Morphological polymorphism of *Desmophyllum dianthus* (Anthozoa: Hexacorallia) over a wide ecological and biogeographic range: stability in deep habitats?

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Abstract

Although zooxanthellate corals are well known for their ecophenotypic variations, there is increasing evidence that azooxanthellate species also harbour a high degree of plasticity. *Desmophyllum dianthus*, a widespread solitary coral, exhibits a high degree of morphological variation in corallum forms that has never been analysed quantitatively. To assess if the clear morphological variation of *D. dianthus* follows a specific pattern based on environmental (or others) variables, this study combines three different morphometric approaches: 1) classical linear external morphology, 2) use of three-dimensional coordinates landmarks, and 3) linear measurements and counts made of cnidocyst features. Comparative morphological characterization of *D. dianthus* specimens shows a pattern of intraspecific variation over a wide ecological and biogeographic range. However, additional future studies on this and other proposed cosmopolitan species, including a similar sampling effort in localities and specimens, will be useful to explore the existence of common global patterns of morphological variability. Hypotheses for intraspecific polymorphism are discussed to explain the incongruence between the obtained results and the seemingly high morphological variability observed within *D. dianthus*.

Keywords: Scleractinia, morphology, 3D landmarks, cnidocysts, phenotypic plasticity, intraspecific variation

1. Introduction

The phylogeny of the order Scleractinia has received renewed attention over the last 20 years due to the availability of new molecular and morphological approaches (Barbeitos et al., 2010; Chen et al., 1995; Fukami et al., 2008; Kerr, 2005; Kitahara et al., 2010; Lin et al., 2014; Romano and Palumbi, 1996; Stolarski and Roniewicz, 2001; Stolarski et al., 2011).

High levels of phenotypic variability and plasticity encrypt species boundaries and complicate the definition of valid taxonomic units (Todd, 2008). Usually, corals do not meet the criteria of conventional species concepts due to extreme phenotypic plasticity and/or instances of geographical restriction or hybridization along the genealogical history of the species. For these reasons, several taxa have been synonymised due to the lack of clear diagnostic morphological characters reflecting species boundaries or to the presence of obvious transitions among morphotypes within an acceptable morphological species range (e.g. Benzoni et al., 2014; Hoeksema, 2014; Vertino, 2003; Zibrowius, 1980). In this renewal scenario, as clearly stated by Budd et al. (2010), “molecular phylogenetic analyses have remarkably changed the understanding of scleractinian evolution at all levels, making it possible to test a wide variety of hypotheses derived from morphological studies - from the issue of monophyly of the order to the role of hybridization in Scleractinia evolution (Budd et al., 2010; Kitahara et al., 2010; Lin et al., 2014; Medina et al., 2006; Stolarski et al., 2011)”. In recent papers, the traditional description of coral species based on the macromorphology of the corallum/corallite, have been combined with “cartesian coordinates of landmarks”, also called two- or three-dimensional methods (e.g. *Favia*, *Pocillopora* and *Psammocora* in Benzoni et al., 2010; Budd et al., 2012; Huang et al., 2014; Kongjandtre et al., 2012; Richards et al., 2013; Schmidt-Roach et al., 2014; van Oppen et al., 2001), where size and shape

coordinates are used as potential characters to discriminate morphospecies (Budd et al., 1994; Budd and Stolarski, 2009). Biometric parameters (e.g. corallite length, width, height) have been also used to assess intraspecific morphological variation in azooxanthellate corals along latitudinal gradient (e.g. Caroselli et al., 2015 and reference therein).

In addition, other authors have highlighted the use of cnidocysts as “organic characters” as a new taxonomic informative source and the phylogenetic implications of using such characters (Martínez-Baraldés et al., 2014; Picciani et al., 2011; Pires, 1997; Terron-Sigler and Lopez-Gonzalez, 2005).

The genus *Desmophyllum* is known since the Cretaceous (Wells, 1956) and Early Miocene to Pleistocene. Mediterranean specimens show skeletal morphotypes indistinguishable from the modern species *D. dianthus* (McCulloch et al., 2010; Remia and Taviani, 2005; Taviani et al., 2005, 2011; Vertino, 2003; Vertino et al., 2014; Zibrowius, 1987). Historically, scleractinian taxonomists based the establishment of new species on a few macromorphological characters (such as size, shape and number of septa). Therefore, unaware of the wide intraspecific variability displayed by modern *D. dianthus*, previous authors have had a tendency to attribute a vast number of new species to the genus. Indeed, 31 taxa established upon fossil records (20) and recent species (11) have been recently synonymised with the cosmopolitan species *D. dianthus* (Cairns, 1995; Vertino, 2003).

As Miller et al. (2011) highlighted in their study, one of the greatest challenges in the delineation of *Desmophyllum* species is the relative paucity of taxonomic features. Where coral populations are potentially isolated, the possibility that allopatric speciation may occur is high, and so it may be that isolated populations of *Desmophyllum* have

diverged or be in the process of speciation. Molecular and morphological studies have shown significant subdivision among populations of *D. dianthus* from three different geographic regions (SE Australia, New Zealand and Chile) and depth ranges (shallow <600 m, mid 1000-1500 m and deep > 1500 m), suggesting that *D. dianthus* populations across the Southern Ocean have a common ancestor but that populations at different depths within the geographic regions are isolated and have begun to diverge (Miller et al., 2011). In contrast, Fillinger and Richter (2013) did not observe any clear pattern of morphological variability related to environmental gradients in a Chilean fjord.

One of the greatest challenges in delineating potential *Desmophyllum* species through skeletal analysis is the relative paucity of taxonomic features and the high level of morphological plasticity of the skeleton (Fig. 1). However, to date, detailed morphometric analyses of the corallum (the aragonitic calcium carbonate skeleton of a scleractinian coral, consisting of only one mouth or calice) of *Desmophyllum* specimens have not been performed, nor has any attempt to identify and measure "organic characters", such as cnidocysts, been made. The main aims of this study were to test the existence of distinct morphological groups within the cosmopolitan species *D. dianthus* and to identify, if any, relationships between morphological groups and oceanographic distribution. To test whether morphological variation in coral polyps has a specific pattern, three different techniques were used: 1) morphometry of macroscopic skeletal features, 2) analysis of three-dimensional coordinates of skeletal landmarks and 3) linear measurements and counts of cnidocyst features.

2. Material and Methods

2.1. Material Examined

The specimens examined in the present study are listed in Table A.1 and consist of 1) material registered at the US National Museum of Natural History; 2) specimens collected during the Mediterranean Sea and Atlantic Ocean cruises ANTARKTIS XIX/5 (LAMPOS) (2002), M70-1 (2006), CORAL-8 (2007), MEDCOR (2009) and EU-FP7 Eurofleets CWC Moira (2012), on board the RRVV Polarstern, García del Cid, Meteor, Urania and Belgica, respectively and 3) specimens collected while SCUBA diving at depths of 12 m at Jaime Island (Pitipalena Fjord, Chile).

Prior to examining the coral skeleton, soft tissues were extracted and preserved in absolute ethanol for molecular analyses (see Addamo et al. 2012, 2015). The corallum was then soaked for 48 hours in a 50% sodium hypochlorite solution at room temperature to remove any remaining soft parts, rinsed in freshwater and dried for microscopic observation. Coral specimens collected for cnidocyst examination were fixed in 4% buffered formalin in seawater, decalcified in a 10% formic acid solution, and then transferred to 70% ethanol.

Specimens of *D. dianthus* were classified here per marine area, provinces, and depth zones using information available in the literature about different biogeographical marine areas, provinces and depth zones (Kelleher et al., 1995; Spalding et al., 2007; Watling et al., 2013) (for more details see Table A.2a-c). Moreover specimens were organized in five size classes, which were defined based on an average *D. dianthus* growth rate of 1 mm year⁻¹ (Adkins et al., 2004). Each size class has been defined as ‘tridecade’ (T) and numbered consecutively, representing the length of corallum reached in 30 years (see Table A.2d). The objective was to compare morphological features of specimens from different marine areas, provinces and depth zones while avoiding an age/size bias.

We examined the skeletal morphology of *D. dianthus* specimens (identified by a corresponding geographic code in Table A.2a-d) collected from five marine areas, 13 provinces and five depth zones. For cnidocyst analyses, three polyps each from the Moira Mounds (Ireland), Jaime Island (Chile), Burdwood Bank (Argentina) and Cap de Creus Canyon (Spain) and two specimens from the coast of Washington (USA) were examined. Hereafter, the samples classified based on marine area, province, depth and size are referred to as PacS/48 (South Pacific), PacN/BY12 (North Pacific), AtlN/BY4 (North Atlantic), AtlS/BY10 (South Atlantic), and Med/BY4 (Mediterranean).

2.2. Skeletal analysis: corallum morphology

A quantitative morphometric approach was used to compare macromorphological skeletal characters of *D. dianthus*. Twenty characters were measured in 174 individuals, inclusive corallum height (H), corallum length (L), corallum diameter measurements (GD1 to GD4 and LD1 to LD4), angle (α), total number of costae (CxN), number of costal cycle (Cx), costae length (CL), presence of discontinuous costae (Cd), theca thickness (TT), fossa diameters (GFD and LFD), total number of septa (SxN), number of septal cycles (Sx), septa exertness (SExV and SEvH), septa width (SW) and septa thickness (ST and SD5T) (Fig. 2a-c and Table 1). The mean values of septa width, extension and thickness and for costae length were calculated from five measurements per corallum following Miller et al. (2011). All characters were measured using a stereomicroscope Wild M8 at 10x ocular micrometre magnification and 10x or 25x objective magnification, with the exception of angle (α), which was measured on digital images using ImageJ64 software (Syed et al., 2009). For each specimen, digital images of calice (front) and corallum (side) were taken with a Nikon D5000 camera. Morphological character ratios (C:S; GCD:L; GCD:LCD; and L: α) were also

considered in order to determine morphological variation among defined biogeographical provinces.

A statistical description of morphological ratios and biogeographical variability in the 13 provinces were represented by boxplots, using ggplot2 implemented in R (Wickham, 2009; RStudio, 2012).

Canonical discriminant analysis (CDA) was performed using marine provinces, depth zones and size classes as dependent variables (the groups) and morphometric characters as independent variables (the predictors). The *a priori* marine province, depth zone and size class groups were used to compare morphological characters among individuals (Table A.2a-d). These analyses were run using SPSS 20.0 (IBM, 2011). The stepwise CDA was used to identify the most influential morphometric characters in the differentiation of the species analysed using the Wilks lambda method. The classification was evaluated by using jackknife validation.

To test for morphological changes during the transition from juvenile to adult form, linear correlation and regression were performed using MASS implemented in the R environment (RStudio, 2012; Venables and Ripley, 2002).

2.3. Skeletal analysis: 3D coordinates of landmarks

A three-dimensional (3D) morphometric method, in which the entire corallum was analysed and the data easily manipulated and converted into two-dimensional (2D) data, was chosen for this study. Morphometric analyses were conducted on the entire skeleton of a solitary coral (corallum). Each corallum consists of a tube (corallum wall) with vertical plates that radiate inward and outward (calice) from the corallum wall (or theca). A complete vertical plate is called a costoseptum (Budd and Stolarski, 2009; Carlon and Budd, 2002). For morphometric analyses, a total of 99 coralla were selected

based on the number of complete septa cycles ($> S_4$). Ultimately only 32 of these were measured, as they represent the most complete skeletons collected, thus providing maximal data per skeleton. Of the 32 specimens, 18 were from the Pacific Ocean, 6 from the Atlantic Ocean and 8 from the Mediterranean Sea (see Table A.1). Juvenile shape and proportions may be similar to other scleractinian corals during development, therefore were avoided when possible. The juvenile condition was established *a priori* and characterized by a corallum length of < 15 mm and septa cycles $\leq S_4$. A combination of type 1 and type 2 landmarks was chosen. Type 1 landmarks are points that can be defined locally, usually an intersection of three structures (Bookstein, 1991). Type 1 landmarks used in this study consisted of the junctions between septa and wall, which are reliably homologous. Homologous points that may have a minimal relationship between shape variation and differences in growth rate were also chosen. Type 2 landmarks are defined by a relative local property, such as maximum or minimum curvature (Bookstein, 1991). Type 2 landmarks used in this study consisted of those that defined septa length and width.

For each corallum, different set of landmarks along costoseptum and the corallum were digitized using 3D Cartesian coordinates (x–y–z) of landmarks (see Budd et al., 1994 for detailed methodology) and a Reflex microscope. Several pairs of landmark endpoints were used to calculate linear distances to identify landmarks that reflect the shape of costoseptum and coralla (Fig. 2d-e, Table 2). Once landmarks were selected, all 3D Cartesian coordinates were then used to construct a wireframe (lengths for each pair of endpoints). This was done in IMP-WireMan7 (Sheets, 2004). Next, Bookstein coordinates calculation and Procrustes superimposition (shape coordinates and centroid size values) were performed using IMP-Simple3D (Sheets, 2004). Variables from the 3D landmark endpoints data matrix were subjected to a principal components analysis

(PCA), which was performed using the Procrustes data with IMP-ThreeDPCA (Sheets, 2004). Finally, a canonical variates analysis (CVA) was performed on the relative warp score in IMP-CVAGen6 to investigate variation between defined groups of specimens from different marine regions. Analyses were carried out using MorphJ (Klingenberg, 2011).

2.4 Tissue analysis: characterization of the cnidom

Squash preparations of tissues from different parts of the polyp (scapus, tentacles, pharynx and mesenterial filament) were made for cnidocyst examinations. Observations, measurements and categorizations of undischarged capsules, using a Leica DMLB and/or Zeiss Axio Scope.A1 microscope with Nomarski interference contrast optics at 1000x magnification, were made following the methodology described in Godknecht and Tardent (1989) and Martínez-Baraldés et al. (2014). Two main types of cnidae, spirocysts (Sp) and nematocysts, were analysed. Following the traditional nomenclature (Weill, 1934) and amendments (Carlgren, 1940), the observed nematocysts were identified as holotrich (H), basitrich (Bs) and microbasic p-mastigophore (MpM). Each type and category was recognized according to differences in size range (length, width of capsule and shafts). Categories from small to large size classes were indicated with consecutive numbers and a letter specifying the tissue the cnidae where found. For the different tissues, the following nomenclature was used: S = scapus, T = tentacle, P = pharynx and M = mesenterial filament. For cnidae comparison and analyses, coefficients of similarity and classifications by hierarchical clustering and discriminant analysis were performed following recognized data analysis procedures (see Martínez-Baraldés et al., 2014 for more details).

Statistical description of cnidom composition in *D. dianthus* and biogeographical variability in the five marine areas were represented by boxplots, using ggplot2 implemented in R (RStudio, 2012; Wickham, 2009).

Similarity relationships among specimens collected at the different sampling stations were obtained by the Bray-Curtis index (Bray and Curtis, 1957), using the average length of each cnida in each tissue type. Original data from Terron-Sigler and Lopez-Gonzalez (2005) and Martínez-Baraldés et al. (2014) were also included to show the interspecific relationship. A hierarchical agglomerative clustering method was applied using the PRIMER v6 program (Clarke and Gorley, 2006).

Phylogenetic relationships were explored using the software Winclada-NONA (Goloboff, 1999; Nixon, 1999-2002), based on the principle of parsimony using the presence-absence matrix obtained for all cnidae in each tissue type. For this analysis, available data from a number of scleractinian, corallimorpharian and actiniarian species were also included (see Martínez-Baraldés et al., 2014). Corallimorpharia and Actiniaria species were used as outgroup. Fitch's parsimony (non-additive) was considered for all characters as it is unknown if any transformation sequence exists among cnidae categories (Fautin, 2009). The consensus clustering was based on a TBR-heuristic analysis, with 100 replicates to test node supports.

CDA was carried out considering the marine areas as dependent variables (the groups) and the cnidae from each tissue as independent variables (the predictors). The objective was to explore the discriminatory capacity of cnidae while trying to classify new observations. The five groups compared in the aforementioned skeletal analysis (Section 2.2) were also used for this analysis. Only cnidae common to multiple tissue types, and present at frequencies recommended for the model, were selected for CDA.

A total of ten cnidae were included in the analysis (Bs1S, MpM1S, SpT, H1T, Bs2P, Bs3P, MpM1P, Bs1M, MpM2M and MpM4M). This analysis was run using SPSS 20.0 (IBM, 2011). The stepwise CDA was used to identify the most influential cnidae tissue in the differentiation of the analysed species or groups (localities) using the Wilks lambda method. However, choosing common cnidocysts caused differences in group size, which was taken into consideration using prior probabilities for each group. The classification was evaluated using jackknife validation.

3. Results

3.1 Skeletal analysis: corallum morphology

Variability and distribution of morphological characters in the 13 provinces studied were compared, but PD and Cd characters were not considered due to the scarcity of data. All characters were found to be constant throughout the marine provinces, except for the morphological ratios GCD:L and $L:\alpha$, which showed relevant differences among provinces (Fig. 3).

CDA was performed on a total of 50 individuals from the five marine areas (groups) also considered in the cnidocyst analysis: PacN/BY12, PacS/BY8, AtlN/BY4, AtlS/BY10, and Med/BY4 (Table A.1-2a). The analysis reached full discriminatory capacity (100%) with three morphological characters: SxN, SExH and SW. Results suggest that SW and SxN contributed the most to differentiation among the five groups (marine areas) (Table A.3a). The coefficients of the Fisher linear discriminant function for each province are shown in Table A.3b. The plot using the first two unstandardized canonical discriminant function coefficients showed the intraspecific variation of individuals and delimitations of groups (Fig. 4a). Some groups (marine areas) partially overlapped due to relatively high intraspecific variability, while other provinces, such as

AtlS/BY10 and PacS/BY8, were clearly defined. The classification, evaluated using jackknife validation for five categories among the dependent variable (marine areas), showed that more than 78.7% of the cases were correctly classified. The percentage of correctly classified cases by groups is as follows: PacN/BY12 77.8%, PacS/BY8 100%, AtlN/BY4 60%, AtlS/BY10 62.5%, and Med/BY4 90% (Table A.3c).

To investigate if the morphological characters revealed a more general and clear biogeographical pattern, a greater number of samples and marine provinces were included in the analysis. A total of 174 *D. dianthus* specimens belonging to 13 provinces were used to perform the CDA (Table A.1). No differences in skeletal morphology were found between specimens when data sets were sorted according to the 13 provinces (Fig. A.1 and Table A.4), the marine areas or depth zones (Fig. A.2b-c). However when sorted by size class, corals clustered as five distinct groups with the variables GD4, α and SxN mostly contributing to differences in skeletal morphology (Fig. 4b). This clear clustering pattern was not found when samples sorted by size class were further classified by marine provinces (Fig. A.3a-d).

Pearson's correlation coefficients among L, GCD, S and TT characters were nearly zero, showing no linear relationship (Fig. A.4).

3.2 Skeletal analysis: 3D coordinates of landmarks

Homologous structures along the corallum wall could not be clearly defined, therefore several sets of landmarks could not be measured accurately. Instead, 19 landmarks per side along costoseptum were successfully found (Fig. 2d), and a total of 44 pairs of landmark endpoints (22 per side) (Table 2) were used to perform the corresponding analysis. Although a slight structure is visible (Fig. 5a), PCA and CVA analyses of datasets using individuals and provinces as *a priori* groups found no significant

differences in landmark endpoints in either nor associated axes positively correlated with specific pair endpoints (Table A.5a-c).

3.3 Tissue analysis: characterization of the cnidom

The following results are based on the study of over 24,000 undischarged capsules. The observed diversity of cnidae in *D. dianthus* included 11 categories: SP, two H, three Bs, and five MpM. The diversity and distribution of the analysed cnidae from the five sampling stations were compared (Table 3) and morphologies (types and categories) represented (Fig. 6-10). The cnidom composition and size of cnidae present in different tissues were constant throughout the sampling areas. Eleven categories were common across the different sampling stations: Bs1 and MpM (scapus); Sp, H1, and MpM3 (tentacles); Bs2, Bs3, and MpM1 (pharynx); Bs2, MpM2 and MpM4 (mesenterial filaments). Each area also showed a lack of cnidae categories per tissue: AtlN/BY4 (Bs3T and MpM1T); AtlS/BY10 (MpM2S, Bs1T, MpM1T, Bs1P and MpM3P); PacN/BY12 (Bs3T and MpM1T); PacS/48 (Bs1T and Bs2T); and Med/BY4 (Bs1T, MpM1T, Bs1P, MpM3P, H2M, MpM1M and MpM5M) (Table 3).

Intraspecific relationships among provinces showed 60% similarity between two distinct groups: one represented by Med/BY4 and another that included all other provinces (i.e. AtlN/BY4, AtlS/BY10, PacN/BY12 and PacS/48) (Fig. 11). The latter group presented two additional groups of provinces, AtlS/BY10-PacS/48 and AtlN/BY4-PacN/BY12, both with over 85% similarity at the inter-individual level. Other scleractinians (Mussiidae, Dendrophylliidae), and corallimorpharians included in the analysis were widely differentiated, with less than 30% similarity.

Parsimony analysis (fast optimization method, minimum number of steps) of the presence-absence matrix based on 55 characters (cnidae tissue) (Table A.6) resulted in a

single tree (Fig. 12). The clade including *D. dianthus* species was weakly supported. With respect to interspecific relationships, the examined species of the family Caryophylliidae showed more affinity with species belonging to the family Dendrophylliidae than to Mussidae. There were three synapomorphies that defined the relationship between the families Caryophylliidae and Dendrophylliidae (according to the sampled species): the presence of Bs2S and the absences of Bs3T and Bs1P (encoded as 2-1, 15-0 and 24-0, respectively). Within this clade, *D. dianthus* showed several differences including a series of autapomorphic (absence of H1S, presence of MpM3T, H2P and MpM1M; encoded as 4-0, 23-1, 29-1 and 43-1, respectively) and homoplastic (presence of MpM1S, H1S, H2S, H1P, MpM1P, Bs4M, H2M, H3M and H5M; encoded as 8-1, 17-1, 18-1, 28-1, 31-1, 37-1, 39-1, 40-1 and 42-1, respectively) characters.

The CDA indicated that H2T and Bs2P were the variables (among all shared cnidocysts) contributing most to the differences among groups (scleractinian species). The coefficients of the Fisher linear discriminant function for each species are shown in Table A.7a-c. The plot using the first two unstandardized canonical discriminant function coefficients showed the intraspecific variation of individuals and delimitations of groups (Fig. 13). Although dendrophylliid species partially overlapped due to relatively high intraspecific variability (by the possible retention of phylogenetic signals in these cnidae characters), *Dendrophyllia ramea*, *D. cornigera* and the caryophylliid *Desmophyllum dianthus* species did not overlap in the same 2D-CDA space.

The CDA suggested that MpM4M/MpM1S and Bs1S were the variables (among all the shared cnidocysts) contributing most to the differences among groups (marine areas). The analysis reached high discriminatory capacity with eight cnidocyst categories (from the eleven chosen *a priori*): Bs1S, MpM1S, H1T, MpM3T, Bs2P, Bs3P, MpM1P and

Bs1M. Results suggested that MpM1S and Bs1M contributed most to differentiation among the five groups (marine areas) (Table A.8a). The coefficients of the Fisher linear discriminant function for each marine area are shown in Table A.8b. The plot using the first two unstandardized canonical discriminant function coefficients showed the intraspecific variation of individuals and delimitations of groups (Fig. A.5). Some groups (marine areas) partially overlapped due to relatively high intraspecific variability, while other provinces, such as AtlS/BY10 and Med/BY4, were clearly defined. The classification, evaluated using jackknife validation for five categories among the dependent variables (marine areas), showed that more than 82.9% of the cases were correctly classified. The percentage of correctly case by provinces is follows: PacN/BY12 67.5%, PacS/48 83.3%, AtlN/BY4 63.3%, AtlS/BY10 98.3%, and for Med/BY4 96.76% (Table A.8c).

4. Discussion

4.1 Skeletal analysis: corallum morphology

Previous studies on morphological variation of *D. dianthus* were conducted at the inter- and intra-population levels, and geographic and environmental conditions were taken into account in the analyses (Fillinger and Richter, 2013; Miller et al., 2011).

In this study, a geographical pattern of morphological differentiation was found among *D. dianthus* populations, consistent with the findings of Miller et al. (2011). However, when a larger number of individuals were examined to measure variation over a wider ecological and geographic range, the pattern was lost. The analyses of morphological features in this study showed the unresolved classification of individuals by potential morphotypes, and morphometric characters did not detect differences among examined

biogeographic areas. In agreement to the conclusion also reached by Cairns (1979, 1982), the results suggested that no morphological divergence is occurring between individuals of *D. dianthus* in the 13 biogeographic provinces and the significant differences in skeletal morphology could simply reflect the high degree of phenotypic variability characterizing the species. Indeed, as Zibrowius (1980) remarked, “the corallum exhibits considerable variation in spite of its rather simple structure (absence of pali and reduced columella on only very young stages), and its shape is largely conditioned by the substrate and micro-environmental factors available for the settling larva”. Similar to reef-building zooxanthellate corals, where the ecomorph concept (combination of high morphological and ecological diversity) is applied as an intraspecific taxonomic approach (Veron and Pichon, 1976), the high levels of intraspecific variation in azooxanthellate corals must be taken into account in species definition (Zibrowius, 1983).

Considering the results from this study and previous studies (see Addamo et al., 2012; Fillinger and Richter, 2013; Miller et al., 2011), and contrary to other solitary corals, (e.g. *Caryophyllia inornata*) whose biometric parameters showed a higher correlation with environmental variables (Caroselli et al., 2015), *D. dianthus* displays a high intraspecific morphological variation that does not seem to be strictly correlated to geographical or ecological domains.

Indeed, in some cases the intraspecific variation is lower among individuals inhabiting different biogeographical regions than among individuals within a province (e.g. specimens from the Subantarctic province-BY8 in Fig.A.1). The first pattern postulates that species occupying a large geographic area while showing little morphological variability may represent old and stable species, whereas highly variable species in the same area may represent young and diversified ones (Wijsman-Best, 1980). However,

the degree of variation within species is possibly not closely related to the age of the genus from an evolutionary viewpoint, but it rather has to do with the extent of genotypic polymorphism within species (Borel Best et al., 1983). The second pattern, on the other hand, postulates that micro-environmental differences may have important impacts that are best exploited by a large phenotypic plasticity response (Bradshaw, 1965). This “silent intraspecific variation” may also be visible at the molecular level. For example, genetic analyses of nuclear (internal transcribed spacer regions, ITS) and mitochondrial (large ribosomal subunit, 16S) genes showed low genetic differentiation and the occurrence of shared haplotypes between Mediterranean Sea and South Pacific populations of *D. dianthus* (Addamo et al., 2012). Conversely, the above mentioned silent intraspecific variation among individuals from different provinces became clear when hypervariable molecular markers (e.g. microsatellites) have been used in order to assess the potential structure among populations of *D. dianthus* (see Addamo et al., 2015, and Addamo pers. obs.).

4.2. Skeletal analysis: 3D coordinates of landmarks in solitary corals

The process of choosing landmarks is one of the most important aspects of geometric morphometric analysis. Landmarks must represent the shape of the object under study and be informative to the specific question being addressed. Three-dimensional morphometric methods are becoming increasingly common among coral taxonomists studying solitary and colonial variability (Budd et al., 1994; Carlon and Budd, 2002; Grass Darrell, 2009; Kongjandtre et al., 2012). This study provided an opportunity to explore the utility of 3D geometric morphometric methods in solitary corals. It represents a first attempt of using morphometric analyses with landmark methods on solitary coral. Due to the nature of the landmark methods employed, only relatively complete specimens could be used. Overall, two main challenges were identified.

Firstly, the lack of type 1 and type 2 landmarks along the corallum wall excluded a set of elements for morphometric studies that otherwise could be potentially informative regarding shape. Secondly, although landmarks could be found along costoseptum, excluding incomplete or deformed specimens from analyses (considered an ideal practice from a morphological standpoint) affected the sample size such that it was too small to perform rigorous statistical analyses. Increasing the number of specimens analysed may overcome this issue. However, there are problems associated with this: the depths at which these species typically inhabit makes collecting numerous samples difficult and current sampling techniques easily damage the septa (one of the most characteristic corallum features of *D. dianthus*). Alternatively, one could exclude or decrease the numbers of landmarks used; however, this would effectively exclude or decrease the number of informative characters. The results presented here showed a slight, but not significant, structure of *D. dianthus* populations. This structure is similar to the pattern observed from the macromorphology and cnidocysts analyses, suggesting that landmarks are potentially informative characters at the intraspecific level and that geometric morphometric methods may be a good alternative for testing phylogenetic hypotheses in scleractinian corals. However, additional future studies on this and other proposed cosmopolitan species, including a similar sampling effort in localities and specimens, will be useful to explore the existence of common global patterns of morphological variability.

4.3 Tissue analysis: characterization of the cnidom at the intraspecific level

Even though intraspecific variability is lower than interspecific variability at the geographical scale (Martínez-Baraldés et al., 2014; Terron-Sigler and Lopez-Gonzalez, 2005), it may be higher at ontogenetic or ecological levels (Williams, 1998). The diversity and distribution of cnidocysts showed qualitative and quantitative differences

among the examined biogeographic units. The Mediterranean Sea, Sub-Antarctic and Chilean Fjords (MED/BY4, AtlS/BY10 and AtlN/48, respectively) were the biogeographic units showing the greatest differences based on cnidocysts analyses. These differences may reflect the environmental heterogeneity of the analysed provinces, corresponding to locations where oceanographic fronts or transitions and/or other environmental variables are known to occur (Watling et al., 2013).

Cnidae diversity could impart sufficient information at the ecological level to group examined individuals by biogeographic areas, as nematocyst morphology may depend on function (Schmidt, 1974). Further studies on the physiology and ecological function of nematocysts may increase their value as phylogenetically informative characters in the Anthozoa as recently discussed by Martínez-Baraldés et al. (2014).

Other authors have also highlighted the importance of nematocyst classification. For instance, Schmidt (1974) considered nematocysts (including type, size and distribution) as important characters for revealing a clear new concept of evolution in the Anthozoa. However, he also argued that the traditional classification of nematocysts and spirocysts (Weill, 1934) is artificial and arbitrary, and could not coincide with a natural or phylogenetic system of the Cnidaria, thus complicating phylogenetic interpretations. The value of cnidae for systematics depends on re-evaluating their diagnostic characteristics, and systematising nematocyst nomenclature may avoid confusion by using synonyms for well-known nematocysts (Östman, 2000). Moreover, as in other morphological characters, the possibility of homoplastic expressions in the cnidae composition cannot be discharged, existing the possibility of different evolutionary ways for arriving to a single cnidae composition as discussed by Martínez-Baraldés et al. (2014). As applied thus far, numeral assignment of categories of a determined type of cnidocyst depends on the number of existing size categories of that type in a

determined dataset. However, when the dataset includes additional species in the comparison, an intermediate category may appear among the previously considered ones. Consequently, the size categories in the matrix are renamed to establish a consecutive sequence for each cnidocysts type (see Martínez-Baraldés et al., 2014, for detailed nomenclature methodology). Therefore, a detailed investigation of the cnidom throughout the anthozoan orders, with a standardized nomenclature and quantitative methodology, may increase their potential as indicators of phylogenetic relationships and ecological conditions.

4.4. Conclusion

This study revealed only a slightly structured pattern of morphological variability in *D. dianthus* populations, likely due to its peculiar and contrasting characteristics: an organism of simple structure with a high degree of phenotypic variability, which could be modelled by punctual environmental conditions (spatial and temporal variations) during larval development rather than by a biogeographical or bathymetric pattern. Moreover, more importantly, cnidocyst characters and 3D landmark coordinates are shown as potentially useful tools for investigating morphological variation at the intraspecific level.

However, the present study highlights the importance to examine a representative size of samples used to perform each analysis, and corroborate the initial hypothesis correctly. The number of specimens examined is a critical issue for deep sea species. In the last 15 years hundreds of oceanographic cruises have focused on deep sea ecosystems and many have them allowed the collection of a large number of cold-water coral species. International standard procedures for collection and storing of these corals

are needed in order to improve our knowledge on the systematics, and phylo-, bio- and paleo-geography of these important organisms.

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References

- Addamo, A.M., Reimer, J.D., Taviani, M., Freiwald, A., Machordom, A., 2012. *Desmophyllum dianthus* (Esper, 1794) in the scleractinian phylogeny and its intraspecific diversity. PLoS ONE 7, e50215.
- Addamo, A.M., García-Jiménez, R., Taviani, M., Machordom, A., 2015. Development of microsatellite markers in the deep-sea cup coral *Desmophyllum dianthus* and cross-species amplifications in the Scleractinia Order. J. Hered. 106(3), 322-330.
- Adkins, J.F., Henderson, G.M., Wang, S.L., O'Shea, S., Mokadem, F., 2004. Growth rates of the deep-sea scleractinia *Desmophyllum cristagalli* and *Enallopsammia rostrata*. Earth Planet. Sc. Lett. 227, 481-490.
- Barbeitos, M., Romano, S., Lasker, H., 2010. Repeated loss of coloniality and symbiosis in scleractinian corals. Proc. Natl. Acad. Sci. USA 107(26), 11877-11882.
- Benzoni, F., Stefani, F., Pichon, M., Galli, P., 2010. The name game: morpho-molecular species boundaries in the genus *Psammocora* (Cnidaria, Scleractinia). Zool. J. Linn. Soc.-Lond. 160, 421-456.
- Benzoni, F., Arrigoni, R., Waheed, Z., Stefani, F., Hoeksema, B.W., 2014. Phylogenetic relationships and revision of the genus *Blastomussa* (Cnidaria: Anthozoa: Scleractinia) with description of a new species. Raffles B. Zool. 62, 358-378.
- Bookstein, F., 1991. Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press, New York, NY.

- Borel Best, M., Boekschoten, G.J., Oosterbaan, A., 1983. Species concept and ecomorph variation in living and fossil Scleractinia. *Palaeontogr. Am.* 54, 70-79.
- Bradshaw, A., 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.* 13, 115-155.
- Bray, R.J., Curtis, J.I., 1957. An ordenation of the upland forest community of Southern Wisconsin. *Ecol. Monogr.* 27, 325-349.
- Budd, A.F., Stolarski, J., 2009. Searching for new morphological characters in the systematics of scleractinian reef corals: comparison of septal teeth and granules between Atlantic and Pacific Mussidae. *Acta Zool.* 90, 142-165.
- Budd, A.F., Johnson, K.G., Potts, D.C., 1994. Recognizing morphospecies in colonial reef corals; I. Landmark-based methods. *Paleobiology* 20, 484-505.
- Budd, A.F., Romano, S.L., Smith, N.D., Barbeitos, M.S., 2010. Rethinking the phylogeny of scleractinian corals: a review of morphological and molecular data. *Integr. Comp. Biol.* 50, 411-427.
- Budd, A.F., Fukami, H., Smith, N.D., Knowlton, N., 2012. Taxonomic classification of the reef coral family Mussidae (Cnidaria: Anthozoa: Scleractinia). *Zool. J. Linn. Soc.-Lond.* 166, 465-529.
- Cairns, S.D., 1979. The deep-water Scleractinia of the Caribbean Sea and adjacent waters. *Stud. Fauna. of Curaçao Caribbean Isl.* 57(1), 1-341.
- Cairns, S.D., 1982. Stony corals (Cnidaria: Hydrozoa, Scleractinia) of Carrie Bow Cay, Belize. *Smithson. Contrib. Mar. Sci.* 12, 272-302.
- Cairns, S.D., 1995. The marine fauna of New Zealand: Scleractinia (Cnidaria: Anthozoa). *NZOI Mem.* 103, 139 pp.
- Carlgren, O., 1940. A contribution to the knowledge of structure and distribution of cnidae in the Anthozoa. *K. Fysiogr. Sällsk. Handl.* 51, 1-62.

- Carlson, D.B., Budd, A.F., 2002. Incipient speciation across a depth gradient in a scleractinian coral? *Evolution* 56, 2227-2242.
- Caroselli, E., Nanni, V., Levy, O., Falini, G., Dubinsky, Z., Goffredo S., (2015). Latitudinal variation in biometry and population density of a Mediterranean solitary coral. *Limnol. Oceanogr.* 60, 1356-1370.
- Chen, C.A., Odorico, D.M., Tenlohuis, M., Veron, J.E.N., Miller, D.J., 1995. Systematic relationships within the Anthozoa (Cnidaria: Anthozoa) using the 5'-end of the 28S rDNA. *Mol. Phylogenet. Evol.* 4, 175-183.
- Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User manual/tutorial. In: PRIMER-E (ed), Plymouth.
- Fautin, D.G., 2009. Structural diversity, systematics, and evolution of cnidae. *Toxicon* 54, 1054-1064.
- Fillinger, L., Richter, C., 2013. Vertical and horizontal distribution of *Desmophyllum dianthus* in Comau Fjord, Chile: a cold-water coral thriving at low pH. *PeerJ* 1, e194.
- Fukami, H., Chen, C., Budd, A., Collins, A., Wallace, C., Chuang, Y., Chen, C., Dai, C., Iwao, K., Sheppard, C., Knowlton, N., 2008. Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). *PLoS ONE* 3, e3222.
- Godknecht, A., Tardent, P., 1989. Discharge and mode of action of the tentacular nematocysts of *Anemonia sulcata* (Anthozoa: Cnidaria). *Mar. Biol.* 100, 83-92.
- Goloboff, P., 1999. NONA (NO NAME) ver.2. Published by the author, Tucumán, Argentina.

- Grass Darrell, A., 2009. Examining ecosystem structure and disparity through time using geometric morphometrics. The University of Iowa, Master Thesis.
- Hoeksema, B.W., 2014. The “*Fungia patella* group” (Scleractinia, Fungiidae) revisited with a description of the mini mushroom coral *Cycloseris boschmai* sp. n. ZooKeys, 371:57-84.
- Huang, D., Benzoni, F., Fukami, H., Knowlton, N., Smith, N.D., Budd, A.F., 2014. Taxonomic classification of the reef coral families Merulinidae, Montastraeidae, and Diploastraeidae (Cnidaria: Anthozoa: Scleractinia). Zool. J. Linn. Soc.-Lond. 171, 277-355.
- IBM Corp. 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.
- Kelleher, G., Bleakley, C., Wells, S., 1995. A global representative system of marine protected areas. Great Barrier Reef Marine Park Authority, World Bank, IUCN (World Conservation Union), Washington (DC).
- Kerr, A.M., 2005. Molecular and morphological supertree of stony corals (Anthozoa: Scleractinia) using matrix representation parsimony. Biol. Rev. 80, 543-558.
- Kitahara, M.V., Cairns, S., Stolarski, J., Blair, D., Miller, D., 2010. A comprehensive phylogenetic analysis of the Scleractinia (Cnidaria, Anthozoa) based on mitochondrial CO1 sequence data. PLoS ONE 5, e11490.
- Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Mol. Ecol. Resour. 11, 353-357.
- Kongjandtre, N., Ridgway, T., Cook, L.G., Huelsken, T., Budd, A.F., Hoegh-Guldberg, O., 2012. Taxonomy and species boundaries in the coral genus *Favia* Milne Edwards and Haime, 1857 (Cnidaria: Scleractinia) from Thailand revealed by morphological and genetic data. Coral Reefs 31, 581-601.

- Lin, M.-F., Kitahara, M.V., Luo, H., Tracey, D., Geller, J., Fukami, H., Miller, D.J., Chen, C.A., 2014. Mitochondrial Genome Rearrangements in the Scleractinia/Corallimorpharia Complex: Implications for Coral Phylogeny. *Genome Biol. Evol.* 6, 1086-1095.
- Martínez-Baraldés, I., López-González, P.J., Megina, C., 2014. Application of cnidae composition in phylogenetic analyses of North Atlantic and Mediterranean dendrophylliid corals (Anthozoa : Scleractinia). *Invertebr. Syst.* 28, 214-230.
- McCulloch, M., Taviani, M., Montagna, P., López Correa, M., Remia, A., Mortimer, G., 2010. Proliferation and demise of deep-sea corals in the Mediterranean during the Younger Dryas. *Earth Planet. Sc. Lett.* 298(1-2), 143-152.
- Medina, M., Collins, A., Takaoka, T., Kuehl, J., Boore, J., 2006. Naked corals: Skeleton loss in Scleractinia. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9096-9100.
- Miller, K.J., Rowden, A.A., Williams, A., Häussermann, V., 2011. Out of their depth? Isolated deep populations of the cosmopolitan coral *Desmophyllum dianthus* may be highly vulnerable to environmental change. *PLoS ONE* 6, e19004.
- Milne Edwards, H., Haime, J., 1857. Histoire naturelle des coralliaires ou polypes proprement dits, Paris.
- Nixon, K.C., 1999-2002. WinClada ver. 1.0000. Published by the author, Ithaca, NY, USA.
- Östman, C., 2000. A guideline to nematocyst nomenclature and classification, and some notes on the systematics value of nematocysts. *Sci. Mar.* 64(1), 31-46.
- Picciani, N., Pires, D.O., Silva, H.R., 2011. Cnidocysts of Caryophylliidae and Dendrophylliidae (Cnidaria: Scleractinia): taxonomic distribution and phylogenetic implications. *Zootaxa* 3135, 35-54.
- Pires, D., 1997. Cnidae of Scleractinia. *Proc. Biol. Soc. Wash.* 110, 167-185.

- Remia, A., Taviani, M., 2005. Shallow-buried Pleistocene *Madrepora*-dominated coral mounds on a muddy continental slope, Tuscan Archipelago, NE Tyrrhenian Sea. *Facies* 50(3-4), 419-425.
- Richards, Z.T., Miller, D.J., Wallace, C.C., 2013. Molecular phylogenetics of geographically restricted *Acropora* species: Implications for threatened species conservation. *Mol. Phylogenet. Evol.* 69, 837-851.
- Romano, S., Palumbi, S., 1996. Evolution of scleractinian corals inferred from molecular systematics. *Science* 271, 640-642.
- RStudio, 2012. RStudio: Integrated development environment for R (Version 0.96.122) [Computer software], Boston, MA.
- Schmidt, H., 1974. On evolution in the Anthozoa. *Proc. 2nd Int. Coral Reef Symp.* 1, 533-560.
- Schmidt-Roach, S., Miller, K.J., Lundgren, P., Andreakis, N., 2014. With eyes wide open: a revision of species within and closely related to the *Pocillopora damicornis* species complex (Scleractinia; Pocilloporidae) using morphology and genetics. *Zool. J. Linn. Soc.-Lond.* 170, 1-33.
- Seguenza, G., 1864. Disquisizioni paleontologiche intorno ai Corallarii fossili delle rocce terziarie del distretto di Messina. *Mem. Reale Accad. Sci. Torino.* (ser 2). 21, 399–560.
- Sheets, D., 2004. IMP-Integrated Morphometrics Package. Department of Physics, Canisius College, Buffalo, New York.
- Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdeña, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A.L., Lourie, S.A., Martin, K.D., McManus, E., Molnar, J., Recchia, C.A., Robertson, J., 2007. Marine ecoregions

- of the world: a bioregionalization of coastal and shelf areas. *BioScience* 57, 573-583.
- Stolarski, J., Roniewicz, E.W.A., 2001. Towards a new synthesis of evolutionary relationships and classification of Scleractinia. *J. Paleontol.* 75, 1090-1108.
- Stolarski, J., Kitahara, M., Miller, D., Cairns, S., Mazur, M., Meibom, A., 2011. The ancient evolutionary origins of Scleractinia revealed by azooxanthellate corals. *BMC Evol. Biol.* 11, 316.
- Syed, R., Sobh, N., Ravaioli, U., Popescu, G., Mohamed, M., 2009. imageJ. <https://nanohub.org/resources/imagej>.
- Taviani, M., Freiwald, A., Zibrowius, H., 2005. Deep coral growth in the Mediterranean Sea: an overview. In Freiwald A, Roberts JM (eds), 2005, *Cold-water Corals and Ecosystems*, Springer-Verlag Berlin Heidelberg, pp 137-156.
- Taviani, M., Vertino, A., López Correa, M., Savini, A., De Mol, B., Remia, A., Montagna, P., Angeletti, L., Zibrowius, H., Alves, T., Salomidi, M., Ritt, B., Henry, P., 2011. Pleistocene to Recent scleractinian deep-water corals and coral facies in the Eastern Mediterranean. *Facies* 57, 579-603.
- Terron-Sigler, A., Lopez-Gonzalez, P.J., 2005. Cnidae variability in *Balanophyllia europaea* and *B. regia* (Scleractinia: Dendrophylliidae) in the NE Atlantic and Mediterranean Sea. *Sci. Mar.* 69, 75-86.
- Todd, P.A., 2008. Morphological plasticity in scleractinian corals. *Biol. Rev.* 83, 315-337.
- van Oppen, M.J.H., McDonald, B.J., Willis, B., Miller, D.J., 2001. The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: Reticulation, incomplete lineage sorting, or morphological convergence? *Mol. Biol. Evol.* 18, 1315-1329.

- Vaughan, T., Wells, J., 1943. Revision of the suborders, families, and genera of the Scleractinia. Spec. Pap. Geol. Soc. Am. 44, 1-363.
- Venables, W.N., Ripley, B.D., 2002. Modern applied Statistics with S. Springer, New York.
- Veron, J.E.N., Pichon, M., 1976. Scleractinia of Eastern Australia. Part I. Families Thamnasteriidae, Astrocoeniidae, Pocilloporidae. Australian Government Publishing Service, Canberra.
- Vertino, A., 2003. Sclerattiniari plio-pleistocenici ed attuali del Mediterraneo. University of Messina, PhD Thesis.
- Vertino, A., Stolarski, J., Bosellini, F.R., Taviani, M., 2014. Mediterranean corals through time: from Miocene to Present. Chapter 14, in: Goffredo, S., Dubinsky, Z. (Eds.), The Mediterranean Sea: its history and present challenges. Springer, Dordrecht, pp 257-274.
- Watling, L., Guinotte, J., Clark, M.R., Smith, C.R., 2013. A proposed biogeography of the deep ocean floor. Prog. Oceanogr. 111, 91-112.
- Weill, R., 1934. Contribution à l'étude des cnidaires et de leurs nématocystes. Laboratoire d'évolution des êtres organisés, Paris.
- Wells, J.W. 1956. Scleractinia, in: Moore, R.C. (Ed.), Treatise on Invertebrate Paleontology. Geological Society of America and University of Kansas Press, Kansas, pp. 328-444.
- Wickham, H., 2009. ggplot2: elegant graphics for data analysis. Springer, New York.
- Wijsman-Best, M., 1980. Indo-Pacific coral species belonging to the subfamily Montastreinae Vaughan & Wells, 1943 (Scleractinia-Coelenterata) Part II. The genera *Cyphastrea*, *Leptastrea*, *Echinopora* and *Diploastrea*. Zool. Med. 55, 235-263, pls. 231-235.

- Williams, R., 1998. Measurements of cnidae from sea anemones (Cnidaria: Actinaria), II: further studies of differences amongst sample means and their taxonomic relevance. *Sci. Mar.* 62, 361-372.
- Zibrowius, H., 1980. Les scléractiniaires de la Méditerranée et de l'Atlantique nordoriental. *Mém. Inst. Océanogr. Monaco* 11, 1-124.
- Zibrowius, H., 1983. Taxonomy in ahermatypic scleractinian corals. *Palaeontogr. Am.* 54, 80-85.
- Zibrowius, H., 1987. Scléractiniaires et Polychètes Serpulidae des faunes bathyales actuelle et plio-pléistocène de Méditerranée. *Doc. Trav. IGAL* 11, 255–257.

Appendices

Fig. A.1. Plot of the first two functions from discriminant analyses of all morphological data, classified by marine province. Key: 48= Magellanic, 54= Southern New Zealand, 55/56= East Centralian/Southeast Australian Shelf, BY3= North Pacific Boreal, BY4= North Atlantic Bathyal, BY6= New Zealand Kermadec, BY7= Cocos Plate, BY8=Nazca Plate, BY10= Subantartic, BY11= Indian Ocean Bathyal, BY12= West Pacific Bathyal, BY13= South Atlantic, BY14=North Pacific Bathyal. *shallow water province (for details see Table A.2.b).

Fig. A.2.a. Plots of the first two functions from discriminant analyses of all morphological data, classified by marine area. Key: Med= Mediterranean Sea, AtlN= Atlantic Ocean-North, AtlS= Atlantic Ocean-South, PacN= Pacific Ocean-North, PacS= Pacific Ocean-South (for more details see Table A.2.a). **2.b.** Plots of the first two functions from discriminant analyses of all morphological data, classified by depth.

Key: SW= shallow water zone, MW= mesopelagic water zone, UB= upper bathyal zone, LB= lower bathyal zone, NA= unknown (for more details see Table A.2.c).

Fig. A.3. Plots of the first two functions from discriminant analyses of all morphological data, classified by size class: 1st tridecade (1T) (**a**), 2nd tridecade (2T) (**b**), 3rd tridecade (3T) (**c**) and 4th tridecade (4T) (**d**).. Key: T= tridecade, Key: 48= Magellanic, 54= Southern New Zealand, 55/56= East Centralian/Southeast Australian Shelf, BY3= North Pacific Boreal, BY4= North Atlantic Bathyal, BY6= New Zealand Kermadec, BY7= Cocos Plate, BY8= Nazca Plate, BY10= Subantartic, BY11= Indian Ocean Bathyal, BY12= West Pacific Bathyal, BY13= South Atlantic, BY14= North Pacific Bathyal (for more details see Table A.2.b). *shallow water province.

Fig. A.4. Scatterplots and Pearson's correlation coefficients of the following morphometric parameters: L, GCD, Sx and TT. Key: L= corallum length, GCD= great calicular diameter, Sx= septa cycle, TT= theca thickness (for more details see Table 2).

Table A.1. Information of specimens used for analyses. USNM= National Museum of Natural History; MNCN= Museo Nacional de Ciencias Naturales; US= Universidad de Sevilla.

Table A.2.a. Legend for marine areas. **2.b.** Legend for marine biogeographical provinces. **2.c.** Legend for marine depth zones. **2.d.** Legend for size classes based on corallum length. Average growth rate = 1 mm/year.

Table A.3.a. Matrix structure of CDA using morphological characters and samples classified by marine area. Combined intra-group correlations between discriminating variables and canonical discriminant functions typified. Variables ordered by size of correlation with function. *Largest absolute correlation between each variable and any discriminant function. (+) This variable is not used in the analysis. **3.b.** Coefficients of

classification of the Fisher's linear discriminant functions for each marine area. **3.c.** Result of classification for each marine area. Of originally grouped cases, 80.9% were correctly classified, and 78.7% were validated by cross-validation. Key: Med= Mediterranean Sea, AtlN= Atlantic Ocean-North, AtlS= Atlantic Ocean-South, PacN= Pacific Ocean-North, PacS= Pacific Ocean-South (for more details see Table A.2.a).

Table A.4. Mean (M), standard deviation (SD) and range of each parameter included in the analyses per marine province. Key: Key: 48= Magellanic, 54= Southern New Zealand, 55/56= East Centralian/Southeast Australian Shelf, BY3= North Pacific Boreal, BY4= North Atlantic Bathyal, BY6= New Zealand Kermadec, BY7= Cocos Plate, BY8=Nazca Plate, BY10= Subantartic, BY11= Indian Ocean Bathyal, BY12= West Pacific Bathyal, BY13= South Atlantic, BY14=North Pacific Bathyal (for more details see Table A.2.b).

Table A.5.a. Summary of statistics of PCA. **5.b.** Summary of statistics of CVA with samples classified by marine area. Key: Med= Mediterranean Sea, AtlN= Atlantic Ocean-North, AtlS= Atlantic Ocean-South, PacN= Pacific Ocean-North, PacS= Pacific Ocean-South (for more details see Table A.2.a). **5.c.** Summary of statistics of CVA with samples classified by marine province. Key: Key: 48= Magellanic, 54= Southern New Zealand, 55/56= East Centralian/Southeast Australian Shelf, BY3= North Pacific Boreal, BY4= North Atlantic Bathyal, BY6= New Zealand Kermadec, BY7= Cocos Plate, BY8=Nazca Plate, BY10= Subantartic, BY11= Indian Ocean Bathyal, BY12= West Pacific Bathyal, BY13= South Atlantic, BY14=North Pacific Bathyal (for more details see Table A.2.b). The dataset contains 32 observations included in the analyses and 38 landmarks in 3 dimensions.

Table A.6. Matrix of 55 characters (presence/absence data) from all tissues for each species. Key: Sp= spirocysts, H= holotrich, Bs= basitrich, MpM= microbasic p-mastigophore (MpM), S= scapus, T= tentacles, P= pharynx, M= mesenterial filaments, C= columella, A= acontia, PD= pedal disc.

Table A.7.a. Matrix structure of CDA using cnidae characters and species. Combined intra-group correlations between discriminating variables and canonical discriminant functions typified. Variables ordered by size of correlation with function. *Largest absolute correlation between each variable and any discriminant function. (+) This variable is not used in the analysis. **7.b.** Coefficients of classification of the Fisher's linear discriminant functions for each species. **7.c.** Result of classification for each species. Of originally grouped cases, 78.6% were correctly classified, and 77.8% were validated by cross-validation.

Table A.8.a. Matrix structure of CDA using cnidae characters and samples classified by marine areas and corresponding marine province. Combined intra-group correlations between discriminating variables and canonical discriminant functions typified. Variables ordered by size of correlation with function. *Largest absolute correlation between each variable and any discriminant function. (+) This variable is not used in the analysis. **8.b.** Coefficients of classification of the Fisher's linear discriminant functions for each marine area. **8.c.** Result of classification for each marine area. Of originally grouped cases, 83.9% were correctly classified, and 82.9% were validated by cross-validation. Key: Sp= spirocysts, H= holotrich, Bs= basitrich, MpM= microbasic p-mastigophore (MpM), S= scapus, T= tentacles, P= pharynx, M= mesenterial filaments, C= columella, Med= Mediterranean Sea, AtlN= Atlantic Ocean-North, AtlS= Atlantic Ocean-South, PacN= Pacific Ocean-North, PacS= Pacific Ocean-South, BY4= North

Atlantic Bathyal, BY8=Nazca Plate, BY10= Subantartic, BY12= West Pacific Bathyal (for more details see Table A.2.a-b).

Figure and Table Captions

Fig. 1. Morphological variability of *Desmophyllum dianthus*. Calice and corallum of specimens from A, B, C, E, J, calice and corallum of specimens from Chile (USNM 106957, USNM 19168.7, USNM 36367.6, USNM 36367.2 and USNM 19168.9, respectively); D, G, calice and corallum of specimens from New Zealand (USNM 47412.3 and USNM 94068); I, calice and corallum of specimen from Ecuador (USNM 84814.2); F, K, calice and corallum of specimens from Japan (USNM 92612.3 and USNM 92612.5); H, calice and corallum of specimen from New Caledonia (USNM 1153987).

Fig. 2. a. Morphometric parameters of corallum. **b.** Morphometric parameters of calice. **c.** Diagrammatic representation of septa cycle. **d.** Landmarks of calice. **e** Landmarks of corallum. Key: H= corallum height, L= corallum length, GCD= great calicular diameter, LCD= lower calicular diameter, α = angle pedicel-calice, CL= costa length, SExV= vertical expertness of septa, SExH=horizontal expertness of septa, GFD= great fossa diameter, LFD= lower fossa diameter, TT= theca thickness, ST= dominant septa thickness, SD5T= dominant septa thickness (5th septa included), SW= maximum dominant septa width, Sx= septa cycle, S= septa. (for more details see Table 2).

Fig. 3. Box plots of ratio of morphological characters throughout marine provinces, which were identified by Spadling et al. (2007) and Watling et al. (2013) (for details see Table A.2a). Key: 48= Magellanic; 54= Southern New Zealand; 55/56= East Centralian/Southeast Australian Shelf; BY3= North Pacific Boreal; BY4= North

Atlantic Bathyal; BY6= New Zealand Kermadec; BY7= Cocos Plate; BY8=Nazca Plate; BY10= Subantarctic; BY11= Indian Ocean Bathyal; BY12= West Pacific Bathyal; BY13= South Atlantic; BY14 North Pacific Bathyal (for more details see Table A.2b).

Fig. 4. a. Plot of the first two functions from discriminant analyses of the partial morphological data (10 individuals per sampling site), classified by marine area. Key: Med= Mediterranean Sea, AtlN= Atlantic Ocean-North, AtlS= Atlantic Ocean-South, PacN= Pacific Ocean-North, PacS= Pacific Ocean-South (for more details see Table A.2a). **b.** Plots of the first two functions from discriminant analyses of the entire morphological data, classified by size class: 1st tridecade (1T), 2nd tridecade (2T), 3rd tridecade (3T), 4th tridecade (4T), and more than 5th tridecade (>5T). Key: T=tridecade (for more details see Table A.2d).

Fig. 5. a. Plots of the first two functions from discriminant analyses of 3D landmarks data in *Desmophyllum dianthus* individuals, classified by marine area. Key: Med= Mediterranean Sea, AtlN= Atlantic Ocean-North, AtlS= Atlantic Ocean-South, PacN= Pacific Ocean-North, PacS= Pacific Ocean-South (for more details see Table A.2a). **b.** Plots of the first two functions from discriminant analyses of 3D landmarks data in *Desmophyllum dianthus* individuals, classified by marine province. Key: 48= Magellanic, 54= Southern New Zealand, 55/56= East Centralian/Southeast Australian Shelf, BY3= North Pacific Boreal; BY4= North Atlantic Bathyal, BY6= New Zealand Kermadec, BY7= Cocos Plate, BY8=Nazca Plate, BY10= Subantarctic, BY11= Indian Ocean Bathyal, BY12= West Pacific Bathyal, BY13= South Atlantic, BY14 North Pacific Bathyal (for more details see Table A.2b).

Fig. 6. Cnidocysts of *Desmophyllum dianthus* from the North Atlantic Ocean (AtlN). Sp= spirocysts, H= holotrich, Bs= basitrich, MpM= microbasic p-mastigophore (MpM).

Fig. 7. Cnidocysts of *D. dianthus* from the South Atlantic Ocean (AtlS). Sp= spirocysts, H= holotrich, Bs= basitrich, MpM= microbasic p-mastigophore (MpM).

Fig. 8. Cnidocysts of *D. dianthus* from the North Pacific Ocean (PacN). Sp= spirocysts, H= holotrich, Bs= basitrich, MpM= microbasic p-mastigophore (MpM).

Fig. 9. Cnidocysts of *D. dianthus* from the South Pacific Ocean (PacS). Sp= spirocysts, H= holotrich, Bs= basitrich, MpM= microbasic p-mastigophore (MpM).

Fig. 10. Cnidocysts of *D. dianthus* from the Mediterranean Sea (Med). Sp= spirocysts, H= holotrich, Bs= basitrich, MpM= microbasic p-mastigophore (MpM).

Fig. 11. Clustering of intra- and inter-specific similarity based on the cnidom mean. Key: Med= Mediterranean Sea, AtlN= Atlantic Ocean-North, AtlS= Atlantic Ocean-South, PacN= Pacific Ocean-North, PacS= Pacific Ocean-South (for more details see Table A.2a).

Fig. 12. Phylogenetic hypothesis based on the presence/absence of cnidocysts in all tissues. Support lower than 50 are not represented in the tree.

Fig. 13. Plots of the first two functions from discriminant analyses of cnidom data in different species; *Dendrophyllia cornigera*, *D. laboreli*, *D. ramea*, *Astroides calycularis*, *Balanophyllia europaea*, *B. regia*, *Desmophyllum dianthus*.

Table 1. Characters included in the macromorphological analysis.

SYMBOL	PARAMETER DEFINITION
H	Corallum height
L	Corallum length
GCD4	Greater calicular diameter (costae included)
LCD4	Lower calicular diameter (costae included)
GCD1-3	Greater calicular diameter section 1-3
LCD1-3	Lower calicular diameter section 1-3
PD	Pedicel diameter
α	Angle pedicel-calice
Cx	Costal cycle
CxN	Total costae number
CL	Costa length
Cd	Presence costae discontinue
TT	Theca thickness
GFD	Greater Fossa Diameter
LFD	Lower Fossa Diameter
Sx	Septa cycle
SxN	Total septal number
SExV	Vertical exertness of septa
SExH	Horizontal exertness of septa
SW	Maximum dominant septa width
ST	Dominant septa thickness
SD5T	Dominant septa thickness 5th septa included
C:S	Ratio of number costae to septa
GCD*:L	Ratio of greater calicular diameter to length of corallum
GCD*:LCD	Ratio of greater calicular diameter to lower diameter of corallum
L: α	Ratio length of corallum to angle pedicel-calice
*GCD=GCD4	

Table 2. 3D Landmarks on corallites of *Desmophyllum dianthus*; endpoint landmarks correspond to Fig. 2d-e.

No	Pair of endpoints	Description	Detailed description
1	1-3	(septa height)	The intercept point between septa and wall on left side of 4th septa and wall to the top outermost point
2	2-5	(distance between septa)	The intercept point between septa and wall on left side of 4th septa to the intercept point between septa and wall on right side of 5th septa (distance between 4th septa and 5th septa)
3	3-4	(septa length)	On 4th septa: length from the endpoint of 4th septa to top outermost point (length of 4th septa)
4	3-2	(septa height)	The top outermost point to the intercept point between septa and wall on right side of 4th septa and wall
5	5-6	(septa height)	The intercept point between septa and wall on left side of 5th septa and wall to the top outermost point
6	6-7	(septa length)	On 5th septa: length from the endpoint of 5th septa to top outermost point (length of 5th septa)
7	6-8	(septa height)	The top outermost point to the intercept point between 5th septa and wall on left side of 2nd septa and wall
8	8-11	(septa height)	The intercept point between 2nd septa and 5th septa on right side of 2nd septa and wall to the top outermost point
9	9-12	(septa length)	On 2nd septa: length from the top middle point of intercept line between septa and wall on left side of 5th septa and septa and wall on right side of 5th septa to the endpoint of 2nd septa (partial length of 2nd septa)
10	10-13	(septa height)	The top outermost point to the intercept point between 5th septa and wall on right side of 2nd septa and wall
11	11-9	(septa length)	On 2nd septa: length from the outermost point of 2nd septa to top middle point of intercept line between septa and wall on left side of 5th septa and septa and wall on right side of 5th septa (partial length of 2nd septa)
12	11-10	(septa height)	The top outermost point to the intercept point between 2nd septa and wall on left side of 5th septa and wall
13	13-14	(septa length)	On 5th septa: length from the endpoint of 5th septa to top outermost point (length of 5th septa)
14	13-15	(septa height)	The top outermost point to the intercept point between septa and wall on right side of 5th septa and wall
15	15-16	(distance between septa)	The intercept point between septa and wall on left side of 5th septa to the intercept point between septa and wall on right side of 4th septa (distance between 5th septa and 4th septa)
16	16-18	(septa height)	The intercept point between septa and wall on left side of 4th septa and wall to the top outermost point

17	18-17	(septa height)	The top outermost point to the intercept point between septa and wall on right side of 4th septa and wall
18	18-19	(septa length)	On 4th septa: length from the endpoint of 4th septa to top outermost point (length of 4th septa)
19	16-17	(septa width)	The intercept point between septa and wall on left side of 4th septa to the intercept point between septa and wall on right side of 4th septa (4th septa thickness)
20	8-10	(septa width)	On 2nd septa: the intercept point between septa and 5th septa on right side to intercept point between septa and 5th septa on left side (2nd septa thickness)
21	11-12	(septa length)	On 2nd septa: length from the endpoint of 2nd septa to top outermost point (length of 2nd septa)
22	1-2	(septa width)	On 4th septa: the intercept point between septa and wall on left side to intercept point between septa and wall on right side (4th septa thickness)

Table 3. Cnidocysts of *Desmophyllum dianthus* from the five marine areas. Mean (M), standard deviation (SD) and range. For abbreviations of cnidae, see main text.

Tissue	Cnidae	<i>D. dianthus</i> AtlS/BY10	<i>D. dianthus</i> AtlN/BY4	<i>D. dianthus</i> PacN/BY12	<i>D. dianthus</i> PacS/48	<i>D. dianthus</i> Med/BY4
		M ± SD (Range)	M ± SD (Range)	M ± SD (Range)	M ± SD (Range)	M ± SD (Range)
SCAPUS	Bs1	9,8 ± 1,1 x 3,0 ± 0,0 (8,0 - 12,0) x (3)	14,9 ± 3,1 x 5,6 ± 0,5 (7,2 - 23,8 x 2,1 - 4,8)	13,4 ± 4,7 x 3,1 ± 0,4 (6,3 - 21,9) x (2,2 - 4,3)	11,5 ± 3,5 x 2,8 ± 0,3 (7,8 - 19) x (2,2 - 3,5)	16,9 ± 2,6 x 3,0 ± 0,1 (10,0 - 20,0) x (2,5 - 3,0)
	MpM1	18,3 ± 2,7 x 6,5 ± 0,9 (15,0 - 26,0) x (5,0 - 9,0)	18 ± 2,5 x 7,3 ± 1,7 (13,3-24,1) x (4,7-10)	16,7 ± 1,7 x 6,9 ± 0,6 (13,3 - 20) x (5,7 - 8)	16,4 ± 1,6 x 6,3 ± 0,9 (10,7-19,2) x (2,9-8,3)	13,3 ± 1,2 x 4,7 ± 0,4 (10,0 - 15,0) x (4,0 - 5,0)
	MpM2		32,9 ± 9,6 x 5,6 ± 0,8 (18,7-54) x (4,2-7,9)	32,2 ± 6,5 x 5,6 ± 0,9 (25,3 - 54,1) x (4,7 - 8,9)	25,0 ± 6,6 x 5,4 ± 1,1 (15,6 - 51,7) x (3,4 - 8,7)	27,8 ± 3,2 x 5,1 ± 0,3 (18,0 - 40,0) x (5,0 - 6,0)
TENTACLES	Sp	38,7 ± 7,0 x 4,5 ± 0,80 (22,0 - 55,0) x (3,0 - 6,0)	36,38 ± 7,24 x 4,18 ± 0,80 (20,5 - 48) x (2,6-6,1)	36,7 ± 7,6 x 4,9 ± 0,9 (21,4 - 48,8) x (2,7 - 6,5)	37,1 ± 8 x 4,7 ± 1,3 (22,1-56,6) x (2,4-7,3)	34,7 ± 6,2 x 4,6 ± 0,9 (22,0 - 49,0) x (3,0 - 6,0)
	Bs1		10,2 ± 1,4 x 2,6 ± 0,3 (7,3-14) x (1,9-3,2)	9,6 ± 0,9 x 2,8 ± 0,4 (7,1 - 11,6) x (2 - 4,2)		
	Bs3	41,3 ± 3,1 x 4,1 ± 0,3 (33,0 - 50,0) x (4,0 - 5,0)			42,8 ± 3,6 x 5 ± 0,5 (33,5 - 55,8) x (4 - 6,4)	30,6 ± 4,1 x 4,9 ± 0,3 (20,0 - 45,0) x (3,5 - 5,0)
	H1	55,7 ± 2,7 x 19,6 ± 0,8 (48,0 - 62,0) x (16,0 - 20,0)	56,2 ± 6,2 x 18,2 ± 1,2 (45,8-69,5) x (15,4-21,1)	50,2 ± 4,2 x 18,6 ± 1,8 (41 - 56,9) x (13,8 - 22,1)	53 ± 3,19 x 17,93 ± 1,7 (47-58,5) x (14,3-22)	53,0 ± 2,1 x 20,0 ± 0,2 (50,0 - 58,0) x (19,0 - 20,0)
	MpM1					
	MpM3	45,3 ± 3,6 x 5,7 ± 0,6 (38,0 - 53,0) x (5,0 - 7,0)	46,8 ± 4,2 x 6,5 ± 0,5 (39,2-58,4) x (5,4-7,4)	49,2 ± 5,3 x 6 ± 0,5 (30,4 - 57,9) x (4,8 - 7,1)	40,7 ± 8,6 x 5,6 ± 0,7 (17,4 - 51,2) x (4,2 - 7,4)	38,32 ± 6,1 x 5,4 ± 0,6 (28,0 - 50,0) x (4,0 - 7,0)

(continued) →

(continued) →		<i>D. dianthus</i> AtlS/BY10	<i>D. dianthus</i> AtlN/BY4	<i>D. dianthus</i> PacN/BY12	<i>D. dianthus</i> PacS/48	<i>D. dianthus</i> Med/BY4
Tissue	Cnidae	M ± SD (Range)	M ± SD (Range)	M ± SD (Range)	M ± SD (Range)	M ± SD (Range)
PHARYNX	Bs1		9,1 ± 1,4 x 2,8 ± 0,3 (6,3 - 13,1) x (2,2 - 3,9)	9,3 ± 1,3 x 2,7 ± 0,4 (6,7 - 12) x (2,1 - 4,2)	8,7 ± 1,2 x 2,5 ± 0,3 (6,4 - 13,2) x (1,9 - 3,2)	
	Bs2	22,7 ± 4,3 x 3,37 ± 0,54 (15,0 - 32,0) x (3,0 - 5,0)	22,2 ± 3,1 x 3,8 ± 0,4 (17,4 - 31,6) x (2,9 - 4,7)	18,5 ± 1,8 x 3,8 ± 0,6 (14,9 - 23) x (3 - 5,6)	20,4 ± 2,9 x 3,3 ± 0,4 (15,2 - 27,8) x (2,4 - 4,8)	23,0 ± 5,5 x 3,8 ± 0,4 (15,0 - 32,0) x (3,0 - 4,5)
	Bs3	45,8 ± 5,6 x 4,7 ± 0,5 (37,0 - 60,0) x (4 - 5,5)	41,3 ± 5,2 x 4,7 ± 0,6 (26,7-51,9) x (3,3-6,3)	40,8 ± 4,9 x 4,6 ± 0,5 (29,8 - 53,3) x (3,4 - 6)	38,4 ± 5,8 x 3,7 ± 0,4 (25,1 - 53,4) x (3 - 5,3)	41,3 ± 5,7 x 4,5 ± 0,5 (33,0 - 60,0) x (4,0 - 5,5)
	MpM1	18,0 ± 2,4 x 6,5 ± 0,6 (14,0 - 25,0) x (5,0 - 8,0)	16,4 ± 2,2 x 5,7 ± 1,4 (11,6-21,8) x (3,6-9)	17,2 ± 2,2 x 6,4 ± 1 (14,2-21,4) x (4,2-8,8)	16,2 ± 1,7 x 5,1 ± 1 (12,1 - 21,3) x (3,8 - 7,2)	17,7 ± 2,1 x 5,3 ± 0,6 (13,0 - 23,0) x (4,0 - 6,5)
	MpM3		38 ± 7,7 x 5,9 ± 0,6 (15,2-61,6) x (3,8-7)	46,7 ± 5,9 x 5,9 ± 0,5 (32,5 - 57,8) - (5 - 7,1)	30,8 ± 8,7 x 5,6 ± 0,8 (17,7 - 51,7) x (3,4 - 7)	
MESENTERIAL FILAMENTS	Bs1	18,9 ± 2,0 x 3,5 ± 0,5 (15,0 - 22,0) x (3,0 - 4,0)	14,4 ± 3,5 x 3 ± 0,6 (6,7 - 24,4) x (2-4)	14 ± 4,9 x 3,2 ± 0,6 (6,3 - 22,8) x (2 - 4)	11,8 ± 4,7 x 2,7 ± 0,5 (6,9 - 20,5) x (1,8 - 3,6)	14,7 ± 2,2 x 3,1 ± 0,3 (12,0 - 23,0) x (3,0-4,5)
	H2	105,7 ± 5,0 x 21,1 ± 2,0 (92,0 - 115,0) x (19-22)	107,7 ± 8,6 x 20,2 ± 1,5 (90,1 - 123,4) x (16,3 - 24,5)	99,1 ± 9,7 x 22,9 ± 2,5 (73,9-119,8) x (13,8-29,6)	92,1 ± 7,4 x 22,6 ± 1,9 (74 - 104,9) x (18,8 - 27,9)	
	MpM1	18,6 ± 2,1 x 6,6 ± 0,7 (13,0 - 23,0) x (5,0 - 8,0)	17,7 ± 2,4 x 7,4 ± 0,9 (12,4 - 23,3) x (5,1 - 9,8)	16,2 ± 1,5 x 7 ± 0,7 (13,7 - 19,3) x (5,3 - 8,2)	16,3 ± 1,6 x 6,4 ± 0,9 (12,6 - 19,1) x (4,1 - 9,7)	
	MpM2	30,2 ± 2,0 x 5,6 ± 0,5 (26,0 - 35,0) x (5,0 - 6,0)	28,8 ± 3,3 x 5,6 ± 0,6 (15,7 - 35,7) x (3,5 - 6,8)	28,5 ± 6 x 5,6 ± 1,2 (15,9- 48,1) x (3,2-8,4)	26,7 ± 2,9 x 5,7 ± 0,8 (22,7 - 38) x (4 - 7,6)	24,8 ± 5,3 x 5,4 ± 0,5 (15,0 - 40,0) x (5,0 - 6,0)
	MpM4	81,7 ± 5,5 x 10,7 ± 0,9 (65,0 - 90,0) x (10, 0-12,0)	67,3 ± 7,1 x 10,2 ± 1 (51,3-78,7) x (8,5-13,9)	63,4 ± 7,4 x 9,9 ± 1,2 (50,5-79,5) x (6,5-12,5)	55,7 ± 4,8 x 11,1 ± 1,5 (50,1 - 66,9) x (8,4 - 14,5)	73,3 ± 13,9 x 11,3 ± 1,3 (60,0 - 89,0) x (10,0 - 13,0)
	MpM5	129,0 ± 8,7 x 15,5 ± 3,6 (105,0 - 140,0) x (12,0 - 20,0)	117,3 ± 10 x 13, 3 ± 0,8 (83,3-138,2) x (11,5-15)	110,7 ± 16,2 x 12,7 ± 1,6 (82,4 - 142,8) x (5,5 -15,2)	104,1 ± 10,7 x 12,5 ± 1,4 (80,4 - 120,6) x (10,4 - 16,4)

a**CDA Morphology** **b****CDA Morphology**

Desmophyllum dianthus (AtIN/BY4)

Desmophyllum dianthus (At/S/BY10)

Desmophyllum dianthus (PacN/BY12)

Desmophyllum dianthus (PacS/48)

Desmophyllum dianthus (Med/BY4)

CDA Cnidom

Resemblance: S17 Bray Curtis similarity