



Research article

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First discovery – and a new species – of *Coelogynopora* (Platyhelminthes, Proseriata) in the Southern Hemisphere

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Abstract. *Coelogynoporidae* (Platyhelminthes) includes comparatively large and slender Proseriata, usually occurring in shallow benthic environments. *Coelogynopora* Steinböck, 1924 is the most frequently reported genus and the one with the highest species diversity. Notwithstanding that, the genus has never been reported from the Southern Hemisphere. A recent analysis of sediment from the Magellan Strait shores (Chile) resulted in the discovery of a new species of *Coelogynopora*, the first representative of the genus to be found in austral waters. The new species is defined by the following combination of characters: sclerotised copulatory system consisting of a slender, ventrally curved stylet with a broad base and three pairs of symmetrically arranged spines, the proximal ends of which are fused laterally to the base of the stylet; distal ends of the three pairs of spines hooked, with apophyses at progressively longer distances from the tip; accessory spines and solar organ absent. Based on the morphological characters, the new species appears to be more related to species from the Pacific Ocean than to those from the Atlantic Ocean. The present work suggests a vast biogeographic disjunction in the genus *Coelogynopora*, which may be described as a bipolar or amphitropical pattern of distribution.

Keywords. Biogeography, Magellan Strait, new species, Subantarctic, turbellarians.

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Introduction

Interstitial (meiobenthic) worms are valuable as the object of ecological, systematic, and biogeographical studies because of their low dispersal ability and strict habitat requirements (Curini-Galletti *et al.* 2012; Armonies 2017). They have also turned out to be helpful in more applied studies, such as the environmental biomonitoring of the direct and indirect impact of human activities (Schratzberger 2012; Armonies 2017; Martínez *et al.* 2020).

Coelogygnoporidae (Platyhelminthes) includes comparatively large and slender Proseriata, usually occurring in shallow benthic environments. Among the representatives of the family, *Coelogygnopora* Steinböck, 1924 is the most frequently reported genus and the one with the highest number of species (Jouk *et al.* 2007, 2019; Armonies 2017, 2018). The highest diversity in the genus is reached at high latitudes, and the 38 previously known, valid species have a strictly Northern Hemispheric distribution (for recent examples, see Curini-Galletti *et al.* 2010; Armonies 2017, 2018; Jouk *et al.* 2019).

A recent analysis of sediment from the Magellan Strait shores (Chile) resulted in the discovery of a new species of *Coelogygnopora*, which is the first representative of the genus found in the Southern Hemisphere. The present paper aims to describe the new species and review the biogeography of the genus in light of this new finding.

Material and methods

Study area

The specimens were collected from a dissipative sandy coast on the northern side of the Magellan Strait, Magellan Province, Chile (53.016667° S, 70.816667° W), on 6 January 2005, 14 January 2005, and 30 January 2010 (collector: R. Ponce de León). Sampling was done along a maximum linear extent of one km on a beach under the influence of lunar tides and predominantly westerly winds of strong intensity. Turbellarians were obtained and processed following Schockaert & Martens (1987). All work was performed according to and within the regulations enforced by the Chilean Animal Welfare Authorities, and no specific permissions were required. The organisms dealt with in the present paper are neither protected nor endangered in Chile.

Study methods and repository

Specimens were first observed alive. Five were whole mounted in Aman's lactophenol, and 11 in Canada balsam. Before mounting, the latter were stained with Semichon's aceto-carmin (Langeron, 1949) and counterstained with 1% fast green solution (in 95% ethanol) following Waikagul & Thakham (2014). Both aceto-carmin and fast green are monochromatic stains; the former binds very strongly to nuclei while the latter has a high affinity for cytoplasm, making it a very effective counterstain (Langeron 1949; Klausner *et al.* 1986). For the histological study, two of the larger specimens were fixed in hot Bouin's solution (Langeron 1949) for 12 h and later washed with 70 % ethanol until all traces of the picric acid were cleared. They were then dehydrated via an ascending series of ethanol, cleared in xylene, and embedded in paraffin. Series of sagittal and transversal sections were cut at 3 µm, stained with hematoxylin-eosin (Langeron 1949), and mounted in Canada balsam. Drawings were made with the aid of a drawing tube, and the distribution map of the genus *Coelogygnopora* was made with raster data from Natural Earth. Averages, standard deviations, and sample sizes follow the ranges between parentheses.

All the whole mounts and slides with sectioned specimens are deposited in the Naturalis Biodiversity Center, Leiden, The Netherlands.

Abbreviations used in the figures

Br	=	brain
Di	=	diverticulum
Ed	=	ejaculatory duct
Fa	=	female atrium
Fo	=	fibrous organ
Gc	=	genital canal

Go	=	gonopore
In	=	intestine
Ma	=	male atrium
Ov	=	ovary
Ph	=	pharynx
Pv	=	prostatic vesicle
Sc	=	sclerotised copulatory system
St	=	statocyst
Sv	=	seminal vesicle
Te	=	testis
Vd	=	vas deferens
Vi	=	vitellaria

Results

Class Proseriata Meixner, 1938
Order Lithophora Steinböck, 1925
Family Coelogynoporidae Karling, 1966
Genus *Coelogynopora* Steinböck, 1924

Coelogynopora kenichii sp. nov.

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Figs 1–6

Diagnosis

Coelogynopora kenichii sp. nov. is defined by the following combination of characters: sclerotised copulatory system consisting of a slender, ventrally curved stylet with a broad base, and three pairs of symmetrically arranged spines, the proximal ends of which are fused laterally to the base of the stylet. Distal ends of the three pairs of spines hooked, with apophyses at progressively longer distances from the tip. Accessory spines and solar organ absent.

Etymology

We dedicate this species to Prof. Dr Ken-Ichi Tajika for his highly relevant contributions to the systematics of Coelogynoporidae and other Proseriata.

Type material

Sixteen whole mounts and two series of sagittal and transversal sections.

Material examined

Holotype

CHILE • Whole mount, stained with carmine and fast green, mounted in Canada balsam; coast of the Magellan Strait, Magellan Province; 53.016667° S, 70.816667° W; 6 Jan. 2005; R. Ponce de León leg.; RMNH.VER.19980.a.

Paratypes

CHILE • 16 whole mounts, 2 series; same collection data as for holotype. RMNH.VER.19980.b to RMNH.VER.19980.k (10 whole mounts, stained with carmine and fast green, mounted in Canada balsam), RMNH.VER.19980.l (a series of sagittal sections stained with hematoxylin-eosin), RMNH.VER.19980.m (a series of transversal sections in four slides, stained with hematoxylin-eosin),

RMNH.VER.19980.n (two whole mounts in one slide, cleared and mounted in lactophenol), RMNH.VER.19980.o (a whole mount, cleared and mounted in lactophenol) and RMNH.VER.19980.p (three whole mounts in one slide, cleared and mounted in lactophenol).

Description

BODY SIZE. Live, adult animals filiform, 5.24–8.82 mm long (6.47, 1.610, 4). Fixed specimens 2.57–5.72 mm long (3.92, 0.981, 11) and 125–286 μm (198, 50.3, 10) wide at the level of the ovaries (Fig. 1A).

SENSORY ORGANS AND GLANDS. Anterior end of the body provided with short sensory bristles and a few long tactile hairs (Fig. 1B). Two types of gland cells open through the anterior epidermis: one that stains green with fast green, and another one, smaller, that does not. Encapsulated brain, anteriorly trilobate, at about 4.4% from the anterior end of the body (Fig. 1B); 54–82 μm (64, 8.8, 9) long, and 40–67 μm (50, 9.4, 9) maximum width. Statocyst with two pairs of statoliths at about 3.2% from the anterior end (Fig. 1B), 11–16 μm (14, 1.7, 7) in diameter. At least three types of parenchymal gland cells, with different affinities for the stains used, open through the epidermis of the general body surface. An aggregation of gland cells is also present in the posterior end of the body. Paracnids absent.

DIGESTIVE SYSTEM. Intestine with an anterior, hollow pre-cerebral diverticulum—“*Kopfdarm*” or “*chorda intestinalis*” sensu Ax (1957) and Ax & Ax (1969)—that arises at the level of the posterior part of the brain, continues anteriorly as a narrow tube, dorsally to the brain and the statocyst, and widens anteriorly to the latter, extending almost to the anterior end of the animal (Fig. 1B). The diverticulum is lined with a thin wall and has a wide lumen except in the region directly above the brain and statocyst; intestinal

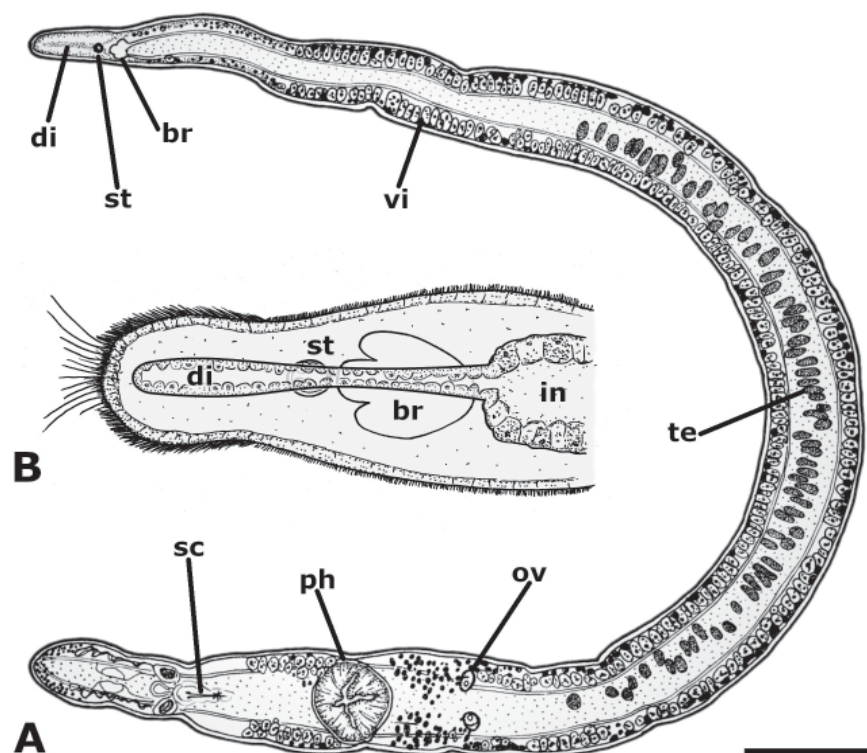


Fig. 1. *Coelogynopora kenichii* sp. nov. **A.** Live specimen. Scale bar = 250 μm . **B.** Schematic depiction of the head, showing the trilobate brain, the statocyst and the anterior intestinal diverticulum.

contents were seen in transversal sections throughout its length. The pharynx, directed ventrally, is at about 75% from the anterior end and has numerous anterior and posterior associated glands (Fig. 1A).

MALE REPRODUCTIVE SYSTEM. An irregular median row of 41–85 (55, 13.4, 10) testicular follicles extends from 26% to 66% from the anterior end (Fig. 1A). Each follicle is 42–63 μm (50, 5.3, 24) long, 15–33 μm (21, 5.2, 24) wide. Two long vasa deferentia extend posteriorly almost to the end of the body, turn 180 degrees, and run anteriorly following a sinuous course, opening into the proximal end of each of the two seminal vesicles (Fig. 2). The seminal vesicles open into a common ejaculatory duct that runs dorsally and enters the prostatic vesicle (Figs 2–3). Several prostatic glands surround the ejaculatory duct and the proximal part of the prostatic vesicle, opening into the latter. Oblique muscle fibres surround the prostatic vesicle, giving a continuous layer that is thicker ventrally. The sclerotised copulatory system lies anterior to the gonopore, is directed downwards and forwards, and opens into the anterior end of the genital atrium (Fig. 3). It consists of a stylet and 3 pairs of symmetrically arranged spines (Fig. 4). The stylet is slender, ventrally curved, and is provided with a broad base (Fig. 4A); it is 77–97 μm long (90, 6.4, 10) and 16–24 μm (20, 2.7, 10) in basal width. The three pairs of spines are 75–90 μm (84, 4.6, 10), 78–93 μm (88, 5.0, 10) and 81–97 μm (91, 5.1, 10) in length; the smallest are the farthest from the stylet. The proximal ends of all three pairs of spines are fused laterally to the base of the stylet (Fig. 4A–B); their distal ends are hooked, and their apophyses are placed at progressively longer distances from the tip (Fig. 4C).

FEMALE REPRODUCTIVE SYSTEM. The vitellaria consist of many follicles in two rows that are lateral to the intestinal sac; they extend from well behind the brain to about the level of the anterior part of the genital atrium (Fig. 1A). Two elliptical ovaries are approximately halfway between the level of the more posterior testes and the anterior end of the pharynx (Fig. 1A); they are 36–75 μm (54, 12.1, 11) long and 24–37 μm (31, 4.0, 11) wide. Two ciliated ovovitelline ducts run posteriorly and open laterally into the ciliated, caudal end of the genital atrium, just behind the gonopore. A winding, narrow genital canal leads from the posterior atrial wall to the intestinal wall; it opens into the intestinal lumen, giving a genito-intestinal connection (Fig. 3). The canal is thick-walled and lined with ciliated epithelium. The distalmost portions of the ovovitelline ducts, the genital canal and the caudal atrial wall are surrounded by glands.

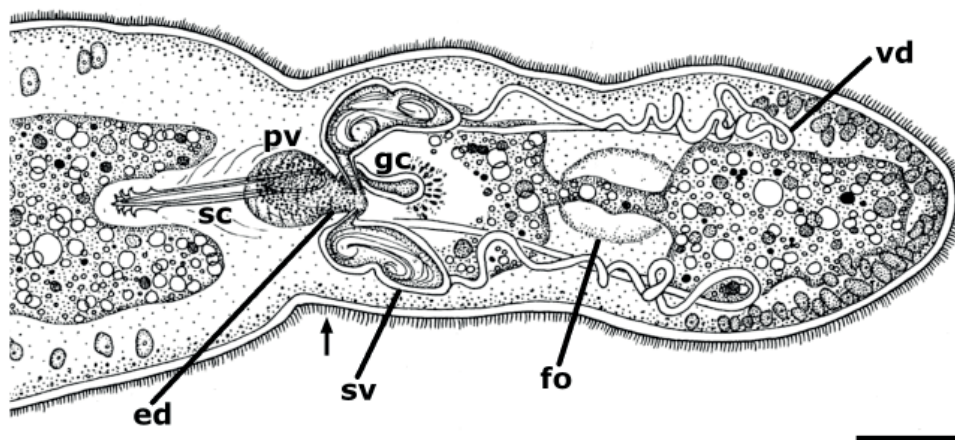


Fig. 2. Posterior end of *Coelogynopora kenichii* sp. nov. in ventral view, from a whole mount (RMNH. VER.19980.a). Scale bar = 50 μm .

The genital pore is at about 80% from the anterior end, at the level of the posterior end of the prostatic vesicle (Figs 1A, 3); it does not have an obvious sphincter.

FIBROUS ORGAN. An unknown structure was observed in every specimen studied, behind the reproductive complex and at the level of a posterior constriction of the body. In whole mounts, it appears as a well-defined, clear area (Fig. 5A); in histological sections, it is evident as a dorsal, fibrous mass between the body wall and the intestine that extends anteriorly for a short distance (Fig. 5B). It does not stain with carmine (compare the seminal vesicles with the organ in Fig. 5A), and only a few filaments are stained with hematoxylin (Fig. 5B), so the presence of sperm cells is ruled out. We cannot entirely exclude the possibility of the presence of muscle cells because of a weak affinity with the fast green stain and because the few fibres that stain with hematoxylin have a strong affinity to the dye, comparable to that of the adjacent circular muscle fibres. However, this seems unlikely because the fibres run in random directions. Interestingly, at this level the longitudinal musculature of the body wall is interrupted, and only a few circular muscle fibres are present. No parenchymal gland cells were seen associated with this structure, either.

This fibrous mass was present in all the specimens, and its position, general organisation, and staining properties were constant; it is, therefore, unlikely to be an artefact. Given the absence of an analogous structure in the relevant bibliography, and for lack of a better term, we call it the 'fibrous organ'.

Taxonomic remarks

The following combination of morphological characters places the new species unequivocally in the genus *Coelogynopora* sensu Karling (1966) and Faubel & Rohde (1998): epidermis entirely ciliated, with intracellular nuclei; sclerotised copulatory system with stylet and spines (note that hard parts are variable among representatives of the genus, to the point that they may be absent); paired seminal vesicles located posteriorly to the gonopore; paired ovaries situated anteriorly to the pharynx, with two

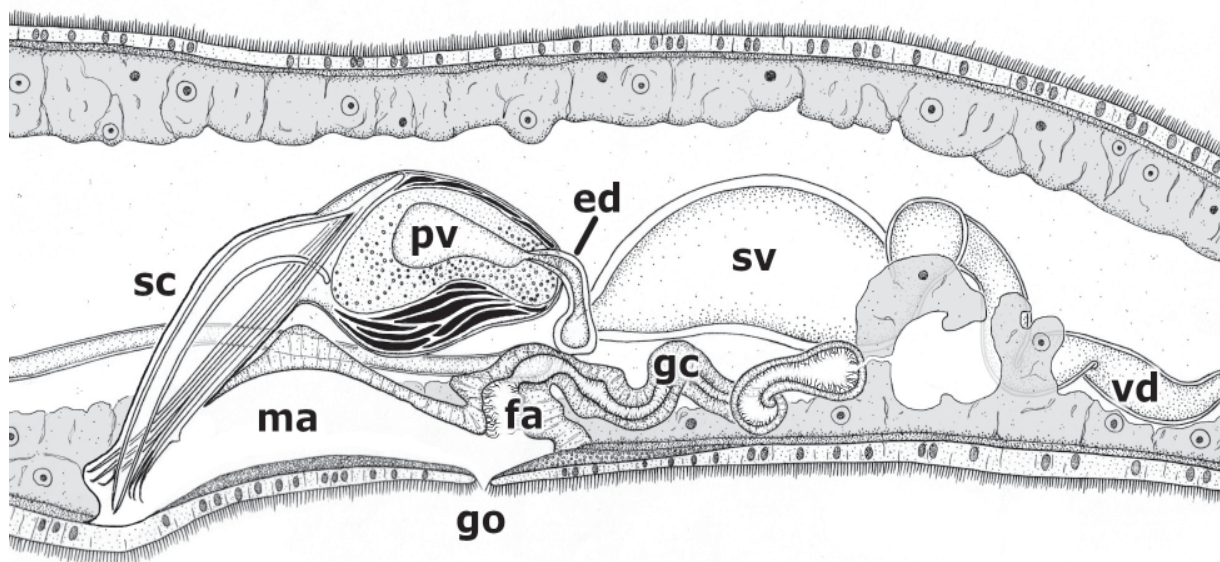


Fig. 3. Reconstruction of the reproductive complex of *Coelogynopora kenichii* sp. nov., based on a series of sagittal sections. For the sake of clarity, a single seminal vesicle and ovovitelline duct were drawn, and gland cells were omitted (RMNH.VER.19980.1). Scale bar = 30 μ m.

ovovitelline ducts that open separately into the posterior part of the genital atrium. While a bursa could not be discerned, a conspicuous female genital canal opens into the intestine through a genito-intestinal connection.

There are currently 39 known species of *Coelogynopora* (Armonies 2018; Jouk *et al.* 2019), but, besides *C. kenichii* sp. nov., only three other species have all the spines fused proximally with the base of the penial stylet, namely *Coelogynopora alata* Tajika, 1981, *Coelogynopora brachystyla* Karling, 1966 and *Coelogynopora coniuncta* Tajika, 1978. *Coelogynopora kenichii* sp. nov. differs from all of them by having a different number of spines and by the shape of the penial stylet. Tajika (1981) reported 36 spines in *C. alata*; in addition, the penial stylet has a more conical shape. *Coelogynopora brachystyla* has a single pair of spines; in addition, the penial stylet has a truncated end and bears a longitudinal slit that has been described as a “channel” (Karling 1966). The closest species to *C. kenichii* sp. nov. is *C. coniuncta*. However, the penial stylet in the latter is longer than in *C. kenichii* sp. nov., and its base is much broader (the proportion of basal length to total length is 66% in *C. coniuncta* and 29% in *C. kenichii* sp. nov.); in addition, in *C. coniuncta* there are only four spines (Tajika 1978, 1981). Besides the differences in the sclerotised copulatory system, in *C. coniuncta* the vasa deferentia enter the seminal vesicles through their anterior, ventral sides and not posteriorly as in *C. kenichii* sp. nov.; furthermore, in *C. coniuncta* the seminal vesicles extend posteriorly and, in most specimens, they were found to be fused at the level of the posterior end of the animal (Tajika 1978).

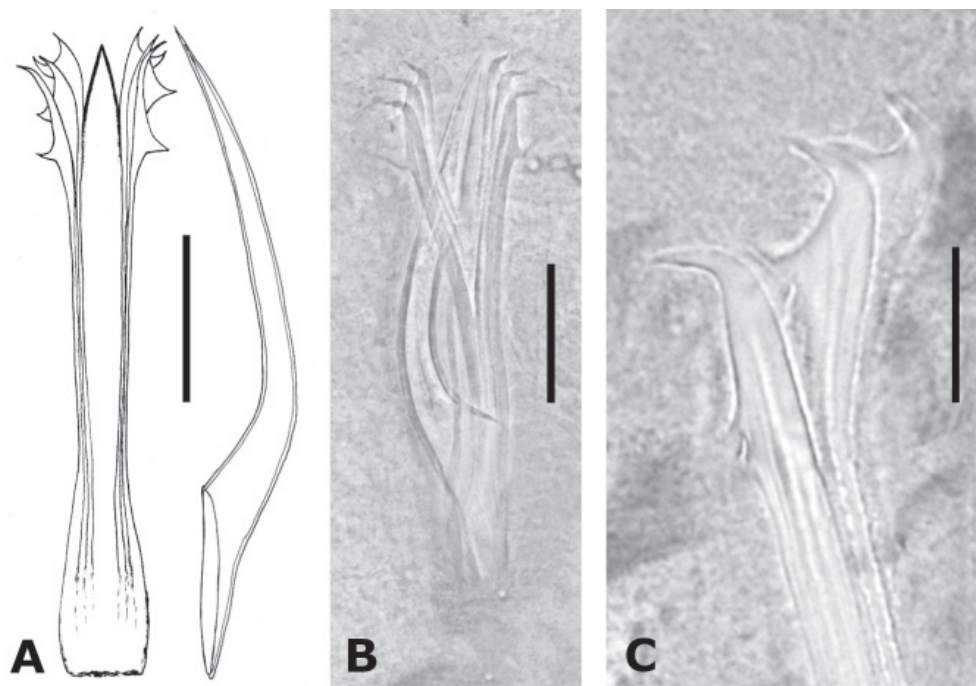


Fig. 4. Sclerotised copulatory system of *Coelogynopora kenichii* sp. nov. **A.** Schematic depiction of the stylet and the three pairs of spines (RMNH.VER.19980.p). **B.** Photomicrograph of the stylet and the three pairs of spines (RMNH.VER.19980.n). **C.** Photomicrograph of the distal portion of the three spines on one side of the stylet, showing their different morphologies (RMNH.VER.19980.o). Scale bars: A–B = 25 μ m; C = 10 μ m.

Discussion

Two out of the three species that are more similar to *C. kenichii* sp. nov. are known only from Japan, and the third one is from the Pacific coast of the United States (Karling 1966; Tajika 1978, 1981). Interestingly, if the observed morphological similitudes reflect a closer relationship between these species, *C. kenichii* would be more closely related to species from the ‘ancient’ Pacific Ocean (Panthalassan Ocean) rather than to those from the ‘younger’ Atlantic Ocean and adjacent seas.

There are many reports of Proseriata from the austral shores of the Atlantic, Pacific, Indian, and Southern Oceans, and even from the Magellan Strait (for recent examples, see Schockaert *et al.* 2009, Curini-Galletti *et al.* 2010, Curini-Galletti 2014). A few representatives of Coelogynoporidae can be found among them: *Parainvenusta* Curini-Galletti 2010 and *Stilivannuccia* Faubel & Rohde, 1998 from Australia, *Vannuccia* Marcus, 1948 from Brazil and *Carenscoilia* Sopott, 1972 from the Galapagos Islands, Ecuador (the latter three are also known from the Northern Hemisphere) (Marcus 1948; Ax & Ax 1974; Faubel & Rohde 1998; Curini-Galletti *et al.* 2010). The genus *Coelogynopora*, however, has never been found south of the Equator. The discovery of *C. kenichii* sp. nov. in the Magellan Strait (Chile) is then the first piece of evidence revealing the presence of *Coelogynopora* in the Southern Hemisphere and adds to the knowledge of the high-latitude diversity observed in the genus.

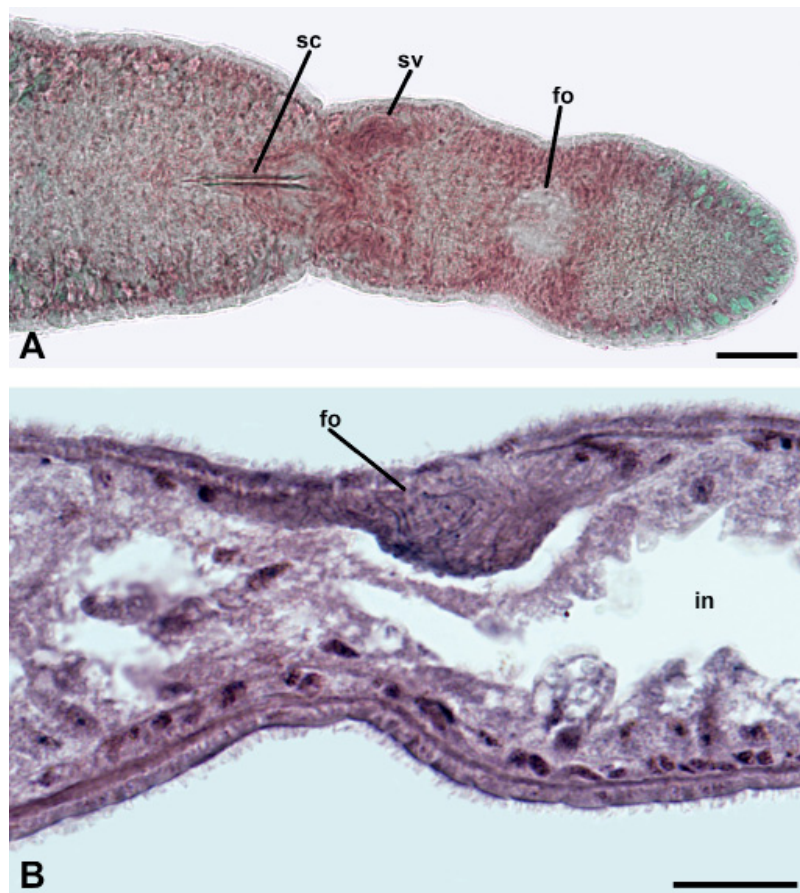


Fig 5. Fibrous organ of *Coelogynopora kenichii* sp. nov. **A.** Posterior end of the holotype stained with aceto-carmine and fast green, where the organ is visible as a well-defined, clear area behind the reproductive complex (RMNH.VER.19980.a). **B.** Sagittal section of a paratype showing the organ above the intestine (RMNH.VER.19980.1). Scale bars: A = 50 µm; B = 20 µm.

The vast biogeographic disjunction observed between the distribution areas of *Coelogynopora* in the Southern and Northern Hemispheres (Fig. 6) suggests a bipolar or amphitropical pattern of distribution (we follow Crame 1993 in that, in practice, the two patterns are inseparable). This is further supported by the fact that *C. kenichii* sp. nov. and its northern congeners are present almost exclusively in cold waters and, interestingly, in only the Northern sector of the Mediterranean Sea (Jouk *et al.* 2019).

Including the new species, there are now six known turbellarians that seem to be bipolar, spread among different high-ranking taxa: *Porrocystis assimilis* (Levinsen, 1879) and *Uncinorhynchus flavidus* Karling, 1947 (Kalyptorhynchia), *Trigonostomum venenosum* (Uljanin, 1870) (Dalytyphloplanida), *Stylostomum ellipse* (Dalyell, 1853) (Polycladida), *Allostoma durum* (Fuhrmann, 1896) (Prolecithophora) and *Coelogynopora kenichii* Volonterio & Ponce de León sp. nov. (Proseriata) (Karling 1952; Westblad 1952; Artois *et al.* 2000; Willems *et al.* 2004; this work). However, it can be argued that the sampling effort in the Southern Hemisphere has not been as extensive as in its Northern counterpart. The current knowledge of the southern distribution of these species is therefore likely to be incomplete, precluding at present any such conclusion.

Unfortunately, the molecular analysis of species of *Coelogynopora* is still in its infancy as a direct consequence of the hitherto limited number of species studied (only three out of the 39 that are now known to science) and by their limited distribution (Curini-Galletti *et al.* 2010; Laumer *et al.* 2014; Scarpa *et al.* 2017). Therefore, it is impossible to confirm whether the new species is more closely related to its Pacific congeners than to the Atlantic ones, as the morphological characters suggest.

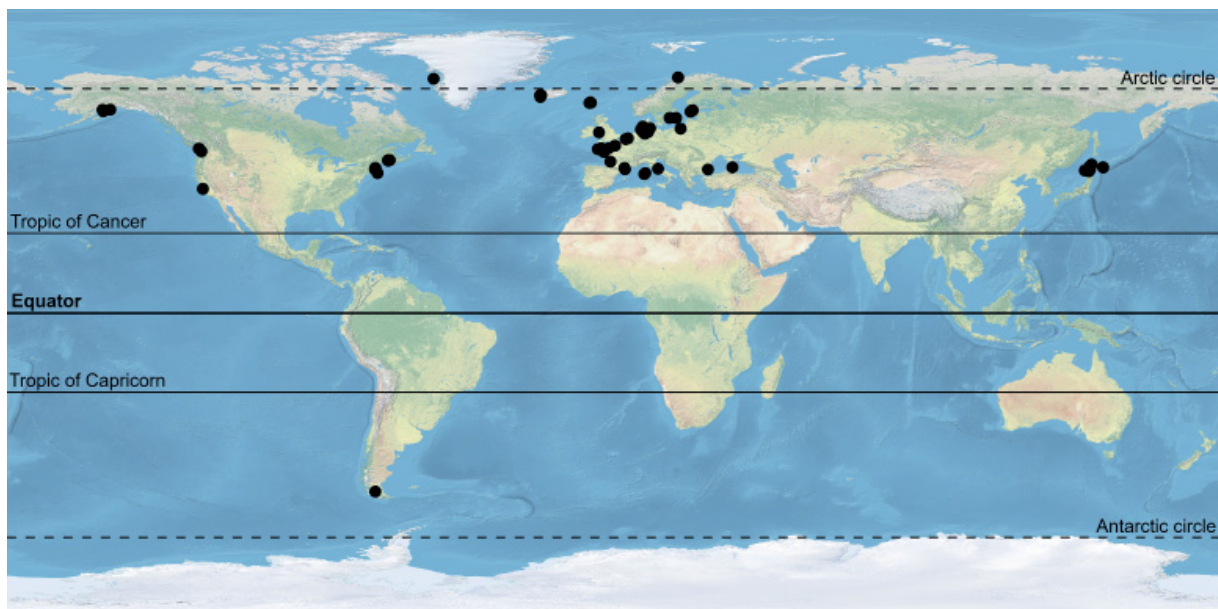


Fig. 6. Map showing the known distribution of the genus *Coelogynopora* based on all the available reports, which may be summarised by Karling (1966), Sopott-Ehlers (1976, 1992), Tajika (1978, 1981), Riser (1981), Ax & Armonies (1987), Ax (2008), Armonies (2017, 2018), Jouk *et al.* (2019), and this work.

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