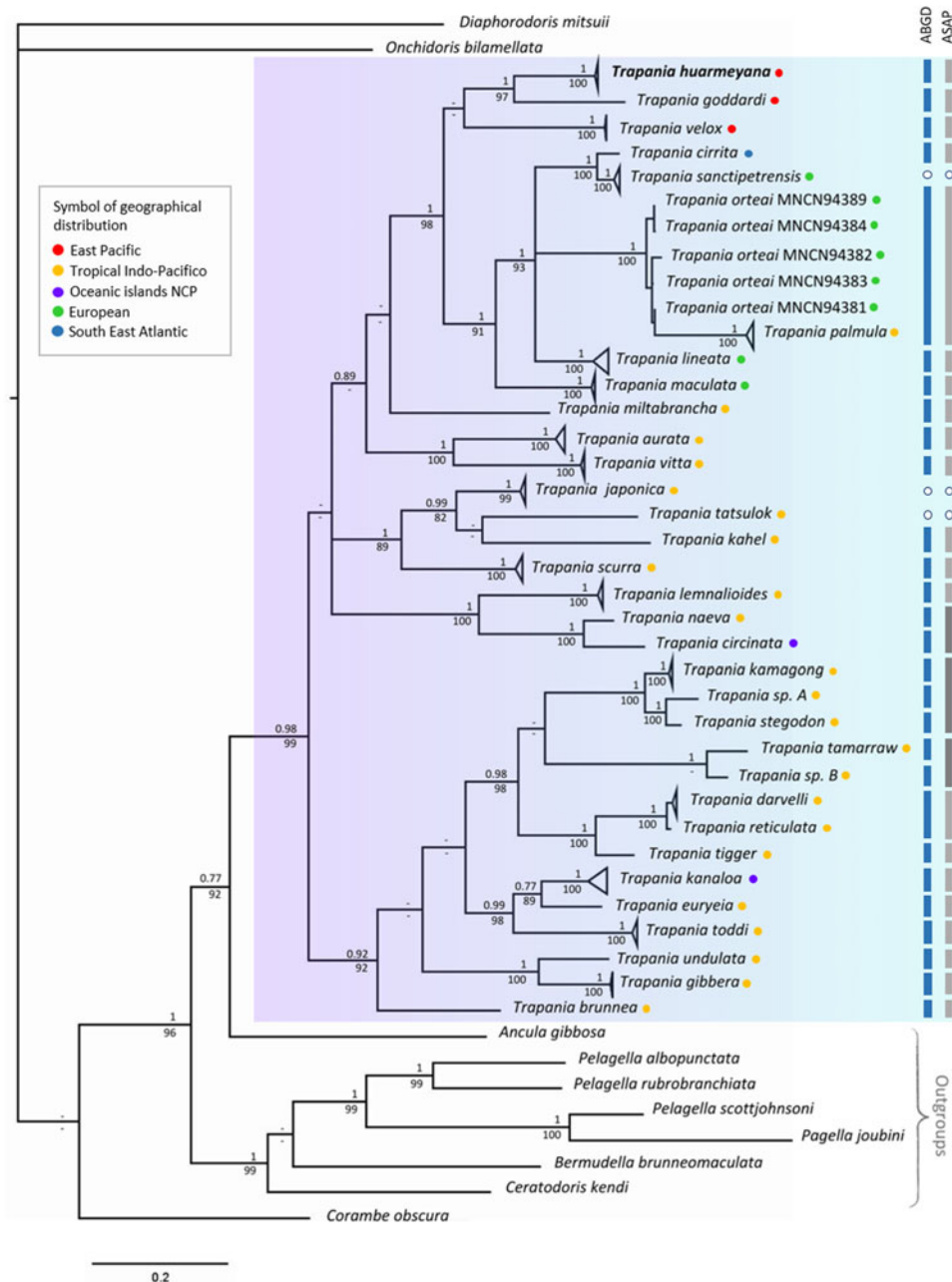
Figure 1. Bayesian consensus phylogenetic tree on the concatenate molecular data (16S + COI + H3) for *Trapania* spp. Values above branches refer to posterior probabilities of BI and values below branches represent bootstrap support values for ML. Only values >0.70 (Bayesian) and >75 (ML) are included. Coloured dots to the right of each species represent the geographic origin. Bars on the right indicate results of species delimitation analyses using ABGD (blue) and ASAP (grey) for COI dataset. White circle: no COI sequence to compare.

identified as *T. goddardi* by Smirnoff *et al.* (2022) showing strong-to-full support (pp = 1; mlb = 96%) as per Muff *et al.* (2022). *Trapania huarmeyana* and *T. goddardi* clustered with *T. velox* from California within an eastern Pacific clade. In addition, the eastern Pacific clade grouped with the European species: *T. cirrita* Gosliner and Fahey, 2008; *T. sanctipetrensis* Cervera, García-Gómez and Megina, 2000; *T. orteai* García-Gómez and Cervera, 1989; *T. lineata* Haefelfinger, 1960; *T. maculata* Haefelfinger, 1960 and the Indo-Pacific species *T. palmula* with strong support (pp = 1; mlb = 98%).

Species delimitation analysis
The ABGD analysis showed a tri-modal pairwise genetic distance (K2P) distribution with a gap located between 3 and 6% of the genetic distance and a second clear and wide barcode gap situated between 9 and 10% of the genetic distance (Supplementary

Figure S1A). ABGD analysis of the genetic pairwise distance in the aligned COI dataset recovered one partition of 28 candidate species with a prior maximal distance = 2.15% and barcode gap distance = 0.049 (Figure 1; Supplementary Figure S1B). Meanwhile, the best ASAP-score (= 4.5) partitioned the COI dataset into 24 putative species (Figure 1). ASAP grouped as the same species those sequences whose genetic distance was < 7.5%: *T. naeva* and *T. circinata*; *T. kamagong, Trapania* sp. A and *T. stegodon*; *T. tamarraw* and *Trapania* sp. B; *T. darvelli* and *T. reticulata* (Figure 1). Although ABGD and ASAP methods show slightly different results, both support the genetic divergence of the new candidate species from other congeners.

Intraspecific variability of *T. huarmeyana* sp. nov. using the COI dataset was 0.2–0.6% (between 1 and 4 polymorphic sites). The genetic divergence of *T. huarmeyana* sp. nov. between its congeners was greater than 11% with a minimum of 11.3%
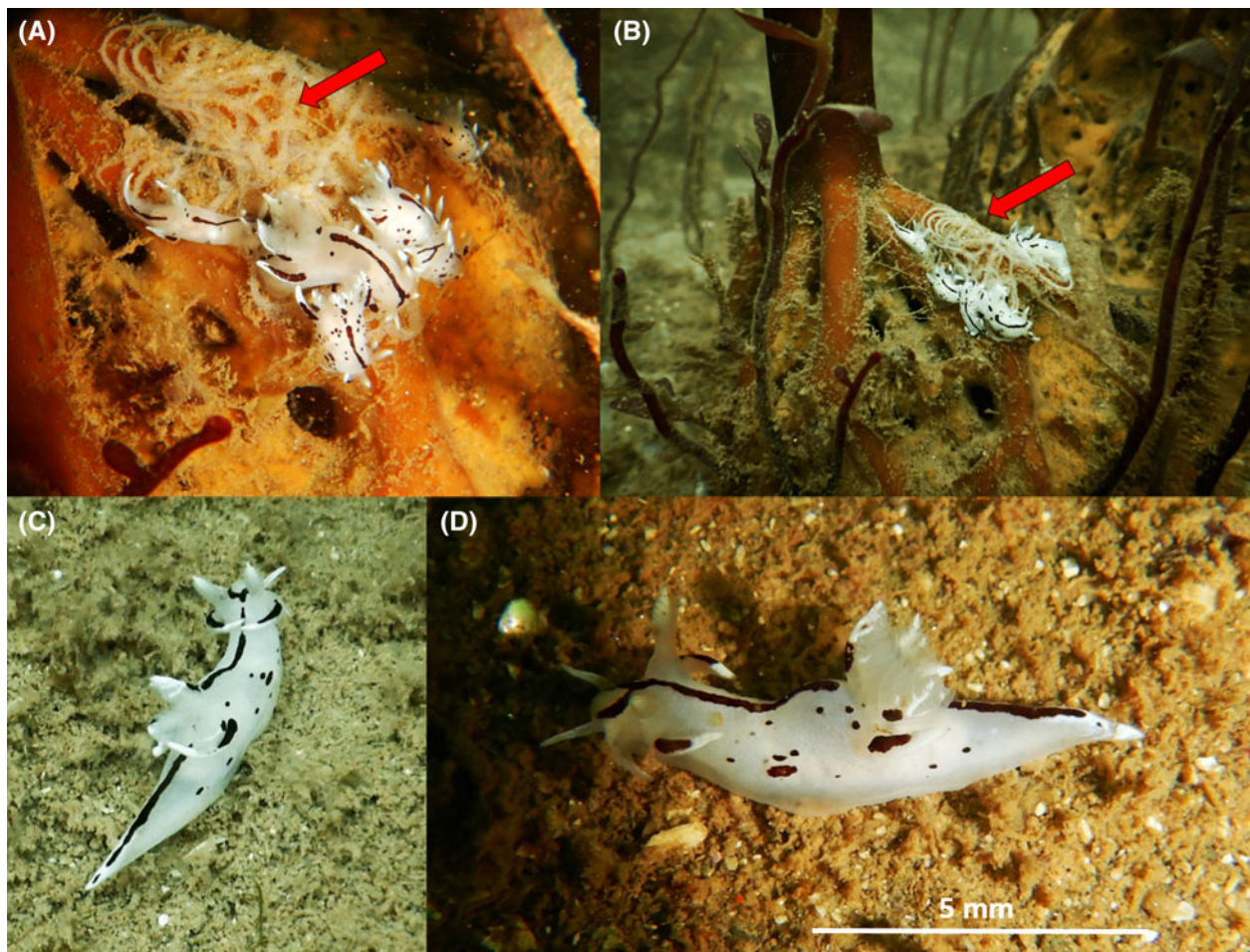
Figure 2. Photographs of living animals. (A, B) Grouped animals and mass egg (red arrow) on the kelp *Eisenia cokeri* holdfast. (C) Dorsal view. (D) Lateral view (photo credits: R. Uribe).

divergence from its closest congeners, *T. goddardi* with 11.3% (Supplementary Table S2). For the 16S dataset, the intraspecific variability for *T. huarmeyana* sp. nov. was 0.6% (two polymorphic sites), with 4.5% divergence from *T. goddardi* with the next closest species being *T. lineata* (average = 6.5% sequence divergence) (Supplementary Table S3). Finally, using the H3 dataset, the two sequences of *T. huarmeyana* sp. nov. were 100% identical; and exhibited 3.4% divergence from *T. goddardi* and 4.5% from *T. lineata* with 4.5% (Supplementary Table S4).

Systematics

Order Nudibranchia Cuvier, 1817
Suborder Doridina
Superfamily Onchidoridoidea Gray, 1827
Family Goniodorididae H. Adams and A. Adams, 1854
Genus *Trapania* Pruvot-Fol, 1931
Trapania huarmeyana sp. nov.

The nomenclatural acts it contains have been registered in ZooBank (http://zoobank.org/). The Life Science Identifier (LSID) for this publication is: urn: lsid:zoobank.org:act: A98A017A-0FE1-44C1-AB4E-78CB132873AB.

Type material

Holotype: 1 specimen, IMARPE 04-002333, Huarmey, Ancash region, Peru, 5.5 mm preserved length, April 2023.

Paratypes: 5 specimens, IMARPE 04-002373–IMARPE 04-002377, Huarmey province, Ancash region, Peru (same coordinates as holotype), 5–7 mm preserved length, April 2023.

Type locality: Huarmey province, Ancash region, Peru (−78.228080°, −9.807092°).

Etymology: The name *Trapania huarmeyana* sp. nov. is dedicated to Huarmey province, the type locality where the specimens were collected.

Diagnosis: Body translucent white, with anterior and posterior midline dark brown stripes and some brown spots on dorsum; with brown blotches on base of extra-rhinophoral processes, extra-branchial processes and gill branches. Radular formula: $28 \times 1.0.1$, lateral teeth without outer denticles and with 9–10 inner denticles. Jaws with several rows of pointed jaw elements. Penis with 10 rows of penial hooks, less developed basally.

External morphology: Living specimens up to ∼8 mm length. Body narrow, elongated, becoming wider and higher at level of gill, narrowing gradually towards elongated and pointed tail. Colour translucent white, with a middorsal dark brown stripe running from rhinophores to anterior margin of gill, with two anterior extensions on base of oral tentacles and a middorsal posterior brown stripe running from posterior margin of gill to sub-distal tail. Body ornamented with elongated to circular dark brown spots, most of them between rhinophores and gill. Additionally, large dark brown blotches on base of extra-rhinophoral processes, extra-branchial processes and gill branches (Figure 2). Anterior margin of foot with a pair of thick propodial tentacles oriented backward (Figure 3: pt). Oral tentacles tapering, oriented forward, smaller than extra-rhinophoral processes, opaque white on distal half (Figure 3: ot). Rhinophores without rhinophoral sheath, with a pointed tip, with 7–8 lamellae decreasing in size distally, opaque white on frontal edge of lamellae and tip
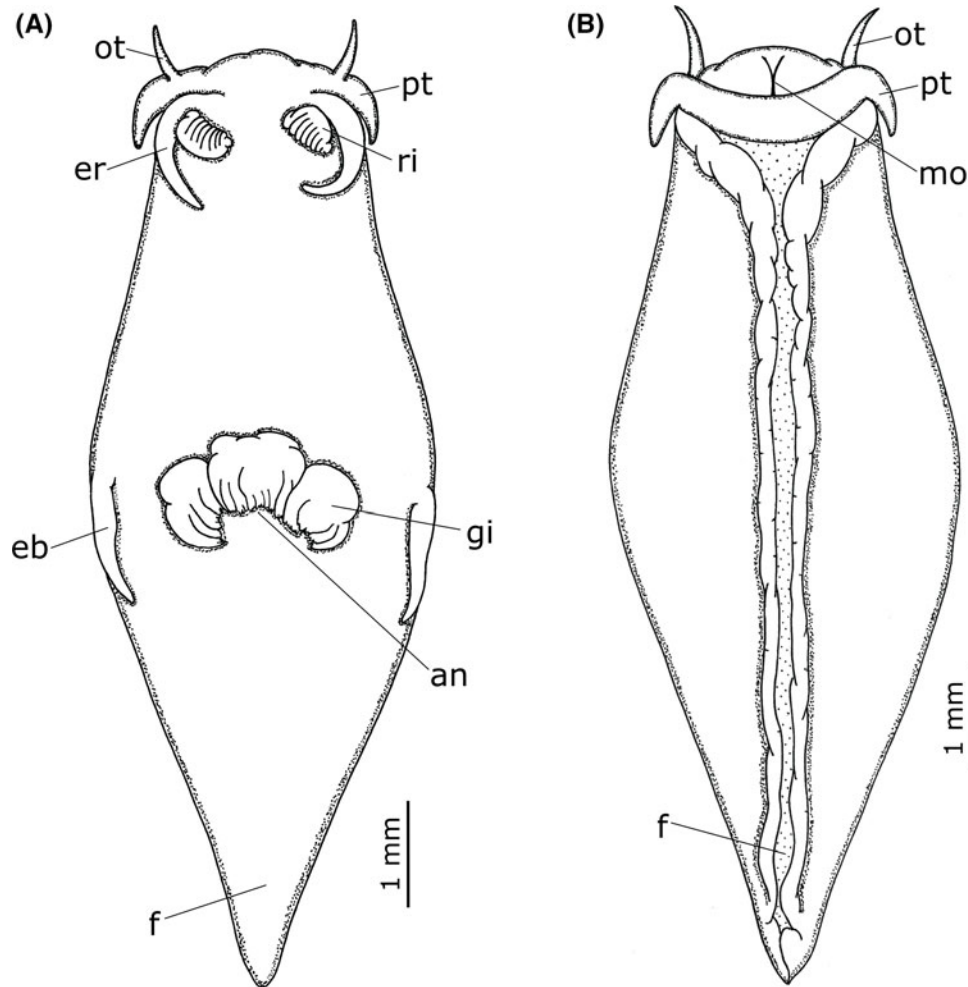
Figure 3. External morphology of *T. huarmeyana* sp. nov. (A) Dorsal view. (B) Ventral view. Abbreviations: eb, extra-branchial processes; extra-rhinophoral processes; f, foot; gi, gill; mo, mouth; ot, oral tentacles; pt, propodial tentacles; ri, rhinophores.

(Figure 3A: ri). Pair of extra-rhinophoral processes digitiform, oriented backward, smaller than extra-branchial processes, opaque white distally (Figure 3A: er). Gill of three tripinnate translucent branches, arranged in a semicircular fashion, opaque white on tip and frontal edge of gill branches (Figure 3A: gi). Pair of extra-branchial processes thick, digitiform, oriented backward, opaque white distally (Figure 3A: eb). Anus surrounded by a gill semicircle (Figure 3A: an). Genital opening on right side of body. Foot elongated, narrow, opaque white on tail (Figure 3B: f).

Reproductive system. Gonad immersed into frontal region of visceral mass. Hermaphroditic duct slender and curved, inserting into ventral surface of ampulla slightly posterior to its midline (Figure 4). Ampulla elongated, proximally pyriform, bifurcating distally into narrow oviduct and vas deferens (Figure 4A: am). Prostate thick and rounded, with a single loop, narrowing into short and slightly curved deferent duct. Penial sac thick and elongated (Figure 4B). Bursa copulatrix ovoid (Figure 4B, C: bc), smaller than prostate, connecting with long and slender vagina (Figure 4B, C: va). Seminal receptacle small and spherical (Figure 4B, C: sr), entering into bursa copulatrix through narrow duct, connected by delicate uterine duct to nidamental gland. Nidamental gland larger than bursa, connecting to genital opening through narrow duct (Figure 4: ng). Penis elongated, with several closely packed rows of hooked spines, thick and elongated distally, becoming smaller, slender and more densely packed proximally (Figure 5).

Central nervous system. Nerve ring dorsal on buccal mass, surrounding oesophagus (Figure 6A, B). Cerebral and pleural ganglia fused forming a cerebro-pleural complex (Figure 6C: clg). Eyes sessile. Cerebral nerves: c1, slender, running anteriorly, innervating oral tentacles; c2, slender, arising posterior to c1, inserting anteriorly into dorsal region of mouth; c3, thick, inserting into rhinophores, rhinophoral ganglion not developed; c4, thick, arising close to c3, inserting into ventral region of mouth. Pleural ganglia on posterior region of cerebral ganglia, with a thick nerve (pl) running posteriorly, inserting into dorsal body wall. Cerebro-pedal connectives short. Cerebro-buccal connectives very long and thin. Buccal ganglia (Figure 6A–C: bg) small, oval, attached on posterior region of buccal mass below oesophagus and salivary glands, with four very slender nerves. Pedal ganglia (Figure 6C: pg) oval, smaller than cerebro-pleural complex, connected by a short pedal commissure. Pedal nerves: p1, slender, running anteriorly, innervating anteriormost foot and propodial tentacles; p2, slender, branched, arising posterior to p1, innervating anterior foot; p3, thick, running posteriorly, bifurcating into two branches, innervating medium and posterior foot, respectively; p4, only arising on right pedal ganglion, dorsal to p2–p3 nerves, running posteriorly inserting into penial sac and genital system. Visceral loop slender (Figure 6C: vl), with a nerve that runs posteriorly inserting into visceral mass.

Circulatory and excretory system. Heart on dorsal body wall inside pericardial cavity. Auricle funnel-shaped, translucent white, with internal longitudinal and transversal fibers. Ventricle pyriform, creamy yellow, thicker and longer than auricle. Blood gland not observed (Figure 6D).
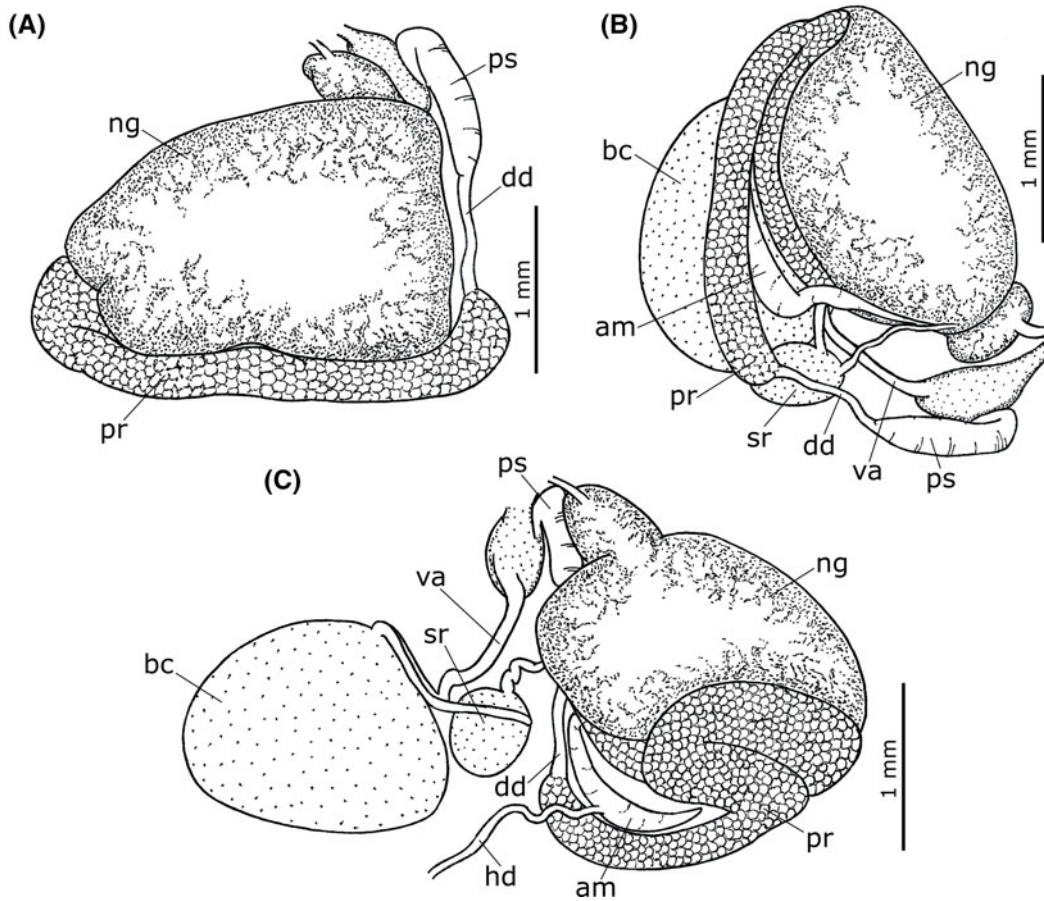
Figure 4. Schematic view of reproductive organs. (A) Dorsal view. (B) Ventral view. (C) Lateral view. Abbreviations: am, ampulla; bc, bursa copulatrix; dd, deferent duct; hd, hermaphroditic duct; ng, nidamental gland; pr, prostate; ps, penial sac; sr, seminal receptacle; va, vagina.

Digestive system. Oral tube short with several oral glands on ventral surface. Buccal mass thick, with a slightly prominent dorsal rounded buccal pump extending anteriorly (Figure 6A, B).

Radula long, widening towards newly developed teeth, translucent white, formula: $28 \times 1.0.1$. Rachidian tooth absent. Lateral teeth with slightly curved, long falcate cusp displaced towards outer
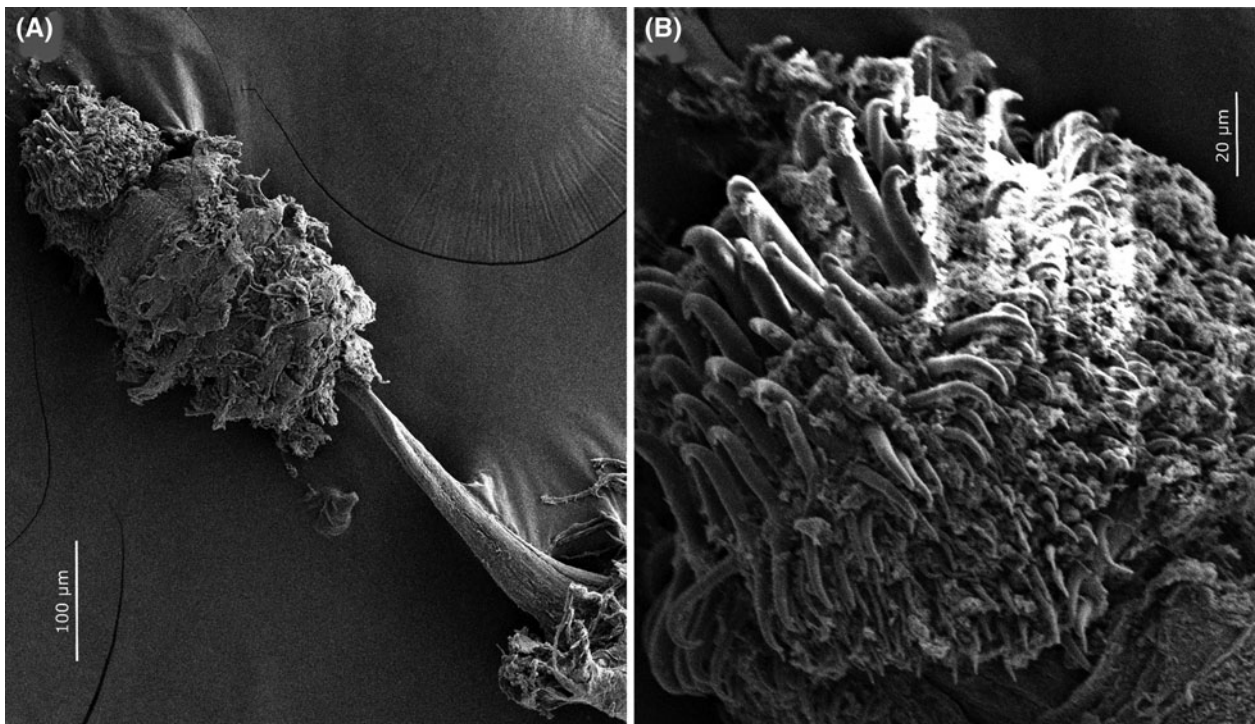

Figure 5. Scanning electron micrographs of penis of the specimen 5 (IMARPE 04-00276) of *T. huarmeyana* sp. nov.
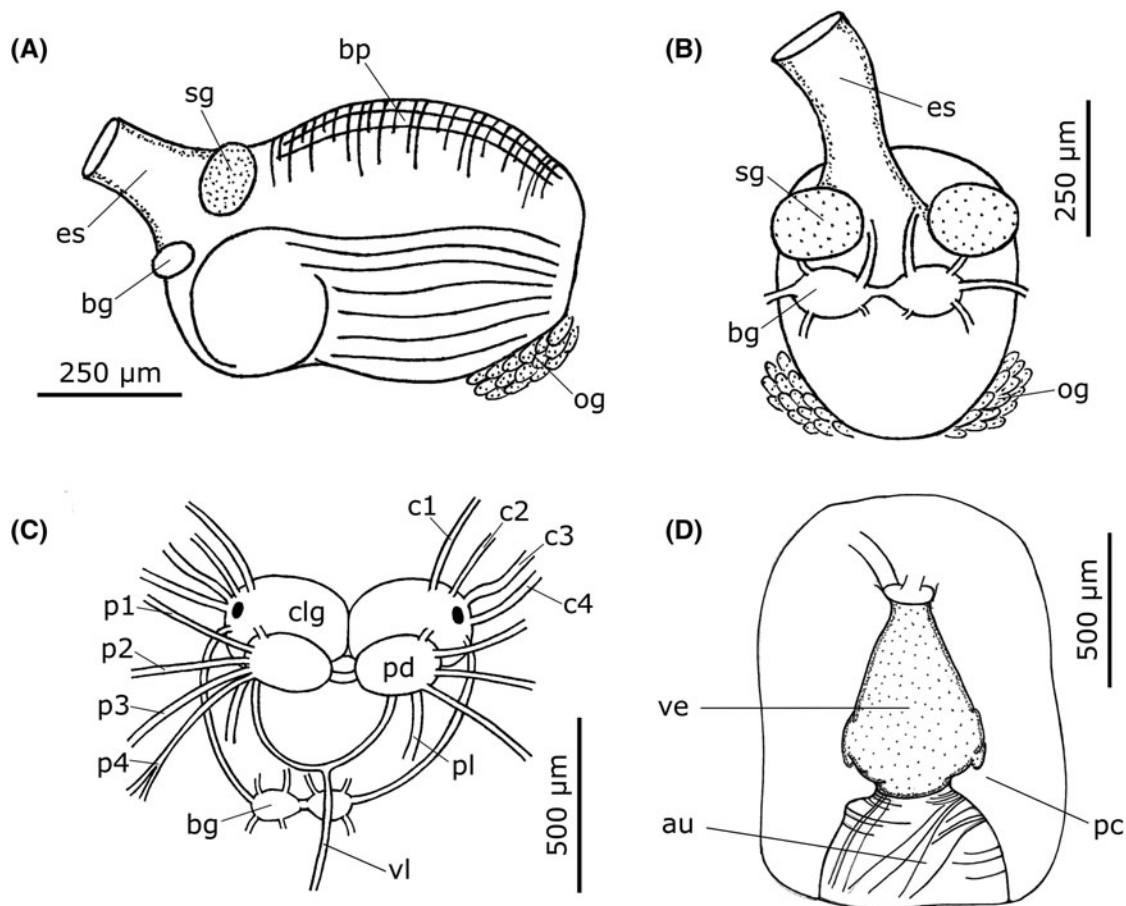
Figure 6. Anatomical details in *T. huarmeyana* sp. nov. (A) Right view of buccal mass. (B) Posterior view of buccal mass. (C) Ventral view of central nervous system. (D) Dorsal view of pericardium. Abbreviations: au, auricle; bg, buccal ganglia; bp, buccal pump; c1–c4, cerebral nerves; clg, cerebro-pleural complex; es, oesophagus; og, oral glands; p1–p4; pedal nerves; pc, pericardium; pd, pedal ganglia; pl, pleural nerve; sg, salivary glands; ve, ventricle; vl, visceral loop.

margin; with 9–10 inner pointed denticles decreasing in size towards radula centre; outer denticles not observed (Figure 7A–D). Pair of triangular jaws, with 3–4 rows of closely packed yellow rodlets on masticatory border, rodlets lanceolated with a pointed tip (Figure 7E, F). Pair of salivary glands very small and rounded, attached at the junction of oesophagus with buccal mass (Figure 8A, B: sg). Oesophagus long, anteriorly swollen, posteriorly narrowing, inserting into frontal side of visceral mass (Figure 8A: es). Digestive gland creamy white, occupying most of visceral mass (Figure 8A: dg). Stomach small, on left surface of visceral mass (Figure 8B: st). Intestine narrow, running one whorl around dorsal surface of digestive gland, curving to right side to open into anus (Figure 8B: in).

Distributional range: Collected only from Huarmey, Ancash region, north-central Peru. *Trapania huarmeyana* sp. nov. probably with affinity to warm waters since it was found during the El Niño 2023/24 event.

Ecology: Specimens of *T. huarmeyana* sp. nov. were observed and photographed on holdfast of the kelp *E. cokeri* covered by Hydrozoa species and a yellow sponge (Demospongiae Sollas, 1885) that probably constitutes their diet (Figure 2A, B). Solitary individuals were observed on rocky platforms inside the *E. cokeri* kelp forest (Figure 2C, D).

Reproduction: *Trapania huarmeyana* sp. nov. deposits spiral and transparent egg masses (Figure 2A, B).

Remarks: Externally, *T. huarmeyana* sp. nov. resembles *T. velox* from California and *T. goslineri* from Baja California and the Gulf of California, based on the white body and dark brown stripes and blotches on dorsum. However, *T. velox* has

five brown stripes on the dorsum (Cockerell, 1901; MacFarland, 1929, 1966) and *T. goslineri* has numerous brown blotches (Millen and Bertsch, 2000), while *T. huarmeyana* sp. nov. has a single median brown stripe along the dorsum. Additionally, the appendages and the tails on these two species have an orange tip which is absent in *T. huarmeyana* sp. nov. Although *T. darwini* from Galapagos Islands also have brown blotches, these are dense and punctuated with cream spots (Camacho-García *et al.*, 2005; Gosliner and Fahey, 2008), while they are solid and scattered in *T. huarmeyana* sp. nov. Two additional species of *Trapania* have been described from the Eastern Pacific, *T. inbiotica* from Central America with dense red patches over the dorsum and appendages with tip yellow (Camacho-García and Ortea, 2000; Camacho-García *et al.*, 2005), and *T. goddardi* from Mexico with irregular brown blotches on dorsum (Hermosillo and Valdés, 2004), but *T. huarmeyana* sp. nov. differs from them by the dorsal brown stripes and the absence of additional marking on appendage tips (Table 2).

The radular morphology of *T. huarmeyana* sp. nov. differs from *T. goslineri*, *T. inbiotica* and *T. darwini* by absence of outer denticles; *T. inbiotica* and *T. darwini* also differing from *T. huarmeyana* sp. nov. in the number of inner denticles. Although *T. goddardi*, *T. goslineri* and *T. velox* show a similar number of inner denticles to *T. huarmeyana* sp. nov., they differ in the number of rows: 17 in *T. goddardi* (Hermosillo and Valdés, 2004), 24 in *T. velox* (MacFarland, 1929), 37–41 in *T. goslineri* (Millen and Bertsch, 2000) and 28 in *T. huarmeyana* sp. nov. Additionally, the jaws of *T. inbiotica* differ from *T. huarmeyana*
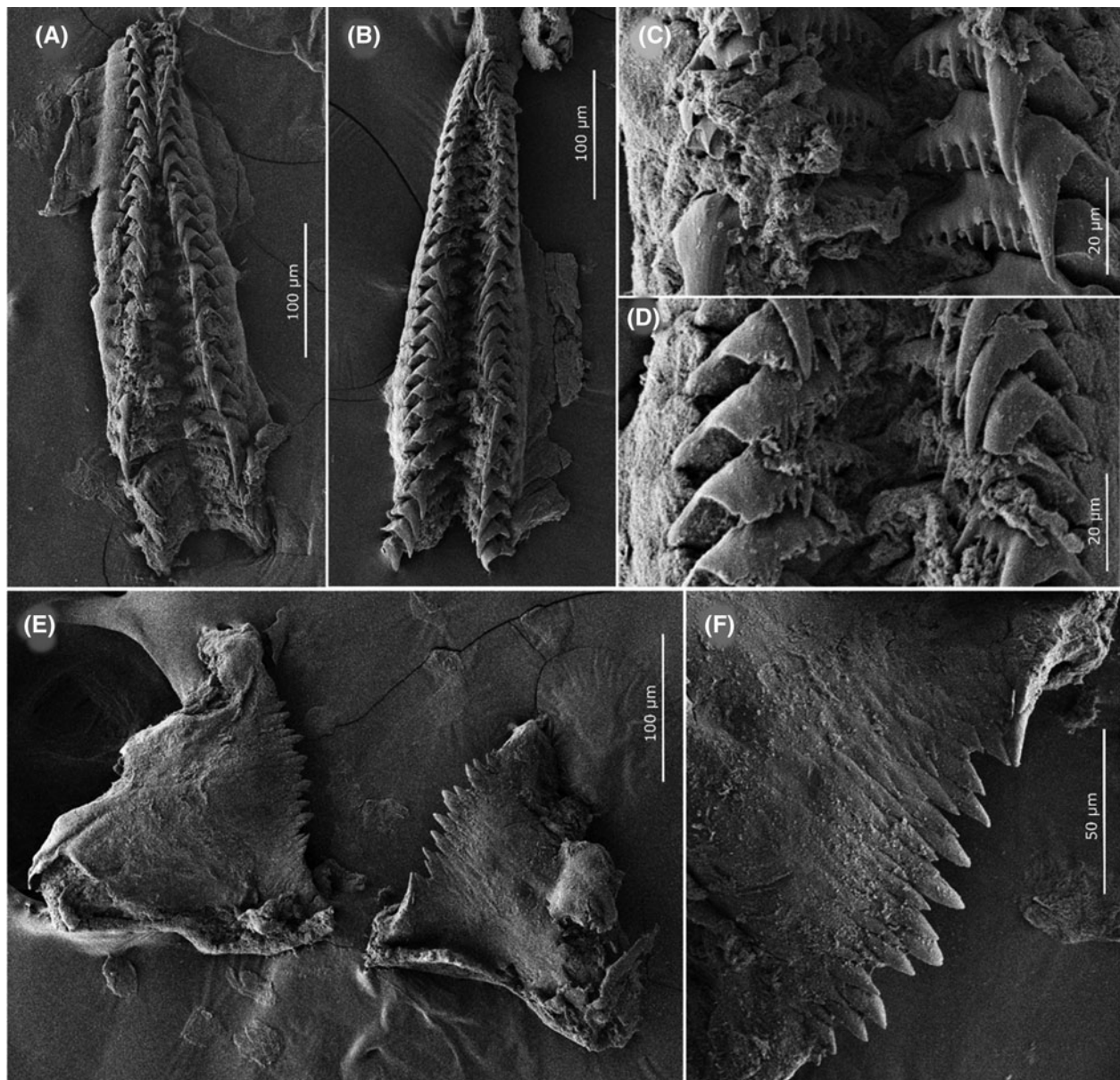
Figure 7. Scanning electron micrographs of radula. (A–D) View of complete rows and innermost teeth of the specimen 5 (IMARPE 04-00276), and specimen 6 (IMARPE 04-002377). (E–F) Detail of the jaws of the specimen 5.

sp. nov. in the presence of irregular elements (Camacho-García and Ortea, 2000) (Table 2).

A comparison of the reproductive system of *T. huarmeyana* sp. nov. shows that the hermaphroditic duct or preampullary duct in these animals enter the ampulla close to its midline, whereas in *T. velox*, *T. goddardi* and *T. inbiotica* it enters on its proximal end. Although in *T. goslineri* and *T. darwini* the preampullary duct enters the ampulla slightly more distally, it is even more distally positioned in *T. huarmeyana* sp. nov. The seminal receptacle in *T. huarmeyana* sp. nov., *T. velox*, *T. goddardi* and *T. inbiotica* enters into the base of the bursa copulatrix, but in *T. goslineri* and *T. darwini* it enters below the bursa (Gosliner and Fahey, 2008). The deferent duct is long and narrow, widening into the thick penial sac in *T. huarmeyana* sp. nov., but this duct (also called ejaculatory duct) is wide in *T. goddardi*, *T. darwini* and *T. inbiotica*. In *T. goslineri*, the prostate narrows into a short deferent duct before widening again into a short penial sac (Millen and Bertsch, 2000), but in *T. huarmeyana* sp. nov. the deferent duct and penial sac are longer. The penial sac is also elongated in *T. velox* (MacFarland, 1966), but the deferent duct is much shorter than in *T. huarmeyana* sp. nov. (Table 2).

Discussion

A combination of morphological and molecular tools represents a significant advance in accelerating the biodiversity knowledge of some groups such as heterobranch sea slugs or which taxonomic data are lacking (Padula *et al.*, 2016; Valdés *et al.*, 2018; Smirnoff *et al.*, 2022; Paz-Sedano *et al.*, 2024b). This approach has been fundamental to increasing the species number in many taxa around the world, including the nudibranch family Goniodorididae from the tropical Indo-Pacific and Atlantic Oceans, with notable discoveries among the genera *Trapania*, *Bermudella*, *Ceratodoris*, *Murphydoris* and *Naisdoris* (Paz-Sedano *et al.*, 2024b). Here, we studied the external and internal morphology, along with DNA sequences, to describe a new species of *Trapania* from the Humboldt province (South-eastern Pacific), a region that is unusually cool despite
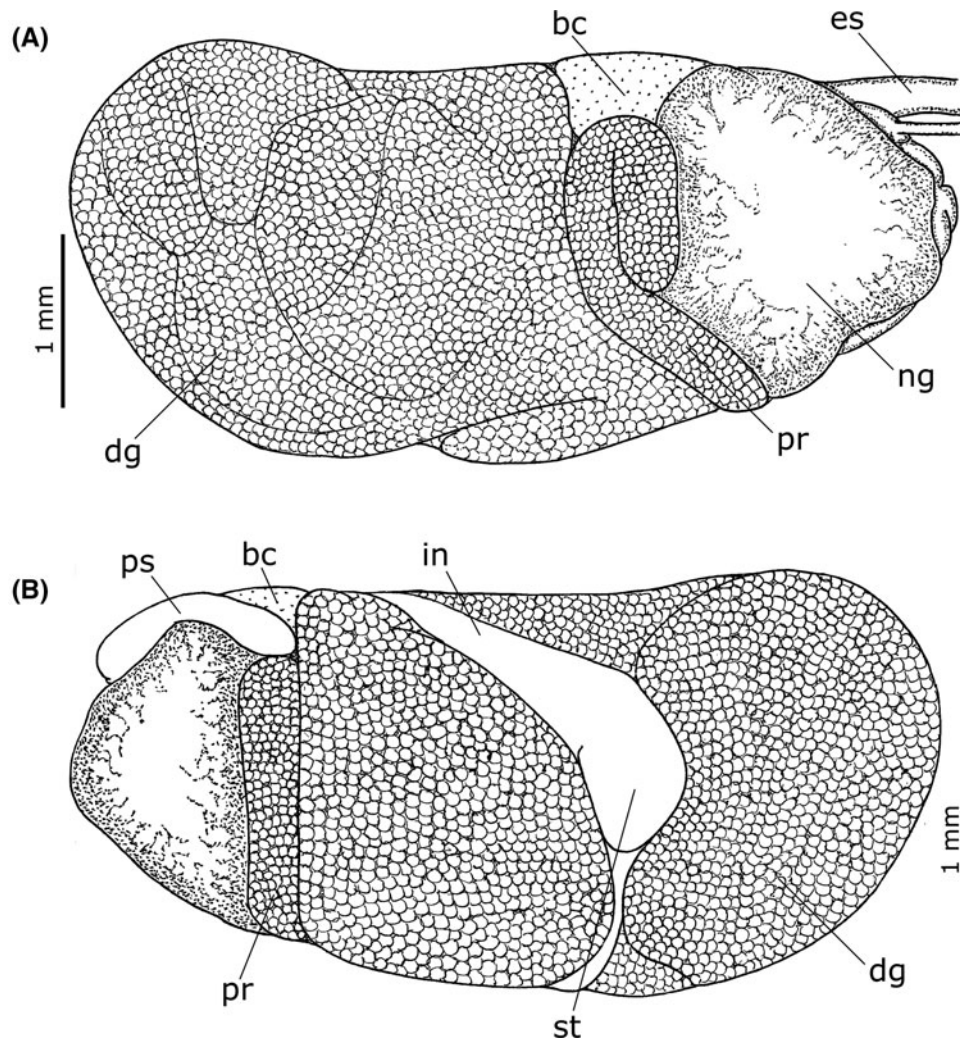
Figure 8. Haemocel organs in *T. huarmeyana* sp. nov. (A) Right view. (B) Left view. Abbreviations: bc, bursa copulatrix; dg, digestive gland; es, oesophagus; in, intestine; ng, nidamental gland; pr, prostate; ps, penial sac; st, stomach.

its close affinity to the equator (Gutiérrez *et al.*, 2016; Ibanez-Erquiaga *et al.*, 2018). During strong El Niño events, many species (e.g. molluscs) extend their distributional range towards high latitudes due to the intrusion of larvae and adults (Goddard *et al.*, 2020). However, when normal conditions are re-established, many may become trapped within coastal ecosystems. Although the genus *Trapania* has been mostly recorded in warm waters around the world, previous studies reported that it thrives in cold water regions with similar climate variability (Cetra and Roche, 2019; Paz-Sedano *et al.*, 2022).

Stressors such as marine heatwaves along of the Eastern Pacific Ocean, produced by warm events, are directly related to habitat loss (e.g. kelp forests) and subsequent alteration of species richness. Many species may survive at specific locations such as caves, bays and kelp forests, that provide refuge during strong warming events (Uribe and Pacheco, 2012; Araya and Valdés, 2016). Recently, high temperatures recorded during the El Niño 2023/24 event have increased the migration of species of tropical origin to the temperate Humboldt province (authors observation), affecting the diversity of some taxonomic groups that inhabit coastal ecosystem, such as the little-known molluscs nudibranchs. *Trapania huarmeyana* sp. nov. may represent a recent migrant or may have been in the region for a long time but simply not been detected due to a lack of research effort, as suggested by Uribe and Pacheco (2012) for other Nudibranchia records in this geographical area.

Our phylogenetic analyses indicate *T. huarmeyana* sp. nov. and *T. goddardi* are sister species, i.e. they have a common ancestor. The sequences from Galapagos, identified as *T. goddardi* by Smirnoff *et al.* (2022) likely correspond to *T. darwini* (Gosliner and Fahey, 2008), since *T. goddardi* was originally recorded in Mexico (Hermosillo and Valdés, 2004). Regardless of the above, the East Pacific species for which genetic sequences were available in this study (= *T. huarmeyana* n. sp, *T. goddardi* and *T. velox*) were grouped with species recorded in Europe, suggesting greater genetic closeness than with the Indo-Pacific species. The biogeographical zone of the Tropical Eastern Pacific extends from the Sea of Cortez (south of ∼29° N) to the northern Pacific coast of Peru (about 5° S) and was isolated from the Caribbean about 3.1 myr with the closure of the Isthmus of Panama (Coates and Obando, 1996). The latitudinal limits of the region are set by western extensions of the continental coastline, where cool currents that flow towards the equator turn offshore towards the central Pacific (Robertson *et al.*, 2004). It is also isolated from the central and western Pacific by the world's widest deepwater marine barrier to the dispersal of marine shore organisms, the 4000–7000 km Eastern Pacific Barrier (EPB) (Ekman, 1953) which may have existed for the past 65 myr (Grigg and Hey, 1992). Consequently, the rise of the Panama isthmus is a more recent barrier to gene flow between the eastern Pacific and Atlantic Oceans. A scenario similar has been documented for the genus *Phyllidiopsis* (Phyllidiidae) and *Hypselodoris*

Table 2. Comparative characteristics of the species of *Trapania* living in the Eastern Pacific

	Trapania velox (Cockerell, 1901)	*Trapania goslineri* Millen and Bertsch, 2000	*Trapania goddardi* Hermosillo and Valdés, 2004	*Trapania inbiotica* Camacho-García and Ortea, 2000	*Trapania darwini* Gosliner and Fahey, 2008	*Trapania huarmeyana* sp. nov.
Body size (living)	Up to 16 mm	Up to 15 mm	7 mm	10 mm	4–9 mm	8 mm
Coloration	Body white with five dark brown stripes on the dorsum and lateral body. Appendages and tail tipped with bright orange	Body white with large brown oval spots and streaks. Appendages and tail tipped with golden orange	Body colour variable, from off-white with irregular small brown blotches on middle and sides of dorsum to tan with darker blotches. Without additional markings	Body white with irregular red patches over the dorsum. Appendages with yellow and red markings	Body white with dense brown patches punctuated with cream spots. Appendages and tail tipped with yellow	Body translucent white with brown stripes and small spots on the dorsum. Brown blotches on the base of extra-branchial, extra rhinophoral and gill branches. Without additional markings
Rhinophores	10–12 lamellae	9–10 lamellae	6–7 lamellae	9–10 lamellae	7–11 lamellae	7–8 lamellae
Gill	Bipinnate branches	Bipinnate branches	Bipinnate branches	Tripinnate branches	Bipinnate branches	Tripinnate branches
Buccal pump	Well developed	–	–	–	Not prominent	Slightly prominent
Jaws	With short, pointed elements	Several rows of pointed jaw elements	A single row of wide pointed elements	With irregular elements	Thickened plate with pointed jaw elements	Several rows of pointed jaw elements
Radula	24 × 1.0.1; without outer denticles; 8–11 inner denticles	37–41 × 1.0.1; 1–2 outer denticles; 7–9 inner denticles	17 × 1.0.1; without outer denticles; 9–10 inner denticles	28 × 1.0.1; 1–3 outer denticles; 21–24 inner denticles	17–32 × 1.0.1; 2–4 outer denticles; 5–8 inner denticles	28 × 1.0.1; without outer denticles; 9–10 inner denticles
Hermaphroditic duct	Enter ampulla on basal region	Enter ampulla one-third of the way from its distal end	Enter ampulla on basal region	Enter ampulla on basal region	Enter ampulla near upper third	Enter ampulla close to midline
Ampulla	Ellipsoidal	Large, oval	Pyriform	Pyriform	Thin, elongate	Pyriform
Deferent duct	Short and narrow, broadening into thick penial sac	Wide?	Wide?	Long and narrow, with no penial sac	Short and wide, with penial sac	Long and narrow, ending in a penial sac
Penis	With regular rows of curved spines, less developed distally	With several rows of slightly curved spines	With several rows	With 20 rows of penial hooks	With rows of curved spines	With 10 rows of penial hooks, less developed basally
Range	California	Lower California and Gulf of California	Mexico	Central America	Galapagos Island	Peru
References	Cockerell, 1901; MacFarland, 1929; MacFarland, 1966	Millen and Bertsch, 2000	Hermosillo and Valdés, 2004	Camacho-García and Ortea, 2000	Camacho-García et al., 2005; Gosliner and Fahey, 2008	This study

(Chromodorididae), where several cases of vicariance between the tropical Indo-Pacific region and the Atlantic-eastern Pacific area followed by vicariance between the eastern Pacific and the Atlantic have been proposed (Valdés, 2001; Alejandrino and Valdés, 2006). To test such biogeographic hypotheses in *Trapania* spp will require more sequencing of species from the eastern Pacific.

This record of *T. huarmeyana* sp. nov. adds support to the predictions of Uribe *et al.* (2013) of undiscovered species heterobranch sea slug species on this region. Although recent investigation efforts have been made in the last decade in the Humboldt province (South-eastern Pacific) (Uribe *et al.*, 2013, 2018; Schrödl and Hooker, 2014; Araya and Valdés, 2016; Hoover *et al.*, 2017; Mendivil and Cardoso, 2022a, 2022b), this finding indicates that further research is necessary to better understand the distribution and variation of this group in specific localities such as the poorly explored kelp forest ecosystems.

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