Chironephthya mediterranea *n. sp.* *(Octocorallia, Alcyonacea, Nidaliidae), the first species of the genus discovered in the Mediterranean Sea*

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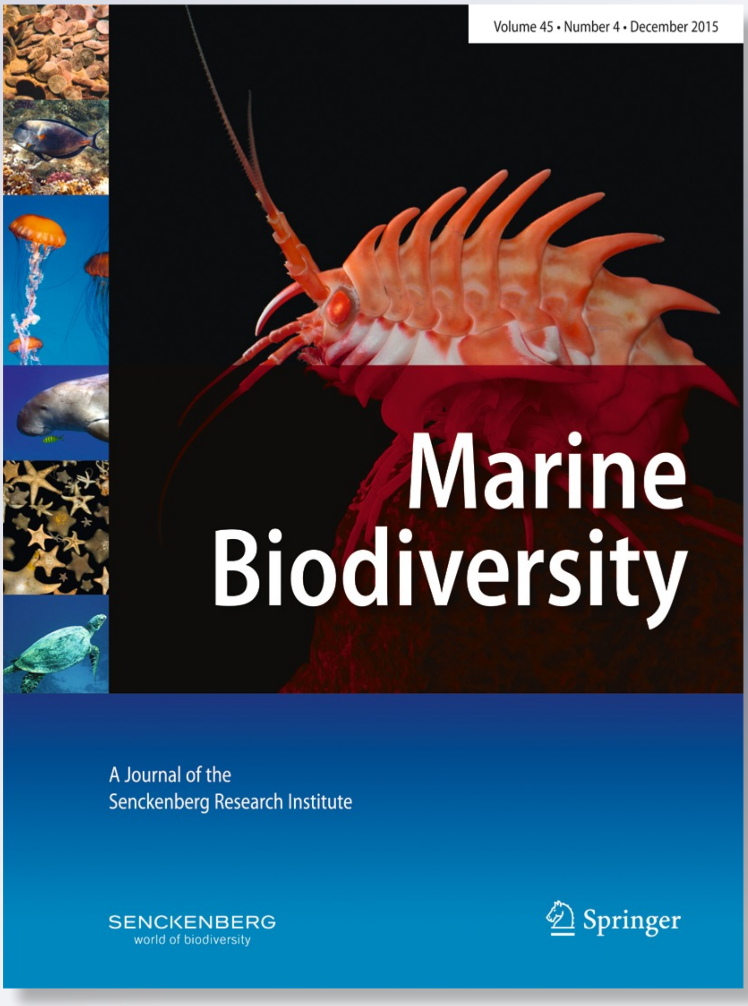

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ORIGINAL PAPER

Chironephthya mediterranea n. sp. (Octocorallia, Alcyonacea, Nidaliidae), the first species of the genus discovered in the Mediterranean Sea

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Abstract In this paper, a new species of the soft coral genus *Chironephthya* from the northwestern Mediterranean Sea is described and illustrated. *Chironephthya mediterranea* sp. nov. is formally described based on morphological, chromatic, molecular, and ecological data. A molecular comparison based on mitochondrial genes (*mtMutS+COI*) and the proposed extended barcode (*mtMutS+Igr1+COI*) relates the Mediterranean species with other *Chironephthya* species. The new Mediterranean species is compared with the available information and type materials from its Atlantic congeners, *Chironephthya agassizii* and *Chironephthya caribaea*. This is the first time that a species of this genus is reported from the Mediterranean Sea. Along with *Nidalia studeri*, this is the second species of the family Nidaliidae found in this biogeographic region. Both nidaliid species were collected in the shelf break area. The present report adds to previous knowledge of *Chironephtya* and its global distribution. Although the molecular analyses carried out do not support the monophyly of the family Nidaliidae, they clearly indicate a close relationship between the genera *Siphonogorgia* and *Chironephthya*, which are both in need of revision.

Keywords *Mediterranean* · *Biodiversity* · Chironephthya · *Nidaliidae* · *Soft corals*

Introduction

Although our current estimates are probably far from the reality, the family Nidaliidae includes more than 70 nominal species distributed in eight genera (e.g., Studer, 1887; Wright and Studer 1889; Kükenthal 1906; Harrison 1908; Kükenthal 1910; Verseveldt and Bayer 1988; Williams 2000; and others). Many species and several genera of the family are in need of revision. The most species-rich genus is *Siphonogorgia* Kölliker, 1874, with about two-thirds of the total species in the family; followed by *Nidalia* Gary, 1835 (about 18 spp.); *Chironephthya* Studer, 1887 (about 11 spp.); *Pieterfaurea* Verseveldt and Bayer, 1988 (5 spp.); *Nephthyigorgia* Kükenthal, 1910 (4 spp.); *Nidaliopsis* Kükenthal, 1906 (3 spp.); *Agaricoides* Simpson, 1905 (1 sp.); and finally, *Orlikia* Malyutin, 1993 (1 sp.). With the exception of *Nidaliopsis*, all genera are monomorphic, and most of them have relatively simple colonies (unbranched torch- to mushroom-shaped, digitated, palmated, or with few rounded lobes). Only *Siphonogorgia* and *Chironephthya* have branched tree-like colonies. Although the genus *Nidaliopsis* is known only from tropical West African coasts, most of the genera of the family have an Indian Ocean or Indo-West Pacific distribution. The genera *Nidalia* and *Chironephthya* are known from Indian Ocean, West Pacific, and West Atlantic localities (e.g., Harrison 1908; Deichmann 1936; Bayer, 1961; Fabricius and Alderslade, 2001).

Recently, two species of the genus *Nidalia* were reported from the southern Azores Islands and the western

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Mediterranean Sea, respectively (López-González and Gili 2008; López-González et al. 2012). In addition, Pérez et al. (2011) reported the genera *Nidalia* and *Chironephthya* from the Brazilian coast. An additional putative species attributable to the genus *Chironephthya* has been reported from the Canary Islands (East Atlantic) (Brito and Ocaña 2004) as *Siphonogorgia* sp. The recent rediscovery of *Nidalia studeri* in the western Mediterranean basin (López-González et al. 2012) represented the first record of Nidaliidae for the whole Mediterranean sea.

During the general community surveys carried out within the LIFE+INDEMARES project in the Menorca Channel, a previously unreported community dominated by whitish tree-like soft corals and gorgonians was discovered on the deep continental shelf and upper continental slope as well as the flanks of a submarine canyon (Grinyó et al., unpublished data) (Fig. 1). During the first cruises, this community was only video-recorded. During subsequent surveys, specimens of the key species were collected. The collection of the relatively large whitish soft coral was performed in August 2012 during the INDEMARES-CSIC VI, MENORCA IV cruise. The preliminary study of the specimens on board confirmed that they belonged to the nidaliid genus *Chironephthya*, not previously reported in Mediterranean waters.

The genera *Chironephthya* and *Siphonogorgia* are considered closely related (Fabricius and Alderslade 2001), and some additional species will likely be reassigned from *Siphonogorgia* to *Chironephthya* after the revision of both genera has been completed. At the moment, both genera are considered separate, at least from a morphological point of view. The goal of the present contribution is the description of a new *Chironephthya* species based on morphological and molecular data; to date, this is the only species is in the Mediterranean Sea. In addition, the possible phylogeographic implications as a link between the previously known set of West Atlantic and Indo-Pacific species is discussed in a global scenario.

Material and methods

The material studied here was collected during the "INDEMARES-CSIC VI, MENORCA IV" cruise on board the R/V *García del Cid*, using the articulated arm of the ROV (Remotely Operated Vehicle) *Nemo*. The main goal of this cruise was to study the distribution patterns and diversity of the major benthic communities of the continental shelf and slope at the Menorca Channel and Cap de Creus peninsula (northwestern Mediterranean Sea). Video materials were examined to establish the geographic and bathymetric distribution of the new *Chironephthya* species in the study area.

Additional information on the communities was obtained with the manned submersible *JAGO* (cruise INDEMARES-CSIC IV, MENORCA II) and the ROV *Nemo* (cruises INDEMARES-CSIC V, Menorca III, and INDEMARES-CSIC VI, Menorca IV) vessels. These cruises were carried out within the framework of the LIFE+INDEMARES project. The relevant information of all video and collecting stations is compiled in Table 1.

The soft coral colonies were fixed in absolute ethanol for morphological examination and molecular analysis. Fragments of different parts of the colony were prepared for study by SEM, employing the usual methodology as described by different authors (e.g., Bayer and Stefani 1988), and permanent slides were made for light microscopy observation. About thirty sclerites from each type and part of the colony and polyp (crown, introvert, points, surface of the stalk, etc.) were measured by optic microscopy. During this process it was attempted to include the complete range of sizes and to identify the different sclerite forms. All sclerite size measurements and illustrations are from the holotype. The colony and sclerite terminology described here mainly follows Bayer et al. (1983). The material studied here has been deposited at the Museu de Ciències Naturals in Barcelona (MCNB).

Total genomic DNA was extracted from the ethanol-preserved holotype and paratype using the E.Z.N.A. DNA kit (Omega Bio-Tek), following the manufacturer's instructions. The *mtMutS* (*MSH1*), and *Igr1+COI* mitochondrial regions were sequenced for comparative purposes as an octocoral barcode proposed by McFadden et al. (2011). The start of the *mtMutS* (MSH1) region was amplified using the primers ND42599F and MUT3458R (France and Hoover 2002; Sánchez et al. 2003). *The Igr1+COI* region was amplified using the primers COII8068F and COIOCTR (France and Hoover 2002; McFadden et al. 2004). Each PCR used 1 U/μl of DNA Stream Polymerase (BIORON), 0.2 mM of dNTPs, 0.3 μM of each primer, and approximately 25 ng of genomic DNA, and was brought to a final volume of 25 μL with H_2O. *MtMutS* PCR was carried out using the following cycle profile: initial denaturation at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The *Igr1+COI* PCR used the same cycle profile but 58 °C as annealing temperature and 40 s for extension duration on each of the 35 cycles. PCR products were purified using the NucleoSpin® Extract II DNA purification kit following the manufacturer's instructions. Purified products were electrophoresed on an ABI PRISM® 3730xl genetic analyzer, and sequence traces were edited using Sequencher™ version 4.0. The obtained sequences were compared with homologous sequences from the EMBL-Bank of Nidaliidae species (Table 2).

In a preliminary *mtMutS* comparison (not shown, ML, T92+G+I, 1000 bootstrap) including more than 300 octocoral species, a *Chironephthya-Siphonogorgia* clade is well-supported (bootstrap 100 %). However, there is not a

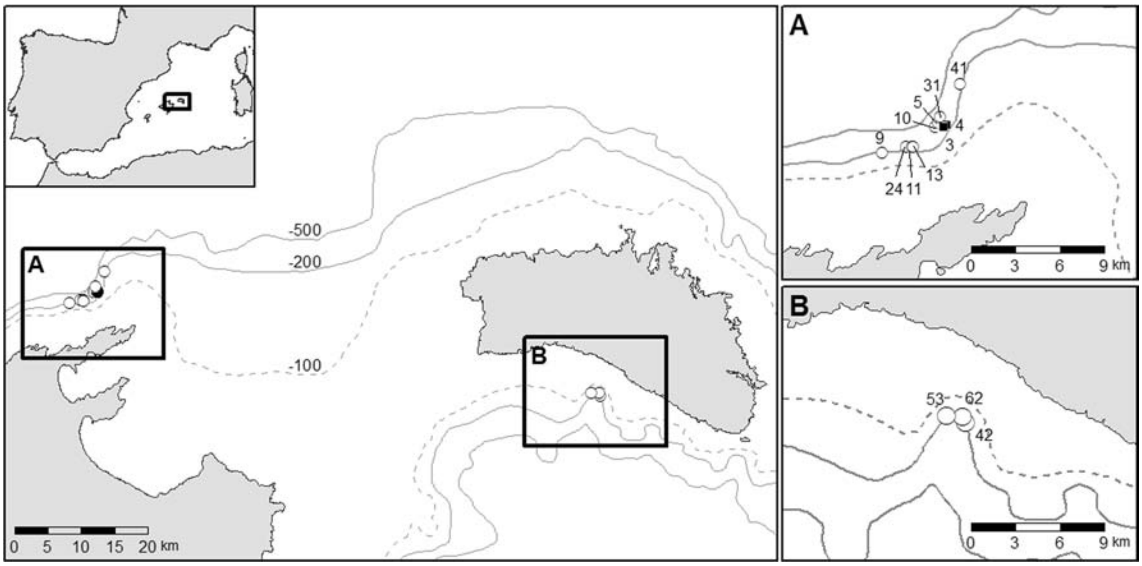

Fig. 1 Distribution of the stations where *Chironephthya mediterranea* n. sp. was video-recorded (*open circles*) or video-recorded and collected (*solid circles*). Numbers in the stations correspond with the station numbers in the different cruises (see Table 1 for all additional sampling data). **a** Cap Formentor area; **b** Son Bou Canyon area

supported sister group to be used as an outgroup for subsequent internal comparison for all nidaliid sequences. This *Chironephthya-Siphonogorgia* clade is not grouped with the other nidaliid genera (*Nidalia*, *Nephthyigorgia*, *Pieterfaurea*) available for this comparison. Thus, the currently considered family Nidaliidae is not supported by this analysis. As an example of this trend, a *mtMutS+COI* analysis on groups of selected soft coral species from the families Alcyoniidae, Paralcyoniidae, Xeniidae, and Nephtheidae is shown here using the divergent sequence of a cornulariid species as outgroup (see Fig. 11, Table 2). Thus, as the goal of this paper is to explore the relationships of the Mediterranean species with their closest nidaliid forms, we use the set of available sequences of *Nidalia-Nephthyigorgia-Pieterfaurea* as outgroup to explore *Chironephthya-Siphonogorgia* species relationships.

The alignment of the concatenated *mtMutS+COI* and *mtMutS+Igr1+COI* sequences were carried out using MUSCLE, implemented in MEGA5 (Tamura et al. 2011). The analyses involved 12 nidaliid nucleotide sequences. All positions containing missing data were eliminated. Positions containing internal gaps were maintained but not considered in the analysis. The *mtMutS+COI* concatenated sequences (11 nidaliidand13 non-nidaliid sequences) had 1,424 positions, with a total of 534 variable and 285 parsimony-informative sites. The *mtMutS+COI* concatenated sequences (only the 11 nidaliid sequences) had 1,414 positions, with a total of 246 variable and 73 parsimony-informative sites. The *mtMutS+*

Table 1 Localities where *Chironephthya mediterranea* n. sp. was video-recorded as well as (in bold) the collected type material used in this study

Cruise	Date	Station	Coordinates	Depth Range (m)
INDEMARES-CSIC IV, MENORCA II	01/04/11	10	40°00′35.880″N 3°08′36.930″E	159–174.4
INDEMARES-CSIC IV, MENORCA II	02/04/11	24	39°59′52.800″N 3°07′12.601″E	145.5
INDEMARES-CSIC IV, MENORCA II	03/04/11	41	40°02′10.980″N 3°09′47.880″E	116.1–126
INDEMARES-CSIC V, MENORCA III	02/11/11	30	40°00′44.220″N 3°08′45.720″E	136.2–186
INDEMARES-CSIC VI, MENORCA IV	**26/06/12**	**3**	**40°00′34.934″N 3°08′59.572″E**	**156–172.2**
INDEMARES-CSIC VI, MENORCA IV	**26/06/12**	**4**	**40°00′40.113″N 3°09′07.176″E**	**171.6 174.2**
INDEMARES-CSIC VI, MENORCA IV	27/06/2012	9	39°59′37.752″N 3°06′03.660″E	145–150
INDEMARES-CSIC VI, MENORCA IV	27/06/2012	11	39°59′52.338″N 3°07′31.056″E	118.1–140.5
INDEMARES-CSIC VI, MENORCA IV	27/06/2012	13	39°59′52.020″N 3°07′33.240″E	118.1–129.8
INDEMARES-CSIC VI, MENORCA IV	29/06/2012	31	40°00′57.000″N 3°08′52.020″E	13004–174.2
INDEMARES-CSIC VI, MENORCA IV	30/06/12	42	39°51′46.806″N 4°02′13.200″E	175.2–181.9
INDEMARES-CSIC VI, MENORCA IV	02/07/12	53	39°52′01.476″N 4°02′04.278″E	133.9–197.9
INDEMARES-CSIC VI, MENORCA IV	03/07/12	62	39°52′04.320″N 4°01′17.220″E	124.4–149.4

Table 2 Octocoral species involved in the molecular comparisons carried out in this paper

Family // Species	Catalog Nos. (or additional information)	*mtMutS* (=*MSH1*)	*Igr+COI*
Nidaliidae			
Chironephthya mediterranea n. sp. (Holotype)	MCNB 2013-0001	KM584053	KM584054
Chironephthya mediterranea n. sp. (Paratype)	MCNB 2013-0002	KM584055	KM584056
Chironephthya caribaea (Deichmann 1936)	USNM 1025683	KM584051	KM584052
Chironephthya sp. (1)	CSM-2010	GQ342513	GQ342440
Chironephthya sp. (2)	NTM-C012426	DQ302830	
Chironephthya sp. 1	ZMTAU CO34223	GU356025	GU356002
Chironephthya sp. 2	ZMTAU CO34222	GU356024	GU356001
Chironephthya sp. 3	ZMTAU CO34226	GU356026	GU356003
Chironephthya sp. A (1)	RMNH Coel. 40888	KF915576	KF955039
Chironephthya sp. A (2)	RMNH Coel. 40892	KF915577	NA
Chironephthya sp. A (3)	RMNH Coel. 40893	KF915578	KF955040
Chironephthya sp. A (4)	RMNH Coel. 40894	KF915579	KF955041
Chironephthya sp. A (5)	RMNH Coel. 40902	KF915580	KF955042
Chironephthya sp. B (1)	RMNH Coel. 40889	KF915581	KF955043
Chironephthya sp. B (2)	RMNH Coel. 40896	KF915582	KF955044
Chironephthya sp. B (3)	RMNH Coel. 40898	KF915583	KF955045
Chironephthya sp. B (4)	RMNH Coel. 40901	KF915584	KF955046
Chironephthya sp. C (1)	RMNH Coel. 40890	KF915585	KF955047
Chironephthya sp. C (2)	RMNH Coel. 40895	KF915586	KF955048
Chironephthya sp. C (3)	RMNH Coel. 40899	KF915587	KF955049
Chironephthya sp. D (1)	RMNH Coel. 40891	KF915588	KF955050
Chironephthya sp. D (2)	RMNH Coel. 40897	KF915589	KF955051
Chironephthya sp. D (3)	RMNH Coel. 40900	KF915590	KF955052
Nephthyigorgia sp. (1)	RMNH Coel.40819	JX203804	JX203864
Nephthyigorgia sp. (2)	NTM-C011345	DQ302831	
Nidalia dissidens Verseveldt and Bayer 1988	LII-10-566	KC984602	KC984638
Nidalia sp.	NTM-C014876	DQ302828	
Pieterfaurea khoisaniana (Williams, 1988)		GQ342510	GQ342437
Pieterfaurea sp.	NTM-C013943	DQ302829	
Siphonogorgia godeffroyi Kölliker, 1874	RMNH Coel.40833	JX203803	JX203860
Siphonogorgia sp.	NTM-C011159	DQ302832	
Siphonogorgia sp. A (1)	RMNH Coel. 41041	KF915768	KF955244
Siphonogorgia sp. A (2)	RMNH Coel. 41044	KF915769	KF955245
Siphonogorgia sp. A (3)	RMNH Coel. 41045	KF915770	KF955246
Siphonogorgia sp. A (4)	RMNH Coel. 41048	KF915771	KF955247
Siphonogorgia sp. A (5)	RMNH Coel. 41051	KF915772	KF955248
Siphonogorgia sp. A (6)	RMNH Coel. 41053	KF915773	KF955249
Siphonogorgia sp. B (1)	RMNH Coel. 41042	KF915774	KF955250
Siphonogorgia sp. B (2)	RMNH Coel. 41043	KF915775	KF955251
Siphonogorgia sp. B (3)	RMNH Coel. 41046	KF915776	KF955252
Siphonogorgia sp. B (4)	RMNH Coel. 41049	KF915777	KF955253
Siphonogorgia sp. B (5)	RMNH Coel. 41050	KF915778	KF955254
Siphonogorgia sp. B (6)	RMNH Coel. 41052	KF915779	KF955255
Siphonogorgia sp. C	RMNH Coel. 41047	KF915780	KF955256
Siphonogorgia sp. D	RMNH Coel. 41054	KF915781	KF955257
Siphonogorgia cf *godeffroyi*	RMNH Coel. 41055	KF915767	KF955243
Alcyoniidae			
Alcyonium acaule Marion, 1878	MAR.9.AA3 (*mtMutS*)	GU355959	GU355942

Table 2 (continued)

Family // Species	Catalog Nos. (or additional information)	*mtMutS* (=*MSH1*)	*Igr+COI*
	BAN.AA1 (*Igr1+COI*))		
Alcyonium roseum van Ofwegen et al., 2007		GQ342468	GQ342383
Lobophytum pauciflorum (Ehrenberg, 1834)	NTM-C014161 (*mtMutS*)	DQ280575	GQ342394
Sinularia leptoclados (Ehrenberg, 1834)	ZMTAU:CO35308	KC542857	KC542874
Sinularia verseveldti van Ofwegen, 1996	RMNH:Coel.40843	KC542860	KC542881
Paralcyoniidae			
Ceeceenus quadrus van Ofwegen and Benayahu, 2006		GQ342514	GQ342442
Studeriotes sp.	*CSM-2010*	GQ342515	GQ342443
Nephtheidae			
Scleronephthya gracillimum (Kükenthal 1906)		GU047879	GU047879
Dendronephthya klunzingeri (Studer, 1888)	ZMTAU CO34109	GU356018	GU355995
Nephthea acuticonica Verseveldt, 1974	ZMTAU CO34070	GU356023	GU356000
Xeniidae			
Heteroxenia fuscescens (Ehrenberg, 1834)		GQ342528	GQ342462
Ovabunda obscuronata (Verseveldt and Cohen, 1971)	ZMTAU CO34077	GU356027	GU356004
Cornulariidae			
Cornularia pabloi McFadden and van Ofwegen, 2012	USNM 1178390	JX203792	JX203847

Materials in bold are species sequenced for this study. Note that all GenBank sequences are considered here with the names as they appear in GenBank and their original publications (including numbers or letters). For sequences with duplicate complete names, we have include (1), (2), (3), etc., for the purpose of correctly identifying the sequence in Figs. 11 and 12 and Suppl. Fig 1. Abbreviations for institutions or personal collections where voucher material from which molecular information was used in this paper is deposited are as follows: CSM, Collection of Catherine S. McFadden; MCNB, Museu de Ciències Naturals de Barcelona; NTM, Museum and Art Gallery of the Northern Territory, Australia; RMNH, Naturalis Biodiversity Center, formerly Rijksmuseum van Natuurlijke Historie, Leiden; USNM, National Museum of Natural History, Smithsonian Institution; ZMTAU, Zoological Museum, University of Tel Aviv.

Igr1+COI concatenated sequences (only the 11 nidaliid sequences) had 1,527 positions, with a total of 279 variable and 79 parsimony-informative sites. After alignment, the best nucleotide substitution model was selected using ModelTest implemented in MEGA5, according to Akaike information criterion (AIC) and hierarchical likelihood ratio test (hLRT) values. The phylogenetic reconstruction was obtained applying maximum likelihood (ML) and Bayesian inference methods. The maximum likelihood method was developed in MEGA5, and based on the T92+G model (Tamura 1992) in all data sets. The bootstrap consensus trees were inferred from 1,000 replicates (Felsenstein 1985). The Bayesian inference was carried out in MrBayes v3.1.2 program (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), using the model GTR+G (lset nst=6 rates=gamma), 10^6 generations, and discarding 25 % initial trees.

An additional exploration of the affinities of the new *Chironephthya* species, but only based on the *mtMutS* marker, was carried out with the alignment of 16 nidaliid sequences (two of them *Siphonogorgia* spp.) (711 positions, 178 variable, and 97 parsimony-informative sites). For this *mtMutS* hypothesis, we used the same phylogenetic procedure as for the previous molecular comparison (ML, T92+G, 1000 bootstrap). In this case, all available nidaliid genera were used as outgroup to explore the relationship between *Chironephthya* and *Siphonogorgia* species.

For comparative purposes, the types and additional material of Caribbean species of *Chironephthya* were consulted, diagnostically described, and illustrated (Suppl. Appendix 1).

Additional subsamples for molecular studies were also analysed, although only *Siphonogorgia caribaea* USNM 1025683 produced a good total DNA extraction and subsequent amplification of both mitochondrial markers examined here.

Results

Order Alcyonacea
Family Nidaliidae Gray, 1869
Genus *Chironephthya* Studer 1887

Diagnosis

Nidaliid with colonies branched, tree-like, and erect. Stalk attached to hard substratum. Branches rigid and brittle due to the presence of densely packed sclerites. Polyps on stem and branches, never restricted to terminal ends of branches. Main stem partially flattened in the preserved state, with wide longitudinal canals internally separated by thin walls. Polyps monomorphic, placed upwards. Calyces prominent, shelf-like, with abaxial side supported by large sclerites split into two groups as two distinct calycular teeth. Anthocodiae completely retractile into calyces, but often partially extended in preserved material. Crown and points well-developed. Aboral side of the tentacles with warty plates. Pinnules with elongated platelets. Pharynx with densely distributed thorny rods.

Remarks

The type species of this genus was originally not designated by Studer (1887: 20). However, when Wright and Studer (1889: 230) again published the diagnosis of *Chironephthya* as "n. gen.", they described *Chironephthya dipsacea* (Wright and Studer 1889), together with two other new *Chironephthya* species, and all three from Japanese waters.

As commented above, the genera *Siphonogorgia* and *Chironephthya* seem to be closely related. As originally stated by Studer (1887) and Wright and Studer (1889: 230), both genera differ in the distribution of the polyps within the colony (restricted to the terminal branches in *Siphonogorgia*, also distributed on the stem and branch surfaces in *Chironephthya*). This is one of the most visible characteristics to distinguish between these two nidaliid genera. Different authors have suggested that both names should be synonymised under *Siphonogorgia* (Chalmers 1929; Bayer 1961), whereas other authors observed enough difference to disagree (Verseveldt and Bayer 1988; Fabricius and Alderslade 2001; Cairns and Bayer 2009). For a detailed review of the history of the genus *Chironephthya* since its original description, see Pérez et al. (2011).

In addition to the distribution of the polyps within the colony, Fabricius and Alderslade (2001) also pointed out the different architecture of the calyces in the two genera. In *Chironephthya* the calyces are distinct, shelf-like, with only lateral and abaxial sides developed, adaxial side nonexistent, and strongly supported abaxially by a set of long spindles that are split into two groups. In the case of *Siphonogorgia*, the calices are formed by small spindles and rods, and are reduced in size, often as a narrow rim around the retracted anthocodia. Harrison (1909) discussed the larger number and larger size of the longitudinal canals in the stem of *Chironephthya* in comparison with those in *Siphonogorgia*. This author also discussed the fact that the stem and branches of *Siphonogorgia* are solid, round, and cylindrical, while the stem and branches in *Chironephthya* have a less solid appearance and become somewhat flattened and deeply grooved, with terminal branches much more slender than those of *Siphonogorgia*. In addition, the interior of the stem and branches are studded with numerous sclerites of different sizes in *Siphonogorgia*, but are practically without sclerites in *Chironephthya* (Harrison 1909). The new species of soft coral collected in the western Mediterranean is consistent with the characteristics assigned to the genus *Chironephthya* by Harrison as well as Fabricius and Alderslade.

The Indo-Pacific species classifications of *Chironephthya* are in need of revision. Harrison (1909) lists up to 18 putative species (not including Caribbean species described later by Deichmann [1936] as *Neospongodes*). Currently, the number of valid species in this genus is uncertain. Recent databases estimate this number to be 11 (Williams and Cairns 2007) or even less, as is the case in the WoRMS database, which currently (June 2014) lists only two Caribbean species (van Ofwegen 2014a). This last record can be explained, however, since most of the Indo-Pacific species are still immersed within the genus *Siphonogorgia* in that database (van Ofwegen 2014b). As mentioned above, the revision of the related genus *Siphonogorgia* is necessary because some *Chironephthya* species may still be cryptic under the former genus. Clearly, the available morphological descriptions are not adequately detailed, lacking measurements of sclerites, especially from the anthocodia, where several specific characteristics have proven useful in other nidaliid genera (such as *Nidalia*).

Chironephthya mediterranea *sp. nov*

(Figures. 2, 3, 4, 5, 6, 7, 8, 9, and 10)

Material examined

Holotype: (MCNB 2013–0001), "INDEMARES-CSIC VI, Menorca IV" cruise, Menorca Channel, Northwestern Mediterranean, stn. 4, 40°00′34.934″N 03°08′59.572″E, 174 m depth, 26 Jun. 2012, collected by ROV, 1 whole colony.

Paratypes: (MCNB 2013–0002), "INDEMARES-CSIC VI, Menorca IV" cruise, Menorca Channel, Northwestern Mediterranean, stn. 3, 40°00′40.113″N 03°09′07.176″E, 154 m depth, 26 Jun. 2012, collected by ROV, 2 colonial fragments.

Description of the holotype

The colony is branched, tree-like, and erect (Figs. 2a and b), 95 mm in length in preserved state. The stalk is attached to hard substratum, not extending beyond the basal stalk dimensions. The branches are rigid and brittle due to the abundance of large and densely packed sclerites in the colony wall. The polyps are distributed along the entire length of the stem and branches, sometimes crowded at the ends but never restricted to them. Some solitary polyps may also be present on the main stem. The polyps are oriented upwards, completely retractile into the calyces, but often partially extended in the preserved material. The main stem is 15 × 8 mm measured 15 mm from

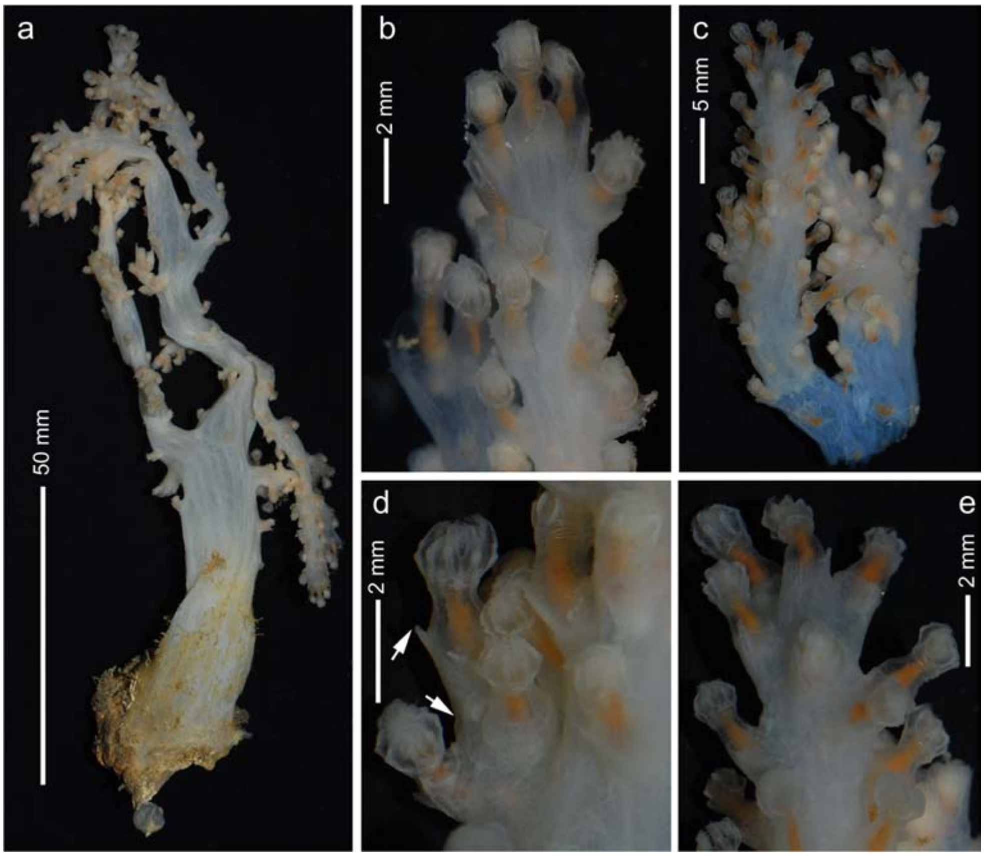

Fig. 2 *Chironephthya mediterranea* n. sp. Holotype (MCNB 2013–0001). **a** Whole colony; **b** detail of distal branches. Paratype (MCNB 2013–0002): **c** Whole fragment; **d** and **e**, details from **c**. White *arrows* in figure **d** indicate the measured aboral side of calyx. All photographs were taken on living materials. Note the orange colour of the pharynx from the transparency of the wall of polyps

substratum, partially flattened in the preserved specimen, and externally covered by a brownish periderm. The stem has about 10 wide longitudinal canals internally (Fig. 3a), and the stem wall is relatively thin, 0.5 mm thickness, with densely packed sclerites (Fig. 3b). Canals are separated by thin walls with scarce sclerites (Fig. 3c). The branches secondary to distal branches are progressively smaller in size with branching order, but in general have a cylindrical aspect. The distal branches are about 5 mm in diameter, including calyces. The density of cortical sclerites in distal branches is lower in comparison with basal parts of the colony. Sclerites are nearly absent in distal branches internally.

The calyces are prominent, shelf-like, up to 2 mm along the abaxial side, on the adaxial side nearly absent (Figs. 2d and 4a, c), only slightly distinct in the distal-most polyps of the distal branches (Fig. 2b). The abaxial side is supported by large sclerites split into two groups as two distinct calycular teeth, with 2–4 (often 3) large sclerites per point, plus

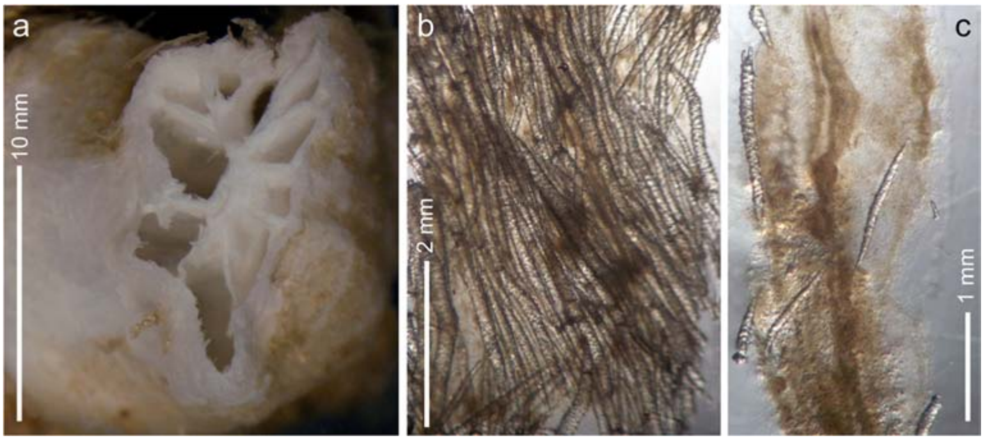

Fig. 3 *Chironephthya mediterranea* n. sp. Holotype. **a** Main stem sectioned transversely about 15 mm from the substratum (note the relatively thin exterior wall and wide longitudinal canals); **b** Portion of the wall showing densely packed warty spindles; **c** Portion of the internal walls between longitudinal canals showing scarce warty spindles

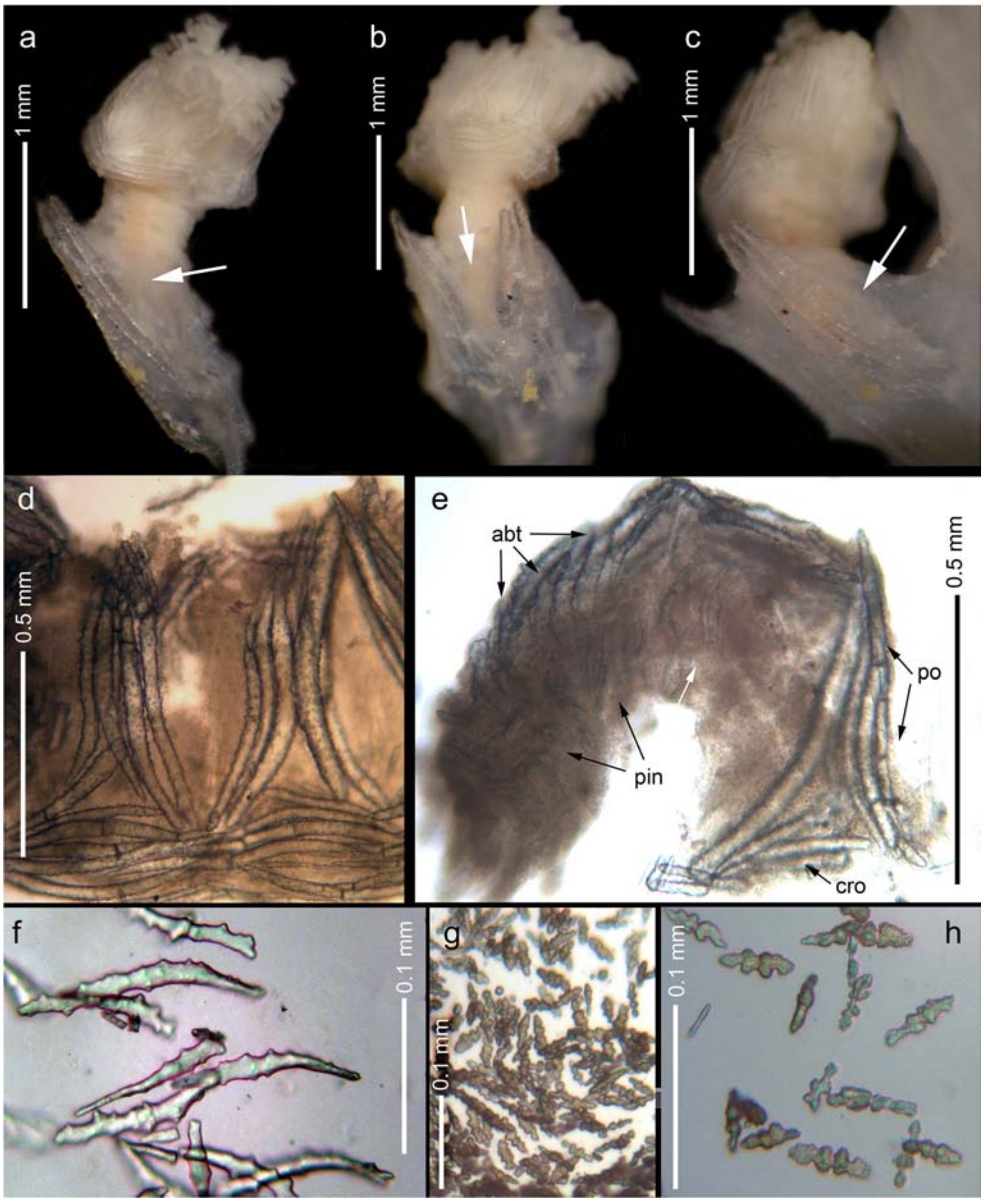

Fig. 4 *Chironephthya mediterranea* n. sp. Holotype. **a**–**c** Polyps showing calycular teeth and anthocodia with crown and points; **d** Crown and points; **e**Dissected tentacle showing (from *right to left*) part of the crown (*cro*), point (*po*), aboral side of tentacle with platelets (*abt*), and pinnules with elongated platelets (*pin*); **f** Elongated platelets from pinnules; **g** Dense group of sclerites from the pharynx; **h** isolated sclerites from the pharynx. *White arrows* in **a**, **b**, and **c** indicate sclerite-less areas in the calyx

additional, shorter and thinner sclerites among them (Figs. 4b and c). In distal branches, the sclerites are thorny spindles up to 2.8 mm in length and 0.15 mm in width (Fig. 5).

In the anthocodiae, the introvert is lacking sclerites, but crown and points are well-developed. The polyps are 1.2 mm in diameter at crown level. The crown has 4–7 rows of transversely placed curved thorny spindles (Figs. 4d and 6a), up to 1.1 mm in length (measured between points, as an arc bowstring), often nearly smooth on the interior (concave) side. The points are 1–3 pairs of curved thorny spindles, blunt basally, up to 1 mm in length (Figs. 4d, e and 6b), some of these smaller, up to 0.25 mm in length. Sclerites as secondary points were observed; sporadic single sclerites of 0.1 mm could be found, but were usually absent. The aboral side of the tentacles has transversely or slightly obliquely placed warty plates up to 0.36 mm in length (Figs. 4e and 7a). The pinnules, largely the basal ones, have elongated platelets up to 0.21 mm in length (Figs. 4e and f and 7b and d).

The sclerites in the pharynx are densely distributed (Fig. 4g) as thorny rods, often with a marked waist and several prominent constrictions (Figs. 4h, 7c, and 8), up to 0.05 mm in length. The wall of the stalk has warty spindles densely packed, primarily arranged longitudinally, but also with some sclerites oriented oblique to the main axis (Figs. 3b and 9a), up to 3.5 mm in length and 0.22 mm in width. The interior of the stalk has a few sparsely distributed spindles (of similar size to those of the surface of the stalk) in the internal walls between longitudinal cavities (Figs. 3c and 9b). The stalk, main stems, branches, and polyps of living and preserved colony are whitish in colour, except for the pharynx, which is orange

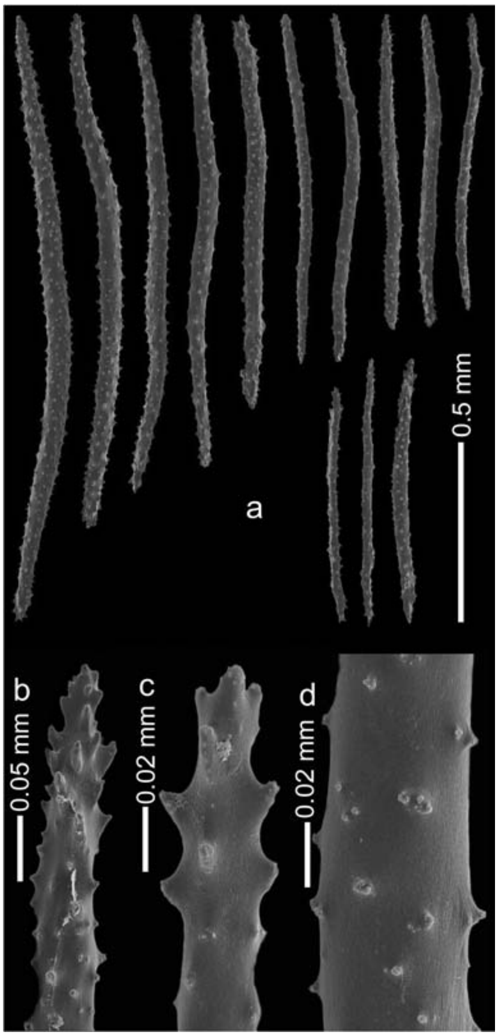

Fig. 5 *Chironephthya mediterranea* n. sp. Holotype. **a** Calycular and coenenchymal sclerites from distal branches; **b**–**c** Distal part of two sclerites showing different orientation of the spines; **d** Surface ornamentation of a spindle

due to the colour of the sclerites (Figs. 2a and b); all other sclerites in the colony are colourless. The basal part of the stalk is brownish due to the presence of a periderm.

Variations

The general structure of the paratype fragments (Figs. 2c, d, and e) is similar to that of terminal parts of the holotype. Paratype fragments were collected nearby site (about 300 m apart) during the same sampling cruise, so we can justifiably consider all types of materials used in the description of this species as belonging to the same population.

Habitat and depth distribution

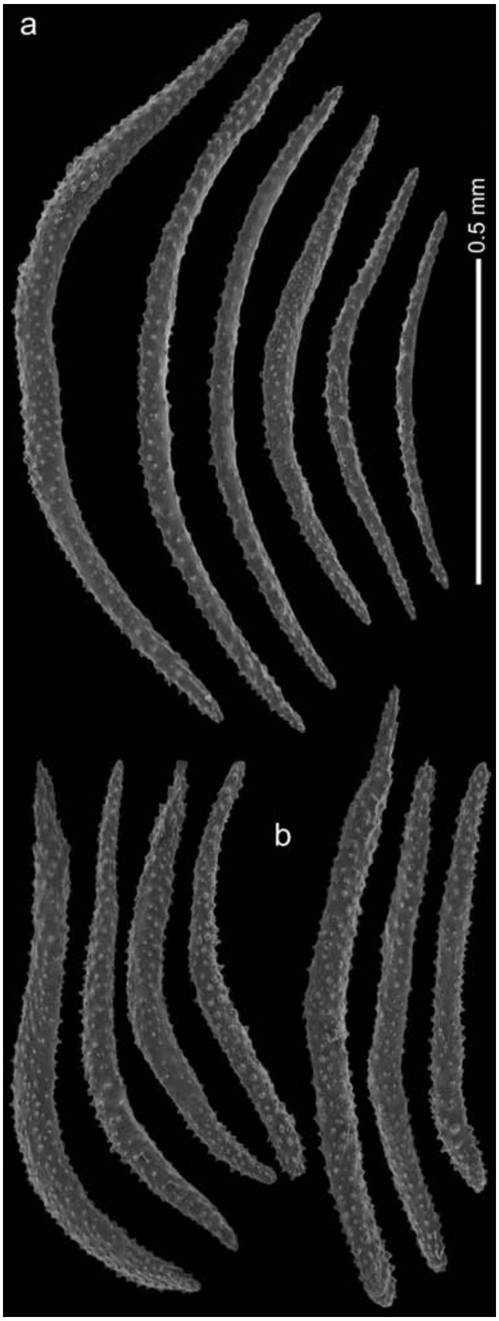

Fig. 6 *Chironephthya mediterranea* n. sp. Holotype. Anthocodial sclerites of the holotype. **a** Crown; **b** Points (note that both set of sclerites are at the same scale

Chironephthya mediterranea n. sp. was found at the edge of the continental shelf, the upper continental slope, and the flanks of the Son Bou Canyon, at a depth range of 115–200 m, although most was present between 150 and 185 m. Colonies were mainly observed growing on rocky bottoms and boulders (Fig. 10a). Both substrates could appear slightly covered by a fine sediment veneer. Occasionally, isolated colonies were encountered on gravelly bottoms and on dead shell debris (Fig. 10b).

Colonies were found to grow over a wide inclination range, from mildly inclined substrates to almost vertical walls.

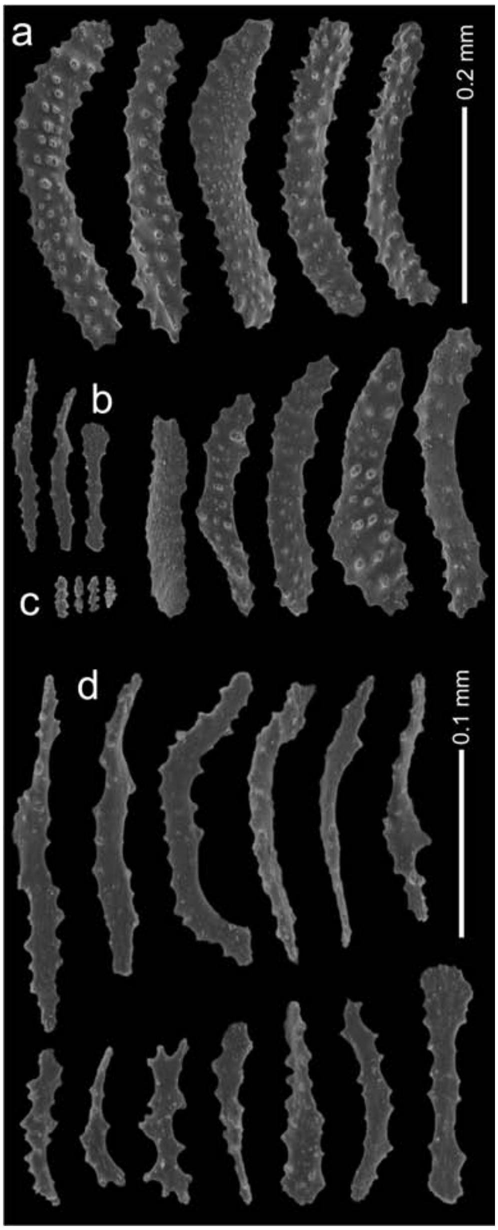

Fig. 7 *Chironephthya mediterranea* n. sp. Holotype. Anthocodial sclerites of the holotype. **a** Aboral side of the tentacles; **b** Three sclerites from pinnules; **c** Four sclerites from pharynx (note that *a*, *b*, and *c* have been presented at the same scale to show the important differences in size in anthocodial sclerites [see also Fig. 6]); **d** Pinnules (note that *a–b–c* and d are not at the same scale)

However, most colonies were observed growing on mild and intermediate inclinations. The species primarily appeared forming small aggregations, although in areas well-exposed to currents it could reach high densities. Based on video material recorded by submersible and ROV, the average density was estimated to be 1–3 individuals m^{-2} and maximum density was 12 individuals m^{-2}.

The benthic species most commonly associated with *Chironephthya mediterranea* n. sp. were the gorgonian *Bebryce mollis* (Philippi, 1842), *Swiftia pallida* (Madsen, 1970), *Viminella flagellum* (Johnson, 1863 [see. Fig. 10c]), the antipatharians *Leiopathes glaberrima* (Esper 1788) and *Antipathes dichotoma* (Pallas, 1766), and two demospongiae species, *Poecillastra compressa* (Bowerbank 1866) and *Phakellia robusta* (Bowerbank, 1866).

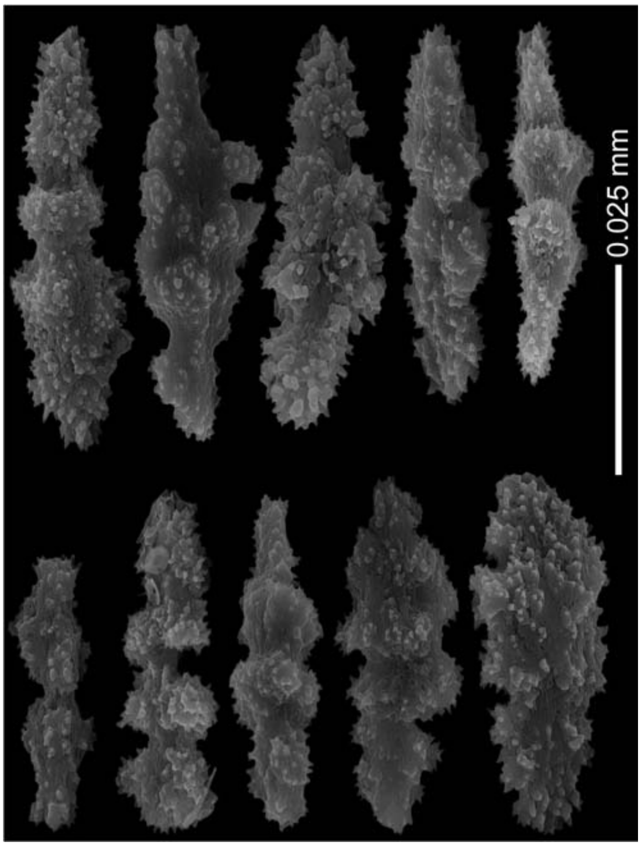

Fig. 8 *Chironephthya mediterranea* n. sp. Holotype. Sclerites from the pharynx

Geographical distribution

At present, *Chironephthya mediterranea* n. sp. is known in the northwestern Mediterranean Sea (Balearic Islands).

Phylogenetic analysis

In preliminary *mtMutS* and *mtMutS*+*COI* comparisons (not shown), the sequences obtained in this work for *Chironephthya mediterranea* and *C. caribaea* were merged into a nidaliidae clade including other *Chironephthya* and *Siphonogorgia* sequences available in GenBank. In that tree, nidaliid genera are not reunited in a single clade. An example with a reduced number of octocorals species and genera is shown in Fig. 11 for *mtMutS*+*COI*. As in the previous tree, the analysis using only *mtMutS* (Fig. 12) and using a select number of nidaliid species as outgroup did not group *Chironephthya* and *Siphonogorgia* sequences together in a

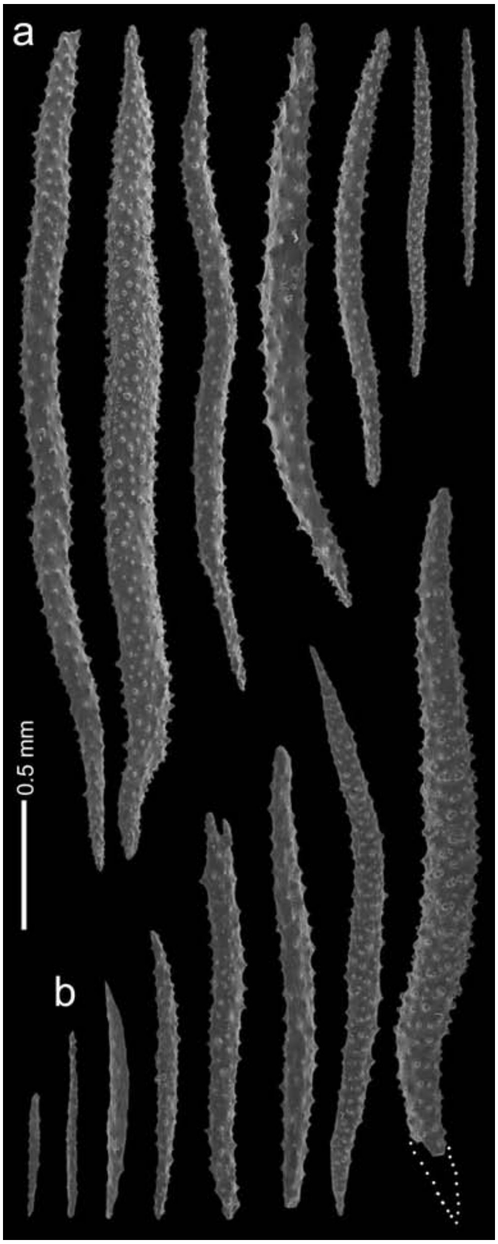

Fig. 9 *Chironephthya mediterranea* n. sp. Holotype. **a** Coenenchymal sclerites exterior wall of the main stem; **b** Coenenchymal sclerites from the interior walls between longitudinal canals (note that dotted lines indicate the limits of a broken spindle)

different clade. No shared sequence was found between these specimens assigned to these two genera.

The analysis of the two mitochondrial genes, *mtMutS*+*COI*, as well as that of the proposed extended barcode for octocorals *mtMutS*+*Igr1*+*COI* (both in Supp. Fig. 1), resulted in the same topology and quite similar support. All analyses supported a close relationship between the Mediterranean and Atlantic forms as sister groups, together referred to as the Atlanto-Mediterrarean clade as sister group of the Red Sea species *Chironephthya* sp.2 with lower support. The remaining Red Sea and Indo-West Pacific sequences were placed in different clades.

Similar species

Despite the difficulty in establishing a priori the taxonomic characteristics in the genus *Chironephthya* useful for species identification , as well as our scarce knowledge of possible intraspecific morphological variability, the molecular approaches carried out for this study strongly support a close relationship between the Mediterranean material described here and the Caribbean species described by Deichmann (1936). The type material of both Caribbean species is diagnostically re-described and illustrated here for comparative purposes. In addition, Table 3 summarizes the main set of morphological characteristics from selected Caribbean and Mediterranean species.

The type colonies of *Chironephthya agassizii* (Deichmann 1936) (MCZ CNID-4069) are branched, tree-like, and erect (Fig. 13a), about 70 mm in maximum length in the examined material. The stalk is attached to hard substratum, not extending beyond the basal stalk dimensions. The main stem and branches are rigid and brittle due to the abundance of large and densely packed sclerites in the colony wall. The branches secondary to distal branches are progressively smaller in size with branching order, but in general have a cylindrical aspect. The distal branches are distinctly spiculose, with large and thick cortical sclerites, observed up to 6.5 mm in length and 0.6 mm in width, as well as many that are smaller and thinner (Fig. 13f). The calyces are prominent, with two distinct calycular teeth (Figs. 13b–d), observed up to 3 mm along the abaxial side, formed by 2–4 large, thick sclerites per calycular teeth, up to 4 mm in length (4.7 mm in the sclerites in a small flask inside the lot identified as "calycular"), often with other, shorter sclerites, as well as other lateral and interteeth sclerites 0.3–0.35 mm in length, difficult to see because of the density and thickness of the large sclerites. In the anthocodiae, the polyps are 1.2 mm in diameter at crown level. The crown has 7–10 rows of curved thorny spindles, up to 0.9 mm in length (Fig. 13e). The points are 1–2 pairs of curved thorny spindles per point, up to 1 mm in length, often one side of a point with two sclerites and the other side with only one, sometimes with accessory smaller spindles among them up to 0.35 mm in length. Secondary points are not clearly visible, sometimes with single sclerites about 0.12 mm in length, but not always present, even in the same polyp. The sclerites of the aboral side of the tentacles are up to 0.32 mm in length. The platelets of pinnules are up to 0.18 mm in length. The thorny rods in the pharynx are up to 0.07 mm in length. The sclerites of the stalk are up to 5.5 mm in length and 0.4 mm in width. The stalk colour is yellowish, the main to terminal branches are light orange, while the anthocodiae are

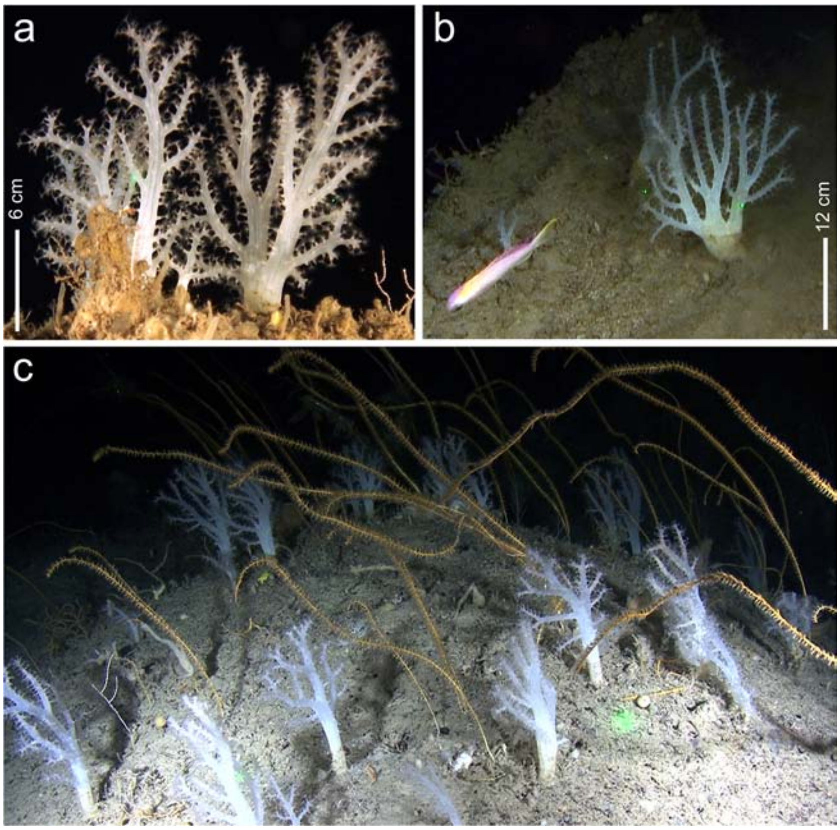

Fig. 10 *Chironephthya mediterranea* n. sp. Living colonies photographed in situ. **a** Colonies from station 11 (*Nemo* dive, cruise INDERAMES VI, Menorca IV); **b** Colonies from station 53 (*Nemo* dive, cruise INDERAMES VI, Menorca IV); **c** Dense field of *C. mediterranea* n. sp. and the ellisellid gorgonian *Viminella flagellum* from station 10 (*JAGO* dive, cruise INDERAMES IV, Menorca II). For additional sampling data, see Table 1.

white, except for the pharynx that is light orange to yellowish. All colours are due to the colour of the sclerites.

The type colony of *Chironephthya caribbaea* (Deichmann 1936) (MCZ CNID-4068) is branched, tree-like, and erect (Fig. 14a). The branches secondary to distal branches are progressively smaller in size with branching order, but in general have a cylindrical aspect. The distal branches are distinctly spiculose, with large and thick cortical sclerites, observed up to 7.5 mm in length and 0.7 mm in width, as well as many that are smaller and thinner (Figs. 14b and c). The calyces are prominent, with two distinct calycular teeth (Figs. 14b, d, and e), observed up to 3.5 mm along the abaxial side in distal parts of branches (but usually smaller, about 2 mm in length, depending upon whether they include some of the larger and thicker sclerites), formed by 2–3 large thick sclerites per calycular teeth, up to 3 mm in length, often with other, shorter sclerites, as well as numerous lateral and inter-teeth sclerites 0.7–1.25 mm long. In the anthocodiae (Fig. 14f), the polyps are 1.5 mm in diameter at crown level. The crown has 11–15 rows of sclerites, up to 1.3 mm in length. The points are 1–2 pairs of curved thorny spindles per point, up to 1.1 mm in length (Fig. 14f), sometimes two sclerites (one of them smaller, up to 0.6 mm in length) on one side of the point, with accessory smaller spindles basally among them, 0.25–0.30 mm in length. Secondary points comprising up to 3–4 shorter sclerites, 0.1–0.15 mm in length, are present. The sclerites on the aboral side of the tentacles are up to 0.45(0.55) mm in length (Fig. 14g). The sclerites of the pinnules are up to 0.22(0.25) mm in length (Fig. 14g). The sclerites of the pharynx are thorny rods, up to 0.07 mm in length. The sclerites of the stalk are up to 4.5 mm in length and 0.45 mm in width. The colour in the main stalk and terminal branches is whitish, while the anthocodiae are white, except for the pharynx that is light orange to yellowish. All colours are due to the colour of the sclerites.

The colony of *Chironephthya caribaea* (USNM 1025683) from which DNA sequences could be obtained is branched, tree-like, and erect, about 110 mm in maximum length in the preserved state (Fig. 15a). The branches secondary to distal branches are progressively smaller in size with branching order, but in general have a cylindrical aspect (Fig. 15b). Distal branches are distinctly spiculose, with large and thick cortical sclerites, observed up to 6.3 mm in length and 0.6 mm in width, as well as many that are smaller and thinner (Figs. 15b and c and 16e). The calyces are prominent, with two distinct calycular teeth (Fig. 15d), observed up to 3.5 mm along the abaxial side in distal parts of branches (but usually smaller, about 2 mm long, depending upon whether they include some of the larger and thicker sclerites), formed by 2–3 large thick sclerites per calycular teeth, up to 3 mm in length, often with other shorter sclerites, as well as numerous lateral and inter-teeth sclerites 0.7–1.25 mm long (Fig. 15c–e).

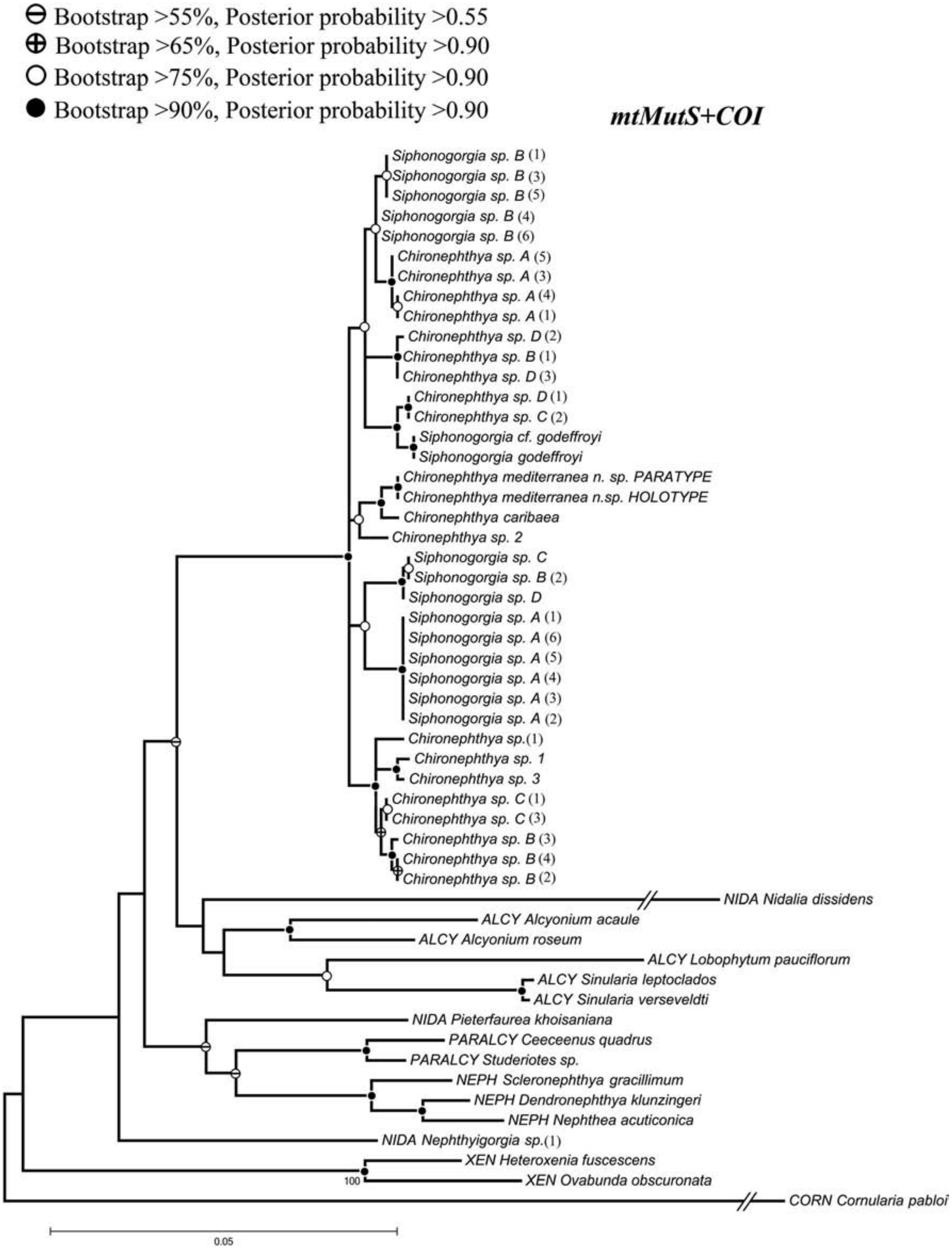

Fig. 11 Molecular analysis by ML method. Relationship of *Chironephthya-Siphonogorgia* species and a set of selected octocorals sequences, hypothesis based in the mitochondrial markers *mtMutS+COI*; note that Nidaliidae sequences are not reunited in this analysis. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. Abbreviations: *NIDA* Nidaliidae; *ALCY* Alcyoniidae; *PARALCY* Paralcyoniidae; *NEPH* Nephtheidae; *XEN* Xeniidae; *CORN* Cornulariidae

In the anthocodiae, the polyps are 1.2 mm in diameter at crown level (Fig. 15e). The crown has 11–13 rows of sclerites, up to 1 mm in length. The points are 1 pair of curved thorny spindles per point, up to about 1 mm in length, sometimes

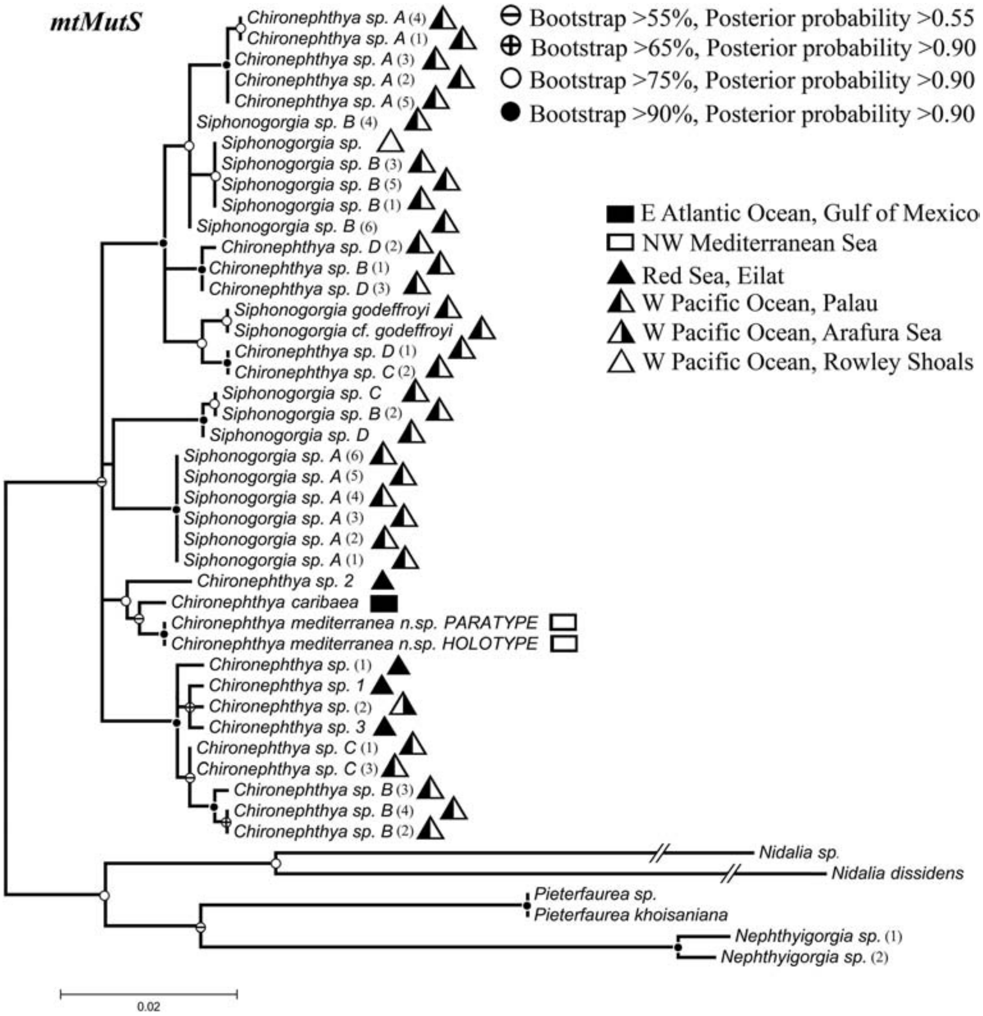

Fig. 12 Molecular analysis by ML method. Relationship of *Chironephthya-Siphonogorgia* species using *Nidaria-Nephthyigorgia-Pieterfaurea* as outgroup; the analysis is based in *mtMutS*. Note the lack of basal resolution in the *Chironephthya-Siphonogorgia* clade (this clade is weakly supported by Bayesian inference [*see* text]). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. The geographical information included in this tree for the voucher specimens sequenced (*see* Table 2) and shown in the *Chironephthya-Siphonogorgia* clade is valid for all trees in this paper. Note that all trees in this clade show the same topology.

with accessory smaller spindles basally among them, 0.25–0.35 mm in length (Fig. 16a). Secondary points comprising2–4 shorter sclerites, 0.15–0.17 mm in length, are present (Fig. 16b). The sclerites on the aboral side of the tentacles are up to 0.4 mm in length. The sclerites of the pinnules are up to 0.16 mm in length. The sclerites in the pharynx are thorny rods up to 0.07 mm in length (Figs. 16c–d). The sclerites of the stalk are up to 4.5 mm in length and 0.5 mm in width. The main stalk is yellowish in colour, and the main to terminal branches are orange, while the anthocodiae are whiten except for the pharynx that is light orange to yellowish. All colours are due to the colour of the sclerites.

Discussion

Our current knowledge of the diversity and distribution of *Chironephthya* is still limited. The genus has been known to be restricted to the Indo-West Pacific, with at least nine nominal species (Wright and Studer 1889; Harrison 1908; Benayahu et al. 2004), and two others from the Caribbean (Deichmann 1936:69–72 as *Neospongodes* Kükenthal 1903; and Bayer 1961:56 as *Siphonogorgia*; Cairns and Bayer 2009) In the near future, a study of the two unidentified Atlantic species (Pérez et al. 2011; Brito and Ocaña 2004) is needed in order to better understand the true distribution and diversity of the genus in the Atlantic Ocean.

Comparison with related species

Fabricius and Alderslade (2001) described the sclerite diversity in the genus *Chironephthya*, likely largely focused on Indo-Pacific species. In this important comprehensive descriptive work concerning the characteristics of shallow-water Indo-Pacific octocoral genera, the authors described the calyces of *Chironephthya* species formed by numerous long spindles and the presence of numerous small rods (at least up to 0.19 mm in length according to the provided figure) on the polyp body (introvert) below the polyp head (crown, point, and tentacles). In the case of *Chironephthya mediterranea* n. sp., calyces seem to be less densely spiculated. Indeed, there is

Table 3 Selected characteristics from sclerites distribution, size, and colour from the type specimens of *Chironephthya agassizii*, *C. caribaea*, and *C. mediterranea*, as well as the specimen of *C. caribaea* from which the extraction and amplification of mitochondrial genes was possible for the molecular comparison in this study

	C. agassizii Syntype (MCZ CNID-4069)	*C. caribaea* Syntype (MCZ CNID-4068)	*C. caribaea* (USNM 1025683) ID: Cairns, 2004	*C. mediterranea* n.sp. Holotype (MCNB 2013–0001)
Surface coenenchymal-calycular sclerites				
-Distal branches	Densely packed, light orange in colour, up to 6.5 mm in length and 0.6 mm in width	Densely packed, whitish in colour, up to 7.5 mm in length and 0.7 mm in width	Densely packed, orange in colour, up to 6.3 mm in length and 0.6 mm in width	Not densely packed, sclerites can be seen individually separated by sclerite-less coenenchymal spaces, colourless, up to 2.8 mm in length and 0.15 mm in width
-Stalk	Densely packed, yellowish in colour, up to 5.5 mm in length and 0.4 mm in width	Densely packed, white in colour, up to 4.5 mm in length and 0.45 mm in width	Densely packed, yellowish in colour, up to 4.5 mm in length and 0.5 mm in width	Densely packed, colourless, up to 3.5 mm in length and 0.22 mm in width
-Calyces	2–4 large thick sclerites per calycular teeth, lateral and inter-teeth areas with smaller sclerites	2–3 large thick sclerites per calycular teeth, lateral and inter-teeth areas with smaller sclerites	2–3 large thick sclerites per calycular teeth, lateral and inter-teeth areas with smaller sclerites	2(3) large thin sclerites per calycular teeth, lateral and inter-teeth areas without sclerites
Crown sclerites	Colourless, (7)8–10 rows, up to 0.9 mm in length	Colourless, 11–15 rows, up to 1.3 mm in length	Colourless, 11–13 rows, up to 1 mm in length	Colourless, 4–7 rows, up to 1.1 mm in length
Point sclerites	Colourless, 1–2 pairs of large sclerites per point, up to 1 mm in length, plus other smaller about 0.35 mm between them	Colourless, 1–2 pairs of large sclerites per point, up to 1.1 mm in length, sometimes with another smaller up to 0.6 mm, but often 0.25–0.30 mm between them	Colourless, 1 pair of large sclerites per point, up to 1 mm in length, plus other smaller 0.25–0.35 mm between them	Colourless, 1–3 pairs per point, up to 1 mm in length, one of them can be distinctly smaller, about 0.25 mm between them
Secondary points (?). (sclerites on the intertentacular mesenterial insertion area)	A colourless sclerites can be present, 0.12 mm, but not always; even variable in a polyp	Present, 3–4 colourless sclerites, 0.1–0.15 mm	Present, 2–4 colourless sclerites, 0.15–0.17 mm	A single colourless sclerite is occasionally observed in one of these spaces, 0.1 mm, but the usual state is complete absence
Tentacle sclerites	Colourless, aboral sclerites up to 0.32 mm, those on the pinnules up to 0.18 mm	Colourless, aboral sclerites up to 0.45(0.55) mm, those on the pinnules up to 0.22(0.25) mm	Colourless, aboral sclerites up to 0.4 mm, those on the pinnules up to 0.16 mm	Colourless, aboral sclerites up to 0.36 mm, those on the pinnules up to 0.21 mm
Pharynx sclerites	Light orange to yellowish in colour, up to 0.07 mm	Light orange to yellowish in colour, up to 0.07 mm	Light orange to yellowish in colour, up to 0.07 mm	Light orange in colour, up to 0.05 mm

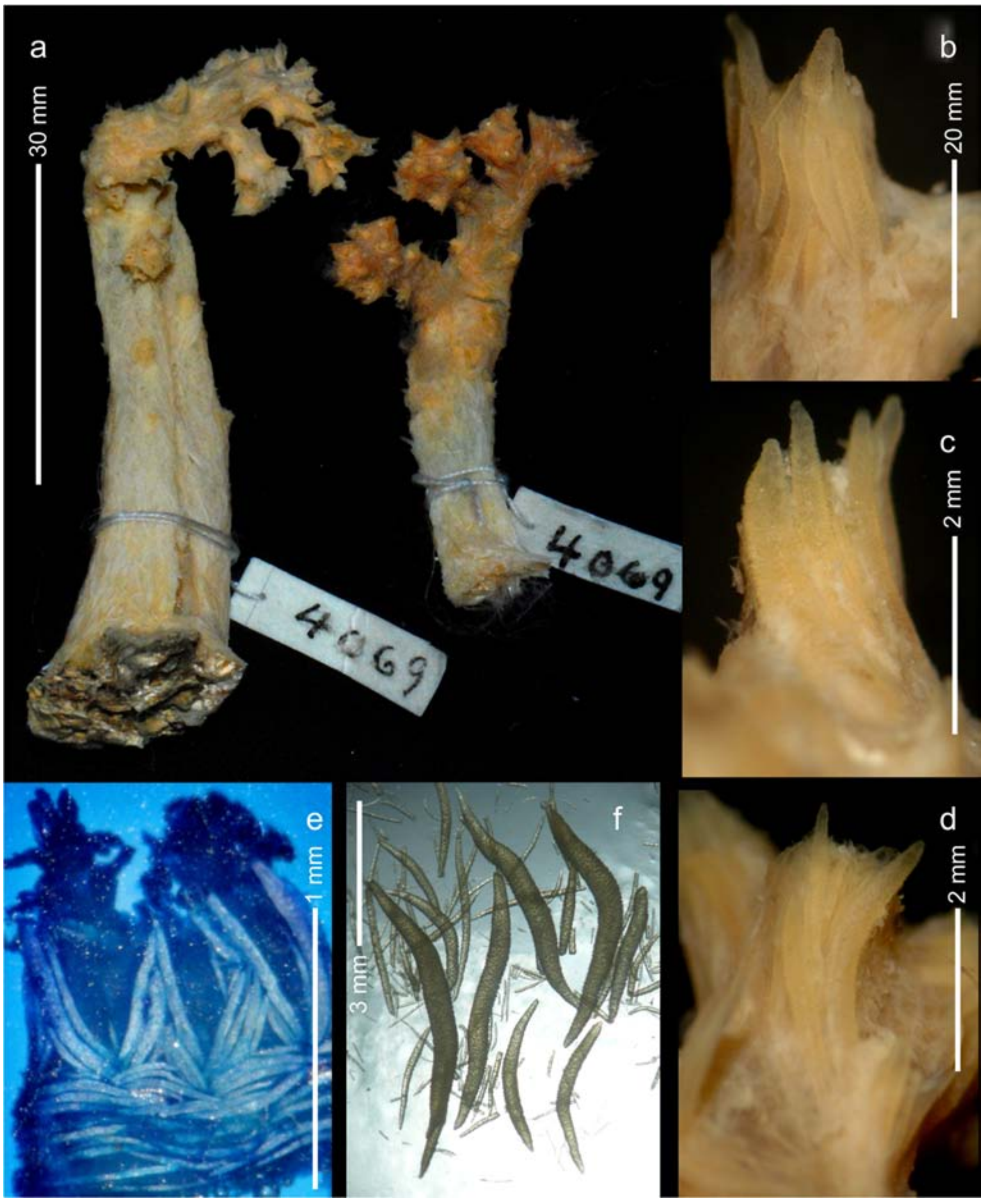

Fig. 13 *Chironephthya agassizii* (syntypes MCZ CNID-4069). **a** Two colonies; **b**–**d** Details of calyces showing large and thick sclerites forming calycular teeth; **e** Anthocodia showing sclerite arrangement at crown and points; **f** Coenenchymal sclerites showing differences in size of the large thorny spindles

a clear sclerite-less triangle between the two distinct points formed by the set of large spindles (Fig. 4b), as well as the lateral sides of the points (Figs. 4a and c). Moreover, sclerites are absent in the introvert. All other skeletal characteristics, such as the presence of minute sclerites on the pharynx, two kinds of sclerites on the tentacles (massive plates on the aboral side, thinner and curved on the pinnules), coenenchymal surface, interior, crown, and points are well-represented in the Mediterranean species.

The two named Atlantic species of *Chironephthya* were initially studied, but not published, by Verrill. Deichmann (1936) respected the specific epithets proposed by the former author in his original typescripts and assigned these species to the nephthyid genus *Neospongodes* (Verrill had the intention to describe a new soft coral genus for these two species). According to Deichmann's description (1936:69), *Chironephthya agassizii* has a greyish stem with branches reddish-brown and polyps pale in colour; crown with four to six rows of sclerites, and the points in two (or three) pairs of curved sclerites; the coenechymal sclerites reaching up to 2 mm in length, those of the crown and points up to 1 mm, and the blunt rods of the tentacles reaching up to 0.3 mm in length. On the other hand, also according to Deichmann (1936), *Chironephthya caribaea* is dull greenish gray in colour (preserved state); branches are about 5 mm in diameter; crown with about six rows of sclerites and points with one (two) pairs of sclerites; coenenchymal sclerites reaching up to 6 mm in length (although a few of them can reach up to 10 mm), those of the crown reaching up to 1.5 mm, those of the points up to 1 mm, and the short "double-cones" in the

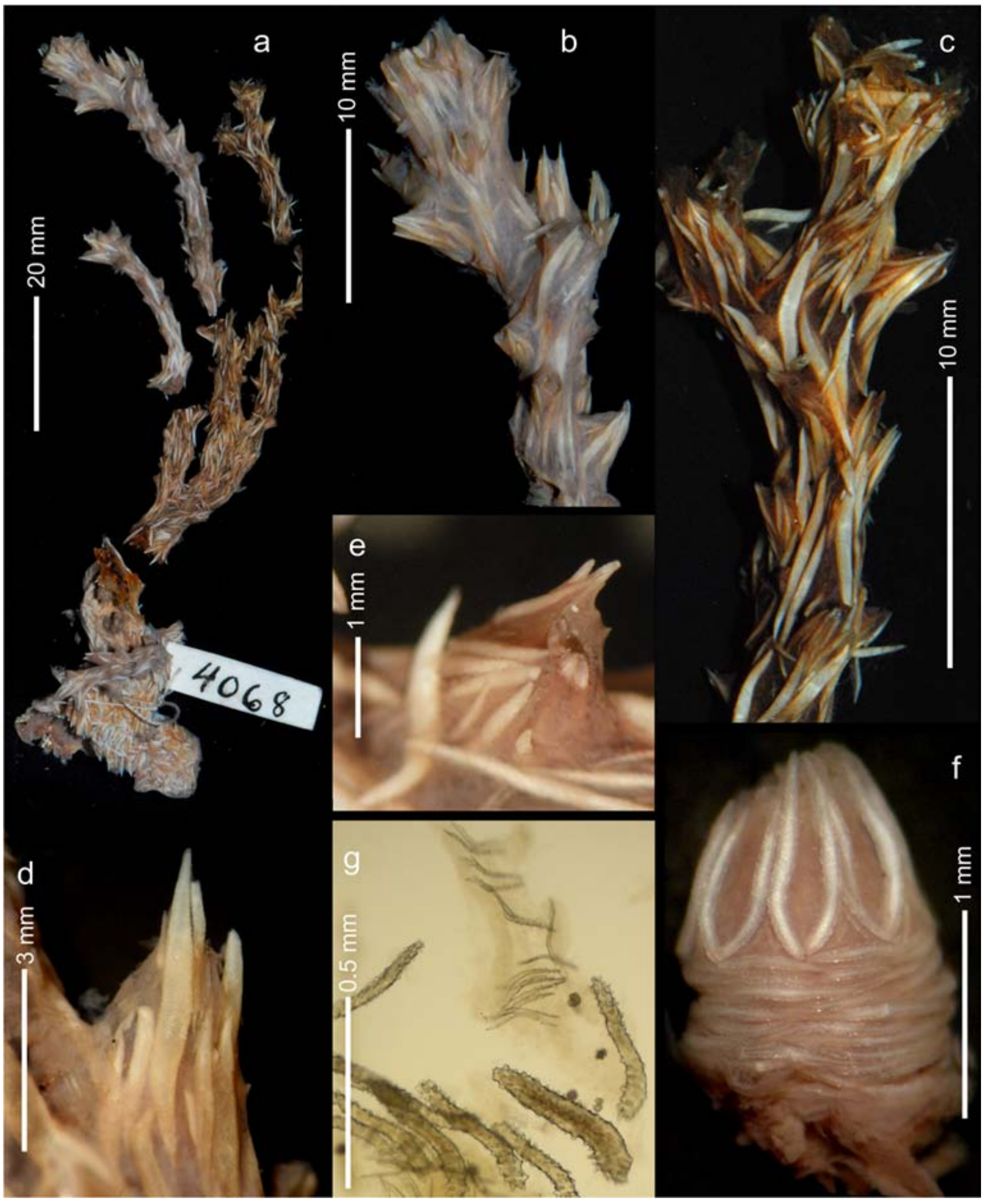

Fig. 14 *Chironephthya caribaea* (syntypes MCZ CNID-4068). **a** Whole fragmented colony (note that some of the fragment probably dried in the past, now having a slightly different appearance); **b**–**c** Detail of terminal branches; **d**–**e** Detail of calyces showing large and thick sclerites forming the calycular teeth; **f** Anthocodia showing sclerite arrangement at crown and points; **g** Tentacular sclerites showing basally those of the aboral side of the tentacle and distally those of the pinnules

tentacles averaging 0.05 mm in length. More than 150 colonies of *C. mediterranea* n. sp. have been observed in the video material examined for an ongoing study of characterization of the benthic community (Grinyó et al. in prep.), and no colour variation has been observed. Living and preserved colonies of the new species are whitish in colour, slightly brownish basally due to the presence of a periderm; the pharynx is distinctly orange due to the colour of the sclerites, and all other sclerites in the colony are colourless.

The revision of the type and additional materials of the Western Atlantic *Chironephthya* species (see Supp. Mat. Appendix 1) revealed a number of discrepancies in the original descriptions provided by Deichmann (1936) (see above for diagnostic re-descriptions and Table 3 for comparison of selected materials).

According to the examined material, both Western Atlantic species bear close resemblance from a morphological point of view, if they are not the same species. Despite the indications from Deichmann, surface coenenchymal sclerites of the type material of *C. agassizii* (MCZ CNID-4068) are distinctly longer than the 2 mm originally reported, reaching 6.5 mm in some sclerites, reducing the differences with *C. caribaea*, type and additional consulted materials. In general, in both Caribbean species, the number of rows of sclerites at the crown is higher than that initially reported, (7) 8–12, including all variation observed in *C. agassizii* specimens, but 10–15 including all variation observed in *C. caribaea* specimens). Additional characteristics such as the number of sclerites present in the calyces or other anthocodial structures (tentacular, pharyngeal, point sclerites) show an overlapping range of

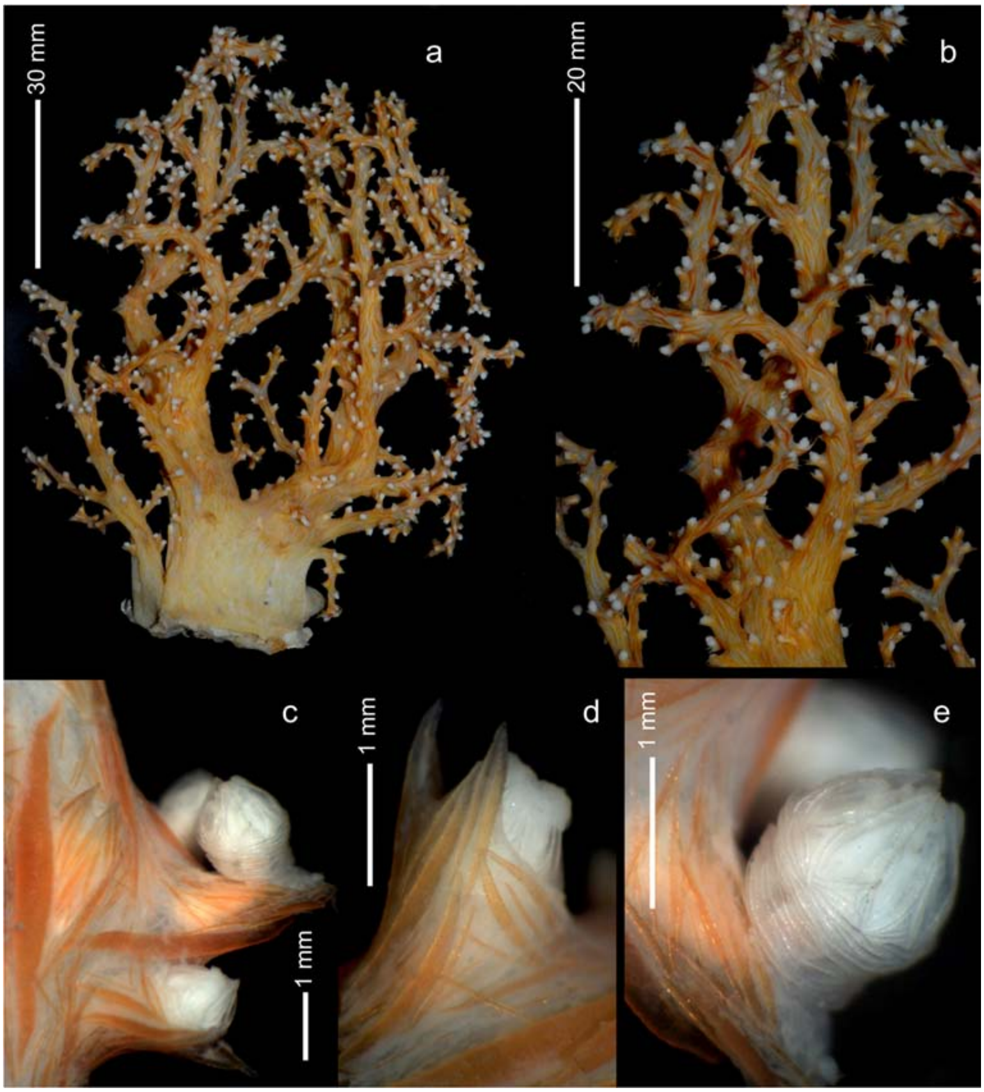

Fig. 15 **a** *Chironephthya caribaea* (USNM 1025683). **a** Whole colony; **b** Details of terminal branches showing the presence of the large and thick sclerites; **c**–**d** Details of calyces and anthocodiae (note the large sclerites in the calycular teeth and the presence of additional sclerites on the lateral areas of the calyces); **e** Detail of an anthocodia showing sclerite arrangement at crown and points

variability in the set of examined colonies, making it difficult to draw a clear difference between the sets of materials, even from the type specimens. Colour information from the original descriptions is mainly related to the colour of the preserved tissues; sclerite colour varies in a single colony from whitish (basally) to orange (distally), but other colonies have light orange polyparia (due to the sclerites' colour). Molecular information appears to be a useful tool for distinguishing *Chironephthya* species. Unfortunately, only the more recently preserved specimens of *C. caribaea* (USNM 1025683 [see also Fig. 14 in this paper]) have provided good DNA extraction and mitochondrial genes successfully amplified. In our opinion, both species are likely synonymous. According to current morphological information, it is difficult to distinguish the limits of the two named Caribbean species. If this is correct, the name *C. agassizii* would have precedence because it was cited first in the original text. More DNA sequences from the Western Atlantic *Chironephthya* specimens are needed to elucidate whether, under this apparently overlapping variability, more than a species is hidden. It remains unclear whether the basal ramification of the colony (producing a short stalk, as in *C. caribaea* type material, and USNM 1025683) or branching distally at the end of a distinct stalk (as in most *C. agassizii* specimens) can be considered specific characteristics or simply varieties.

There are clear morphological differences among the Caribbean species and the new Mediterranean species described here. In the Caribbean specimen examined, surface coenenchymal sclerites can reach up to 7.5 mm in length (maximum values per colony varied from 4.5 to 7.5, usually about 6 mm) and 0.7 mm in width (maximum values per colony varied from 0.3 to 0.7, usually about 0.5 mm), while in the Mediterranean species these sclerites reach up to only 2.8 mm in length and 0.14 mm in maximum width (usually 0.7–0.10 mm). Maximum dimensions of surface coenenchymal sclerites of the stalk show a similar pattern, with Caribbean species ranging from 3.5 to 5.5 mm in length and 0.38–0.55 mm in width, and the Mediterranean species

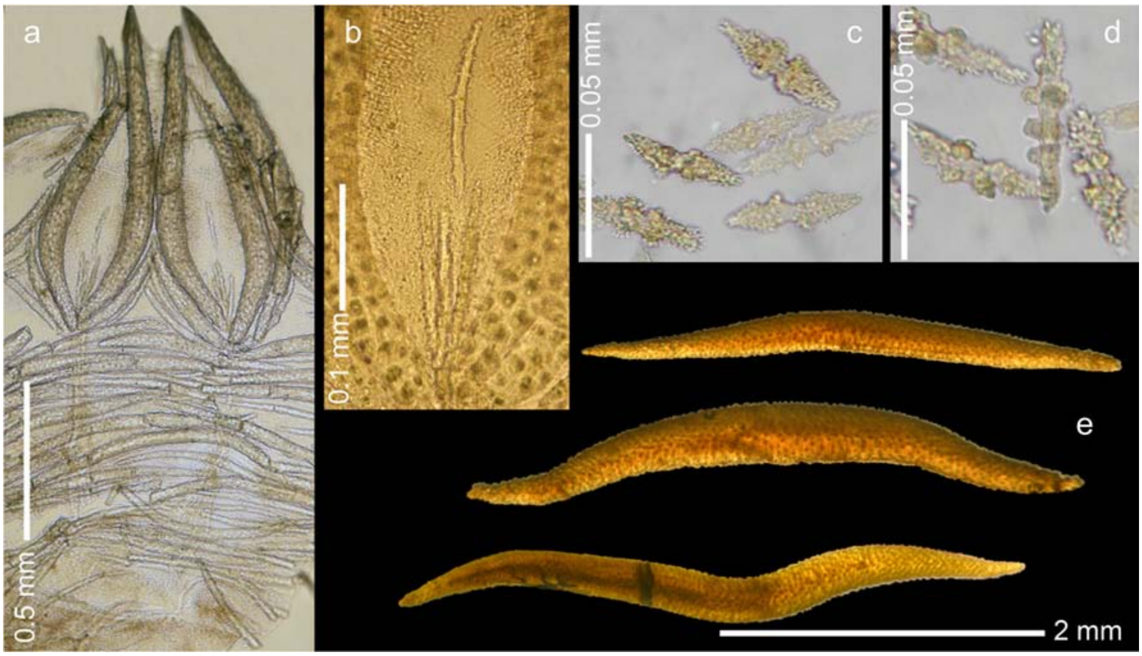

Fig. 16 **a** *Chironephthya caribaea* (USNM 1025683). **a** Detail of anthocodial sclerite arrangement, showing crown, points, and some sclerites in the inter-tentacular areas (secondary points ?); **b** Detail of a group of those sclerites in the inter-tentacular areas between two consecutive points; **c**–**d** Pharyngeal sclerites; **e** Three of the large and thick coenenchymal sclerites from terminal branches

3.5 mm and 0.22 mm, respectively. In general, coenenchymal sclerites in the Caribbean species can reach longer and wider sizes, but not to the detriment of shorter and thinner sclerites. Caribbean species show a wider range of coenenchymal sclerites, consisting of distinctly larger and thicker sclerites, in addition to those sizes in common with the Mediterranean form. The density of coenenchymal sclerites in distal branches is clearly different in Caribbean and Mediterranean species, the former being more densely spiculated.

The number of sclerites conforming each of the two calycular teeth vary from two to five in the observed Caribbean forms: two when sclerites are large and thick, but up to five when sclerites are shorter. When large sclerites are poorly represented in stems or branches, a higher number of shorter sclerites forming the calycular teeth are present. Conversely, when large and thick sclerites are present, they often participate in the formation of the calycular teeth, and a few additional sclerites are added to it. In general, shorter sclerites are found covering lateral areas of the calyces (after the original Verrill's plates compiled by Bayer and Cairns 2004: plt 69, Figs. 8 and 13 in this paper). Although two to three sclerites can be found in the calycular teeth of *Chironephthya mediterranea*, these sclerites are thinner and shorter than 3 mm in length, and lateral and inter-point calycular spaces are devoid of sclerites (see Figs. 3a, b, and c).

Regarding the sclerites present in the polyp, the number of rows of sclerites in the crown of Caribbean and Mediterranean species is different, four to seven (this variability is observed in different polyps of the same colony) in the new species, but higher in the Caribbean species (data, commented above). Other features such as tentacular, point, or pharyngeal sclerites show similar dimensions and shapes. The information for *C. caribaea* provided by Deichmann (1936:71) stating that "The short double cones in the tentacles average 0.05 mm in length"surely refers to the pharyngeal rather than tentacular sclerites, which can reach 0.55 mm (axial tentacular sclerites) or 0.25 mm (pinnules), and are not consistent with the "double-cone" shape described.

In the above molecular phylogenetic hypotheses based on *mtMutS* and on the combination of two mitochondrial genes (*mtMutS*+*COI*), the Mediterranean species is different from the only available Caribbean sequence, its sister group, suggesting a common ancestry between the Atlantic and Mediterranean species.

The comparison based on the extended proposed barcode for octocorals (*mtMutS*+*Igr1*+*COI*) appears to show a good resolution for species and genera currently included in Nidaliidae. More sequences and species need to be added to these analyses, especially for the speciose genus *Siphonogorgia* as well as other nidaliid genera for which molecular information is still lacking. This molecular information could be used in combination with the morphological and chromatic descriptions of the different species.

Biogeographic approach

Previously, species of the genus *Chironephthya* were known from several localities in the Indo-Pacific Ocean, Caribbean

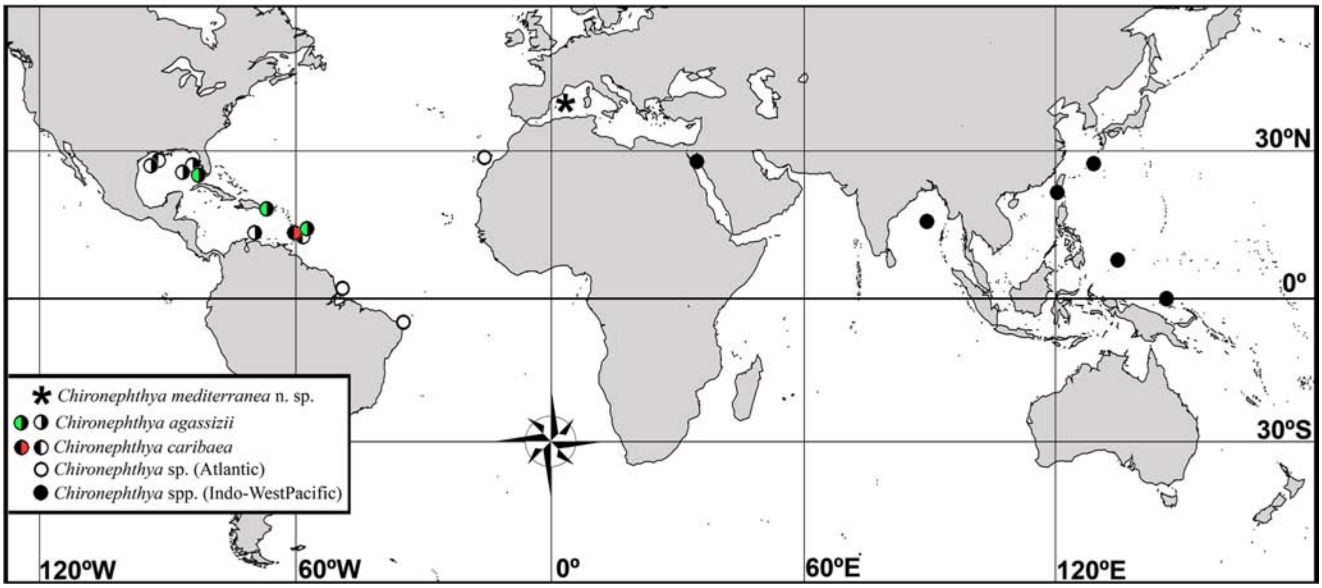

Fig. 17 *Chironephthya* spp. distribution, modified and updated from Pérez et al. (2011), with special emphasis on Atlantic and Mediterranean records and species. * known localities of *Chironephthya mediterranea* n. sp.; *half open-solid circle*, known localities of *Chironephthya agassizii*; *half solid-open circle*, known localities of *Chironephthya caribaea*; *open circle*, known Atlantic records as *Chironephthya* sp.; *solid circle*, Indo-West Pacific records of *Chironephthya* spp. *Colour in symbols* indicates type localities for the respective species

Sea, and tropical Atlantic off the Brazilian coast (Pérez et al. 2011). This is the first time that a species of this genus has been found and described in the Mediterranean Sea. A similar situation recently occurred with another soft coral genus. The re-description of *Nidalia studeri* represented the first species of this genus described in the Mediterranean. The genus *Nidalia* shows some biogeographical parallels with the genus *Chironephthya*. Most species of *Nidalia* are distributed in the western sector of the Indo-Pacific Ocean and the Caribbean Sea.

This peculiar biogeographic distribution was also found in extensive studies on deep-water medusa fauna in the Mediterranean (Gili et al. 1998, 2000; Bouillon et al. 2000). These authors proposed that the Indo-Pacific and Mediterranean species, in particular, could have diverged from a common ancestor after being isolated in their respective region by geological events in conjunction with the Messinian salinity crisis (Hsü 1973).

The current distribution of the genus *Chironephthya* (Fig. 17) suggests that in the past, species of this genus were distributed continuously across the Tethys Sea, and went through allopatric speciation as tropical provincialism gradually consolidated. It is plausible that the Mediterranean and Atlantic *Chironephthya* species is of a Tethyan lineage that was subsequently isolated during the Early Miocene with the closure of the eastern channels and diversified in the Atlantic and Mediterranean Sea. Although it has been postulated that the Tethyan marine fauna disappeared completely during the Messinian salinity crisis (Hsü 1973), several faunistic studies from recent decades have suggested that the Tethyan fauna could have survived in scattered refuges throughout the Mediterranean basin (Boury-Esnault et al. 1992; Boero and Bouillon 1993; Gili et al. 1998, 2000; Jaume and Boxshall 1996). In fact, recent studies related to deep North Atlantic medusa fauna (Hosia and Pagès 2007) have marked the discovery of a set of new species belonging to a genus initially described as endemic to the Mediterranean Sea. This may suggest that the Tethys Sea fauna colonized the Atlantic Ocean after the Early Pliocene (van der Spoel 1996), and when the Strait of Gibraltar reopened, the Mediterranean Sea level was reestablished and the Mediterranean outflow water (MOW) circulation pattern began (Maldonado and Nelson 1999). Today, the MOW flow moves to the north along the Iberian slope and to the southwest as far as the Canary Islands, and then westward (Iorga and Lozier 1999). The MOW may have allowed Mediterranean species to repopulate North Atlantic waters. Although most colonization events have occurred from the Atlantic to the Mediterranean (Bianchi and Morri 2000), there are few cases in which the opposite may have occurred (Henry and Roberts 2006).

In the event that the Messinian salinity crisis resulted in the disappearance of all Tethyan marine fauna, the presence today of *Chironephthya* (and likely *Nidalia*) species in the western Mediterranean should be seen as a result of further immigration from remaining Atlantic lineages. In any case, whether Mediterranean emigrants colonizing the Atlantic or Atlantic emigrants re-colonizing the Mediterranean areas (both hypotheses according to the current available molecular information), Atlantic and Mediterranean species of *Chironephthya* are closer to each other than to any Red Sea or Indo-Pacific species. Indeed, it is noteworthy that in the different relationships of the three available sequences from the Red Sea (only identified at generic level, as sp.1 to sp.3 in McFadden et al. 2011), two of them (sp.1 and sp.3) fall into a clade with Indo-Pacific species, whereas *Chironephthya* sp.2 is, in this study, the sister group of the Atlantic-Mediterranean forms.

The discovery of species such as *Chironephthya mediterranea* n. sp. in the Mediterranean reinforces the hypothesis that the Tethyan component of deep Mediterranean fauna is more important than previously thought (Pérès 1985). Further studies on deep areas may confirm this hypothesis and perhaps lead to a confirmation that during the Messinian salinity crisis, the Mediterranean was probably not completely dry, and some areas remained flooded, preserving marine conditions. Under that hypothesis, therefore, the current distribution of some sessile organisms in the Mediterranean probably represents relict populations of lineages that have a wider distribution in the Mesozoic Tethys (Logan et al. 2004). *Chironephthya mediterranea* n. sp. has been found in one of the least explored marine habitats in the Mediterranean Sea, the shelf break and upper continental slope communities (Pérès 1985). We believe it is highly unlikely that this species is an invasive one, since most invasive species in the Mediterranean have been associated with surface warm water (Laubier et al. 2003; Por 2009; Raitsos et al. 2010). *Chironephthya mediterranea* n. sp. is found in stable cold water environments (13 °C), far from harbours, aquaculture plants, or warm shallow waters, where invasive species are commonly first detected (Zibrowius and Ramos 1983; Galil 2000; Fine et al. 2005; Hoeksema and Ocaña 2014).

Our molecular data indicates a close relationship between Atlantic and Mediterranean forms, with this clade related to Indo-Pacific sequences. Moreover, *Chironephthya* species are suspected to be present in the Atlantic Ocean (Brito and Ocaña 2004; Pérez et al. 2011). Morphological and molecular data should shed some light on the biogeography of the genus *Chironephthya* as well as provide additional information about the origin of *Chironephthya mediterranea* n. sp.

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