

The phylogeny of *Pacifigorgia* (Coelenterata, Octocorallia, Gorgoniidae): a case study of the use of continuous characters in the systematics of the Octocorallia

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ABSTRACT

The Octocorallia constitute a group of organisms that inhabit a wide spectrum of habitats ranging from abyssal to shallow marine environments and from tropical to polar waters. As a subclass, the group is clearly defined and the clade is considered monophyletic based on a series of morphological and molecular synapomorphies. In contrast, the systematic treatment of octocorals below the subclass level is complex given the continuous nature of many of the characters used in their taxonomy. The use of continuous characters for the deduction of phylogenies generally has been considered a philosophical and methodological challenge; within the Octocorallia it has led researchers to rearrange genera, to consider character analysis problematic, or to prefer molecular data sets, which avoid morphological characters, for the study of octocoral phylogenetic relationships. In this study, we assessed the role of continuous morphological variation on the deduction of octocoral phylogenies. Specifically, we analyzed the morphologically diverse genus *Pacifigorgia* Bayer, 1951 and show that continuous variation, after adequate coding, represents a valuable source of information suitable for phylogenetic inference.

KEY WORDS

Coelenterata,
Octocorallia,
Gorgoniidae,
Pacifigorgia,
character analysis,
continuous characters,
gap weighting,
tropical eastern Pacific.

RÉSUMÉ

La phylogénie de Pacifigorgia (Coelenterata, Octocorallia, Gorgoniidae): un cas d'étude de l'utilisation de caractères continus dans la systématique des Octocorallia.

Les Octocorallia constituent un groupe d'organismes occupant un large spectre d'habitats allant des abysses aux environnements marins peu profonds et depuis les tropiques jusqu'aux eaux polaires. En tant que sous-classe, le groupe est clairement défini et le clade est considéré comme monophylétique d'après une série de synapomorphies morphologiques et moléculaires. Au contraire, le traitement systématique des octocoralliaires en-dessous du niveau de la sous-classe est complexe étant donnée la nature continue d'un grand nombre des caractères utilisés dans leur taxonomie. L'utilisation de caractères continus pour la déduction des relations phylogénétiques a généralement été considérée comme un défi philosophique et méthodologique; chez les Octocorallia ceci a mené les chercheurs à réarranger les genres, à considérer l'analyse des caractères comme problématique, ou à préférer les jeux de données moléculaires, ce qui évite les caractères morphologiques, pour l'étude des relations phylogénétiques des octocoralliaires. Dans ce travail nous estimons le rôle de la variation morphologique continue sur les phylogénies d'octocoralliaires. Nous analysons en particulier le genre *Pacifigorgia* Bayer, 1951, morphologiquement diversifié et montrons que cette variation continue, après un codage adéquat, représente une source d'information valable pour l'inférence phylogénétique.

MOTS CLÉS

Coelenterata,
Octocorallia,
Gorgoniidae,
Pacifigorgia,
analyse de caractères,
caractères continus,
pondération des «gaps»,
Pacific oriental tropical.

INTRODUCTION

Octocorals (subclass Octocorallia) constitute a diverse group of organisms that inhabit marine ecosystems ranging from shallow water to deep-sea environments (Bayer 1961; Fabricius & Alderslade 2000; Breedy & Guzman 2002, 2003a; Sánchez *et al.* 2003b; Guzman *et al.* 2004; Williams & Breedy 2004; Sánchez 2005). The systematic status of the group at the subclass level is fairly clear, and the clade is considered monophyletic based on the shared presence of polyps with eight tentacles and eight complete septa dividing the gastrovascular cavity (Bayer 1961; Wirshing *et al.* 2005). In contrast, the systematics within the subclass, at the ordinal, familial, and generic levels, remains obscure (but see Sánchez *et al.* 2003a; McFadden *et al.* 2006a, b; Aguilar & Sánchez 2007; Grajales *et al.* 2007).

The deduction of octocoral phylogenies has proven difficult for a number of reasons. The continuous nature of many of the characters used in the taxonomy of the Octocorallia has been a particularly important

and controversial problem for the systematic study of the group and has led some authors to consider octocoral characters difficult to code or to polarize (Sánchez 2001, 2005; Wirshing *et al.* 2005), to rearrange or discard genera (e.g., *Antillogorgia-Pseudopterogorgia* [Bayer 1961]; *Lophogorgia-Leptogorgia* [Grasshoff 1988]; *Leptogorgia-Pseudopterogorgia* [Williams & Lindo 1997]), or to prefer molecular data sets for the study of the systematics of different octocoral groups (Sánchez *et al.* 2003a, b; Wirshing *et al.* 2005; McFadden *et al.* 2006a, b; Aguilar & Sánchez 2007; Grajales *et al.* 2007).

In general, continuously varying traits represent a challenge to traditional coding techniques, as these methodologies depend on the existence of gaps between character states to transform observed variation into discrete codes suitable for the deduction of phylogenies (but see Goloboff *et al.* 2006). In recent years, several novel methodologies have been proposed to overcome these coding difficulties and to explore the role of continuous characters in the deduction of phylogenies of dif-

ferent groups (Thiele 1993; Strait *et al.* 1996; Rae 1998; Wiens 2001, 2004; Goloboff *et al.* 2006). In octocoral systematics, continuous characters provide reliable information for the identification and delimitation of many groups and have been used regularly by taxonomic workers for the description of new octocoral species (Bayer 1961; Williams 1992; Williams & Lindo 1997; Breedy 2001; Sánchez 2001, 2005; Breedy & Guzman 2002, 2003a, b, 2004, 2005, 2007). In contrast, the use of continuously varying traits for the deduction of phylogenies of the group has received little attention.

Sánchez *et al.* (2003b), for example, used gap coding to map continuous morphological characters over a molecular phylogenetic hypothesis of the Caribbean octocoral fauna. Vargas *et al.* (2010) introduced a novel image analysis technique, which in combination with finite mixture modeling (*sensu* Strait *et al.* 1996) allowed them to assess the number of statistically different sclerite classes and the validity of traditional sclerite classifications in the genus *Pacifigorgia*. These studies represent initial efforts to apply methodological advances in character analysis to octocoral systematics. Neither study, however, used continuous characters for the deduction of octocorals phylogenetic hypotheses, nor did they attempt to test the performance of continuous traits in comparison to discrete characters.

In this study, we explored the performance of continuous characters, relative to discrete characters, in the deduction of octocoral phylogenies. We used a matrix composed of continuously varying and discrete morphological characters to evaluate the performance of continuous versus discrete characters in the reconstruction of the phylogenetic relationships within the eastern Pacific genus *Pacifigorgia* Bayer, 1951. *Pacifigorgia* is a morphologically diverse genus, which has been the subject of major systematic (Breedy 2001; Breedy & Guzman 2002, 2003a, b, 2004; Williams & Breedy 2004; Vargas 2008) and biogeographic work (Vargas *et al.* 2008). The relative stability of the taxonomy of *Pacifigorgia* makes the genus suitable for cladistic research. This study provides the first cladistic analysis of an octocoral genus inhabiting eastern Pacific shallow (< 50 m) waters and represents a further effort towards a

TABLE 1. — List of species used in the phylogenetic analysis of *Pacifigorgia* Bayer, 1951 with their geographic distribution. Abbreviations: **CI**, Cocos Island; **CM**, Cortez-Mexican Province; **GA**, Galápagos Archipelago; **P**, Panamic Province; **WA**, Western Atlantic.

| Species | Geographic distribution |
|---|-------------------------|
| <i>P. adamsii</i> (Verrill, 1868) | CM, P |
| <i>P. agassizii</i> (Verrill, 1864) | CM |
| <i>P. bayeri</i> Breedy, 2001 | P |
| <i>P. cairnsi</i> Breedy & Guzman, 2003 | P |
| <i>P. catedralensis</i> Breedy & Guzman, 2004 | P |
| <i>P. curta</i> Breedy & Guzman, 2003 | CI |
| <i>P. dampieri</i> Williams & Breedy, 2004 | GA |
| <i>P. darwinii</i> (Hickson, 1928) | GA |
| <i>P. elegans</i> (Milne Edwards & Haime, 1857) | WA |
| <i>P. exilis</i> (Verrill, 1870) | CM |
| <i>P. eximia</i> (Verrill, 1868) | P |
| <i>P. ferruginea</i> Breedy & Guzman, 2004 | P |
| <i>P. firma</i> Breedy & Guzman, 2003 | P |
| <i>P. gracilis</i> (Kükenthal, 1924) | CM |
| <i>P. irene</i> Bayer, 1951 | P |
| <i>P. media</i> (Verrill, 1864) | CM |
| <i>P. rubicunda</i> Breedy & Guzman, 2003 | P |
| <i>P. rubinoffi</i> Breedy & Guzman, 2003 | P |
| <i>P. rubripunctata</i> Williams & Breedy, 2004 | GA |
| <i>P. samarensis</i> Breedy & Guzman, 2003 | P |
| <i>P. sculpta</i> Breedy & Guzman, 2004 | P |
| <i>P. senta</i> Breedy & Guzman, 2003 | CM, P |
| <i>P. smithsoniana</i> Breedy & Guzman, 2004 | P |
| <i>P. stenobrochis</i> (Valenciennes, 1846) | CM, P |
| <i>P. symbiotica</i> Williams & Breedy, 2004 | GA |

better understanding of the diverse eastern Pacific octocoral fauna.

MATERIALS AND METHODS

ABBREVIATIONS

| | |
|-------|---|
| BMNH | The Natural History Museum (formerly British Museum [Natural History]), London; |
| CASIZ | California Academy of Science, Invertebrate Zoology, San Francisco; |
| MCZ | Museum of Comparative Zoology, Harvard University, Cambridge, USA; |
| MNHN | Muséum national d'Histoire naturelle, Paris; |
| STRI | Smithsonian Tropical Research Institute, Panama; |
| UCR | Museo de Zoología, Universidad de Costa Rica, San Pedro, Costa Rica; |
| USNM | National Museum of Natural History (formerly United States National Museum), Smithsonian Institution, Washington, D.C.; |
| YPM | Yale Peabody Museum of Natural History, New Haven. |

TABLE 2. — Continuous characters used in the phylogenetic analysis of the genus *Pacifigorgia* Bayer, 1951 with their maximum and minimum values.

| Character code and name | Minimum (min) and maximum (max) values |
|---|--|
| 0 Branch thickness (mm) | min = 0.7 max = 7.0 |
| 1 Spindle form (spindle max length/max width) | min = 1.67 max = 4.00 |
| 2 Number of whorls observed in the spindles | min = 2 max = 10 |
| 3 Capstan form (capstan max length/max width) | min = 1.25 max = 2.50 |
| 4 Anthocodial rods form (anthocodial rods max length/max width) | min = 2.33 max = 9.00 |
| 5 End branchlets length (mm) | min = 1.0 max = 30.0 |
| 6 Colony form (colony width/colony height) | min = 0.34 max = 2.34 |
| 7 Mesh form (mesh length/mesh width) | min = 1.0 max = 10.0 |
| 8 Mesh density (meshes/cm ²) | min = 2 max = 32 |

SPECIMENS

Table 1 lists the *Pacifigorgia* species (n = 25) used for the phylogenetic analysis. We acquired type specimens and comparative reference material on loan from the BMNH, CASIZ, MCZ, MNHN, USNM, and YPM; we also examined material from UCR and STRI. We obtained sclerites from fragments of the colonies after treating them with sodium hypochlorite (household bleach) following Bayer (1961) and Breedy & Guzman (2002, 2005).

CONTINUOUS CHARACTER CODING

We used gap weighting to code the continuous characters (Table 2) used for the phylogenetic analysis of the genus *Pacifigorgia*. Thiele (1993) proposed gap weighting as a method for continuous character coding that retains information about the rank order of the character states and the gap size between them. The procedure weights the transformations between the character states proportionally to the size of the gap separating them, increasing the cost of the transformation as the difference between the values of the character states increases.

In gap weighting, all differences between character states are taken as informative and the differentiation between informative and spurious transformation series is done *a posteriori* after the most parsimonious tree has been obtained. Therefore, the method avoids *a priori* decisions on the informativeness of a character in accordance with Hennig's auxiliary principle (Wiley *et al.* 1991).

The basic methodology for gap weighting is as follows. Raw data are expressed as an ordered set of states, arranged according to their means, medians, maximum, or minimum values. Once the data have been ordered, the values are range standardized using the following formula:

$$x_i = \frac{(x - \text{min})}{(\text{max} - \text{min})} \times n$$

where *n* is the maximum number of character states allowed by the algorithm used (i.e. 32 in PAUP and 10 in Hennig86 and NONA), *max* and *min* are the maximum and minimum values recorded for a character, and *x* refers to the value to be standardized. After range standardization, the integer part of each of the new standard values is used as the character state in an ordered multistate transformation series.

DISCRETE CHARACTER CODING

We coded discrete characters as binary or multistate characters; we included each recognizable character state in the transformation series. We treated all discrete characters in the matrix (Table 3) as unordered transformation series. We represented sclerite colour combinations as multistate polymorphic characters.

As suggested by Wiens (2001), many morphological characters, independent of whether they are coded in a quantitative or qualitative way, describe variation in quantitative (continuous) traits. In this study, this was true for the characters mesh pattern and dominant sclerite form, which we treated as binary (0, 1) characters even though they represent continuously varying traits. For instance, mesh pattern could be represented quantitatively as the proportion of incomplete meshes occurring in a given area of the fan. Dominant sclerite form could be treated as the proportion of spindles (or capstans) occurring in a species' random sclerite sample.

Although these two characters can be treated quantitatively, sampling is somewhat difficult. Determining mesh pattern depends on the general shape of the fan, its size, and its ramification pattern; thus, it is difficult to have comparable samples between and within species. Similar problems occur when sclerites are sampled to determine the dominant sclerite form, as this would require the characterization of three different levels of sclerite variation (i.e. the single sclerite forms, the combination of forms, and the variation of sclerite forms and their combinations in a number of independent samples [Grasshoff 1988]). Because of these sampling problems, we coded these two characters as discrete binary transformation series. For this purpose, we used sclerites from the holotype and two distinct specimens for the determination of the dominant sclerite form in the 25 *Pacifigorgia* species studied. We prepared microscope slides and analyzed three visual fields per specimen; afterwards, we scored each species as being spindle dominant or capstan dominant. The score represents an overall estimation of the proportion of sclerite classes present in the species under consideration, and it is supported by Grasshoff (1988) statement:

“The classical method of presenting spiculation of species by drawings of sclerite forms, species by species, *does not give any impression of the typical sclerite features. However, the typical differences* [i.e. between species] *become apparent when we see all sclerite types of a specimen side by side in an unsorted mixture in a slide preparation*” (our emphasis; our comments in brackets).

Similarly, we treated and coded mesh pattern as a binary discrete character. We used complete specimens, fragments, or pictures of the specimens to score each species as having a regular mesh pattern or an irregular mesh pattern (as defined by Breedy & Guzman 2002). The score is an overall estimation of the mesh pattern observed in each of the species sampled.

PHYLOGENETIC ANALYSIS

We used the program NONA (Goloboff 1999) to deduce a maximum parsimony tree for the genus *Pacifigorgia*. The final matrix (Appendix 1) consisted of 18 characters: nine were continuous and nine

TABLE 3. — Discrete characters used in the phylogenetic analysis of the genus *Pacifigorgia* Bayer, 1951 with their character states.

| Character code and name | Character states |
|---------------------------|---|
| 9 Anastomosis | 0. absent 1. present |
| 10 Mesh pattern | 0. irregular 1. regular |
| 11 Colony colour | 0. pink 1. red 2. purple 3. orange 4. yellow |
| 12 Dominant sclerite form | 0. spindles dominant 1. capstans dominant |
| 13 Colony form bushy | 0. absent 1. present |
| 14 Midrib | 0. absent 1. present |
| 15 Sclerite grooves | 0. absent 1. present |
| 16 Swollen polyp aperture | 0. absent 1. present |
| 17 Sclerite colour | 0. white 1. red 2. yellow 3. purple 4. orange |

were discrete. Before the phylogenetic analysis, we scaled each transformation series to the maximum number of character states present in the matrix (i.e. 10). The scaling process equalizes the total cost of the transformation series, thereby avoiding undesired overweighting of the transformation series with more character states.

After scaling the matrix, we set NONA to perform a total of 1500 independent tree bisection-reconnection (tbr) replicates, after which we retained all most parsimonious (i.e. minimum length; MP) trees. Trees were treated as unrooted networks. To assess nodal support, we used 15 000 suboptimal trees up to 50 steps longer than the MP tree found to calculate the decay index (DI; Bremer 1988, 1994) for each node in the tree.

CHARACTER PERFORMANCE

AND PARTITIONED PHYLOGENETIC ANALYSES

To evaluate the performance of the characters used in the phylogenetic analysis of *Pacifigorgia*, we recorded the consistency index (CI) of each

character in the data matrix for 15 000 random trees and for the MP tree. Afterwards, we determined a random CI frequency distribution for each character in the matrix. The frequency distribution represents the distribution of CI values expected for a given character under the assumption of random phylogenetic relationships between taxa. We used this frequency distribution as a phylogenetic null model to calculate the probability of finding at random a CI value equal to or higher than the CI value obtained for a character in the MP tree; if this probability was less than 0.05 for a given character, we interpreted it to mean that the selected character performed better on the MP tree than on a set of trees indicating random phylogenetic relationships.

In order to assess the contribution of each character type (i.e. continuous and discrete characters) to topological resolution of the *Pacifigorgia* tree, we ran two separate parsimony analyses using 1) only continuous characters, and 2) only discrete characters. After each analysis we visually compared the topology of the tree(s) obtained for each partition with the topology obtained from the total evidence (i.e. continuous+discrete characters) matrix. We pursued, this way, to identify shared groupings between the topologies derived from each character type and the total evidence hypothesis. To evaluate the congruence between continuous and discrete character, we calculated the Incongruence Length Difference Index for the partitioned dataset.

TREATING CONTINUOUS CHARACTERS AS SUCH

Finally, we used the program TNT (Goloboff *et al.* 2008) to deduce a cladistic hypothesis of the genus *Pacifigorgia* treating continuous characters as such (i.e. avoiding discretization). We stabilized the consensus 10 times using a driven search consisting of 500 sequence additions followed by a sectorial search using default options, and finally by three rounds of tree fusing. We scaled the characters using implied weights (Goloboff 1993) with a concavity constant $k = 4$. After the analysis, we compared visually the resulting tree with the tree obtained using gap weighting discretization to find similar groupings in both trees.

RESULTS

PHYLOGENETIC ANALYSIS

We found nine MP trees of 380 steps, a CI of 52, and a retention index (RI) of 68. The strict consensus (Fig. 1) revealed the existence of four main clades within the genus. The first grouped two species, namely *P. senta* and *P. sculpta*, with relatively high DI value ($DI = 3$). The sclerites of both *P. senta* and *P. sculpta* are remarkably similar in their general shape, size and colour (i.e. a mixture of red/white sclerites). The general shape of the colony, as well as their colour, differs between these two species although both show irregular mesh patterns (Breedy & Guzman 2004).

The second clade grouped, with high support values, species with red/yellow sclerites (*P. exilis*, *P. dampieri*, *P. agassizii* and *P. media*; Fig. 1). This group of species also presents sclerite grooves in the thicker colony branches, and fills sections of their mesh with coenenchyme. The clade includes two species (*P. exilis* and *P. agassizii*) that occur in the Cortez-Mexican Province, one species (*P. media*) that occurs in the Cortez-Mexican and in the Panamic provinces, and one species (*P. dampieri*) that occurs exclusively in the Galápagos Archipelago.

The third clade had low basal DI values ($DI = 1$) and grouped together *Pacifigorgia* species with red/white or red/yellow sclerites. Within this clade, *P. rubicunda* and *P. samarensis* – two species with complex morphological differentiation – appeared as a sister pair, but the support value for this pair was low ($DI = 1$). The other species group within this clade was highly supported ($DI = 4$) and consisted of *P. adamsii* and *P. irene*. These two species, *P. adamsii* and *P. irene*, present high mesh densities and generally resemble each other in their general colony morphology.

Finally, the fourth clade found in our analysis grouped together a diverse array of species that occur mostly in the Panamic Province and *P. elegans*, the only *Pacifigorgia* species occurring in the southern Caribbean and western Atlantic. Within this group all species but *P. rubinoffi* share the presence of purple sclerites. The DI values within this clade were low for some internal nodes, such as that for the *P. darwinii*, *P. firma*, *P. cairnsi*, and *P. stenobro-*

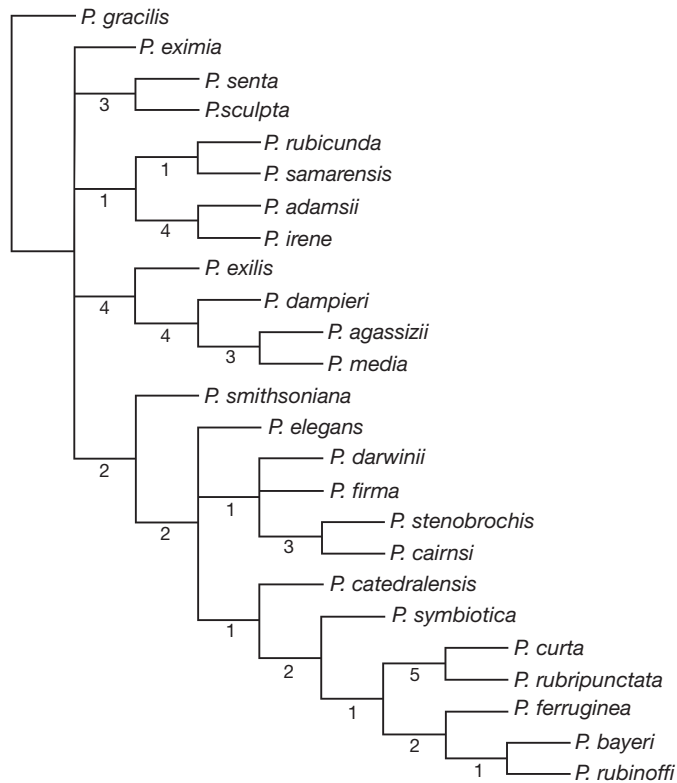


FIG. 1. — Unrooted tree of the eastern Pacific genus *Pacifigorgia* Bayer, 1951. Strict consensus tree of nine most parsimonious hypotheses. Decay index (DI) values for each node in the tree are indicated below branches.

chis group (DI = 1), and high for others, like the *P. symbiotica*-*P. curta* sister group (DI = 5).

CHARACTER PERFORMANCE AND PARTITIONED ANALYSES

Fourteen characters used in the phylogenetic analysis of *Pacifigorgia* performed significantly better over the MP tree than over a random sample of trees. Of these 14 characters, eight were continuous and five were discrete, binary, or multistate (Table 4). The only characters found to perform as well as, or worse on the MP tree than on a sample of trees indicating random phylogenetic relationships were mesh pattern and colony form; interestingly colony form had the second higher CI (CI = 45) value among continuous characters. The characters anastomosis, colony form bushy, and swollen polyp aperture were not included in the performance analysis because they represent

either plesiomorphies for *Pacifigorgia* or apomorphies for some of the species. For instance, anastomosis is a plesiomorphy for the genus. Colony form bushy and swollen polyp aperture are apomorphies for *P. curta* and *P. symbiotica*, respectively.

To further explore the role different character sets had in resolving the phylogenetic relationships of the genus *Pacifigorgia*, we divided the matrix in two partitions corresponding to the discrete and continuous characters in the dataset and ran two independent searches in NONA. Analysis of discrete characters resulted in 94 MP trees of 150 steps, CI = 80 and RI = 91. The strict consensus of these trees resulted in a complete bush, the 50% majority rule consensus resulted in a tree showing two groupings that correspond roughly with the *P. exilis*, *P. dampieri*, *P. agassizii* and *P. media* clade, and with the major clade including *P. elegans*,

TABLE 4. — Character performance. Probability of finding a character performing better or equally better over a set of random trees than over the most parsimonious tree found. Abbreviation: **n.s.**, non significant.

| Character | Character type | Probability |
|--|----------------|-------------|
| Branch thickness | continuous | 0.0011 |
| Spindle form | continuous | 0.0197 |
| Number of whorls observed in the spindles | continuous | 0.0023 |
| Capstan form | continuous | 0.0003 |
| Anthocodial rods form | continuous | 0.0366 |
| End branchlets length | continuous | 0.0001 |
| Colony form | continuous | n.s. |
| Mesh form | continuous | 0.0197 |
| Mesh density | continuous | 0.0001 |
| Mesh pattern | discrete | n.s. |
| Colony colour | discrete | 0.0001 |
| Dominant sclerite form | discrete | 0.0001 |
| Midrib | discrete | 0.0021 |
| Sclerite grooves | discrete | 0.0003 |
| Sclerite colour | discrete | 0.0001 |

P. darwinii and *P. bayeri*, among others (Fig. 2). Analysis of continuous characters resulted in 162 MP trees of 183 steps, CI = 44 and RI = 56. Despite the considerable number of MP trees, the strict consensus showed four species pairs also present in the total evidence tree, and the 50% majority rule tree shows three more groupings compatible with clades found in the total evidence tree (Fig. 3). Incongruence length difference (ILD = 0.1237) for our dataset was not significant (i.e. the partitions were not significantly incongruent).

TREATING CONTINUOUS CHARACTERS AS SUCH

The analysis of continuous characters as such, without discretization, resulted in a single MP tree of 406.92 steps long, CI = 51.92 and RI = 67.40 (TNT uses floating point arithmetics). The tree was topologically equivalent to the total evidence hypothesis except for the position of *P. eximia* which was basal to the *P. adamsii*, *P. irene*, *P. samarensis* and *P. rubicunda* clade, and to the major clade including *P. smithsoniana* and *P. bayeri* among others (Fig. 4). Note that the CI and RI values obtained when continuous characters were treated as such are practically equal to those obtained using gap weighting; the lengths differ, however the values are not directly comparable as both analyses use different weighting schemes (i.e.

fixed weights for gap-weighting and implied weights for TNT analysis).

DISCUSSION

Due to their great morphological diversity, octocorals present a major challenge for cladistic research. The intra- and interspecific variation in many of the morphological characters important for the taxonomy of the group have led researchers to consider them difficult to code or polarize (Sánchez 2001, 2005) and therefore difficult to use for the deduction of phylogenetic hypotheses of the Octocorallia. However, attempts to deduce octocoral phylogenetic relationships at different taxonomic levels from morphological data sets have been successful, despite all of the coding problems (Bayer 1953; Williams 1997; Sánchez 2001, 2005).

In general, coding morphological variation (i.e. the process of character analysis and character argumentation) is a controversial procedure hindered by the plethora of methodological decisions (e.g., how to code intraspecific variation, how to order and weight the resultant transformation series, etc.) necessary to transform the observed variation into discrete codes (Wiens 2001). Many methods have been proposed to solve different aspects of the coding process and to increase the repeatability of this important step of systematic research. In octocoral systematics, despite the acknowledged morphological complexity (*sensu* Kirchoff *et al.* 2004) displayed by the group, the application of such character analysis methods has been explored only recently (Sánchez *et al.* 2003b; Sánchez 2005; Vargas *et al.* 2010).

In this study, we attempted to deduce a phylogenetic tree for the genus *Pacifigorgia* from a matrix composed of both discrete and continuous morphological characters. We used gap weighting (Thiele 1993) to code the quantitative variation observed in several characters of importance for the systematics of the genus. The phylogeny we obtained revealed the existence of four major clades that grouped species roughly based on sclerite colour mixtures. Previous research reported the importance of sclerite colour for the taxonomic determination of *Pacifigorgia* species (Breedy & Guzman 2002, 2003a, b, 2004;

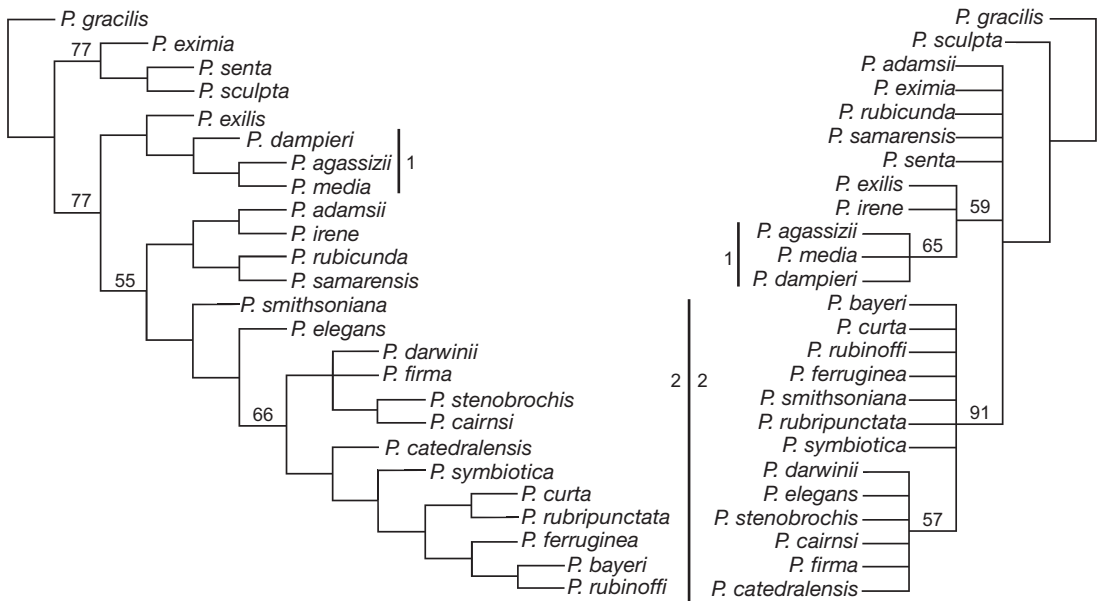


FIG. 2. — Unrooted tree of *Pacifigorgia* Bayer, 1951 using discrete characters only. Left side: majority rule consensus tree of nine most parsimonious trees obtained using all characters. Right side: majority rule consensus tree of 94 most parsimonious trees obtained using discrete characters only. Node frequency values are indicated above branches. Vertical bars and numbers show common groupings between trees.

Williams & Breedy 2004); it also appears to be an important taxonomic character in other gorgoniid octocorals, such as *Leptogorgia* spp. (Bayer 1961; Grasshoff 1988; Breedy & Guzman 2007). In members of the gorgoniid genus *Eugorgia*, sclerite colour is the product of acidic organic compounds (probably carotenoid acids) embedded in the crystalline calcium carbonate (calcite) sclerite structure (Fox *et al.* 1969). The origin of the sclerite colour in other gorgoniids, including *Pacifigorgia*, remains unknown and deserves further study given the importance of this trait for the systematics of the group.

Other morphological characters of importance for the taxonomy of the genus – such as the relative proportion of sclerite types (i.e. dominant sclerite form) or the continuous characters branch thickness, end branchlet length, mesh density, and colony form – that performed better (Table 5) over the MP tree were also important for the definition of clades within the genus. Breedy & Guzman (2002) previously reported that these characters, despite of being continuous, were essential for establishing the

identity of many *Pacifigorgia* species. In this respect, our analyses showed how continuous characters increased the resolution of the cladistic hypotheses of *Pacifigorgia* (Fig. 3). Continuous characters defined relationships between closely related species, but had little impact in the definition of major clades within *Pacifigorgia*. In contrast, discrete characters defined broader groupings within the genus but were unable to resolve the relationships of species pairs (Fig. 2). Both character types (i.e. discrete and continuous) were congruent (ILD = 0.1237), and together yielded a well-resolved hypothesis of the genus relationships (Fig. 1).

A rather different approach to the use of continuous characters in the inference of phylogenetic relationships of taxa has been recently implemented in the computer program TNT (Goloboff *et al.* 2008). We used this method to avoid character discretization, and deduced a tree for the genus *Pacifigorgia* generally equivalent to the hypotheses deduced after gap-weighting the continuous characters (Fig. 4). In conjunction, our results suggest

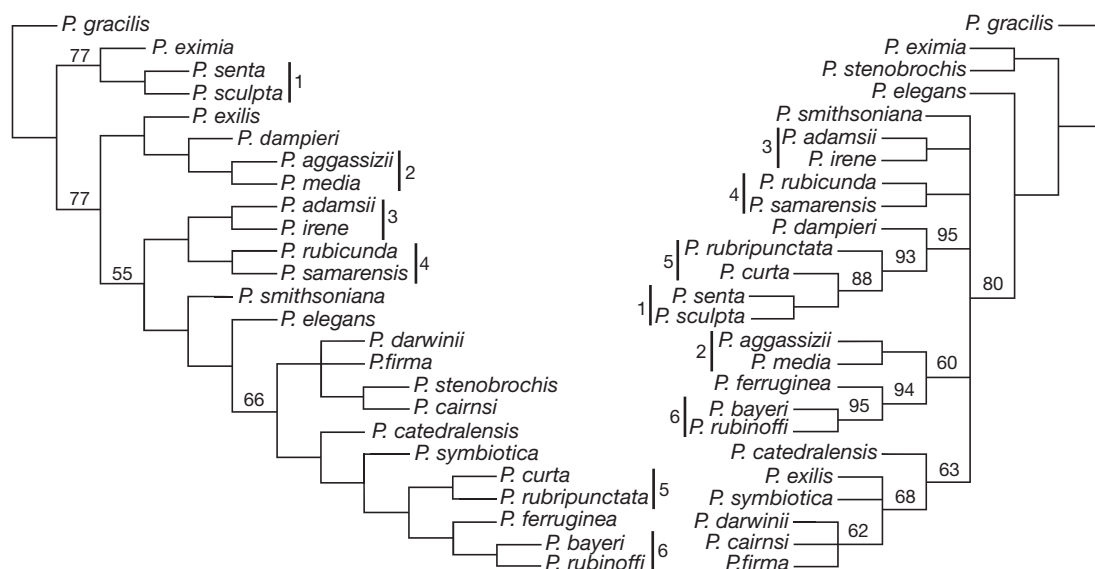


FIG. 3. — Unrooted tree of *Pacifigorgia* Bayer, 1951 using continuous characters only. Left side: majority rule consensus tree of nine most parsimonious trees obtained using all characters. Right side: majority rule consensus tree of 162 most parsimonious trees obtained using continuous characters only. Node frequency values are indicated above branches. Vertical bars and numbers show common groupings between trees.

that continuous variation provides suitable information for the deduction of intrageneric phylogenetic relationships among octocorals. This is particularly important because continuous characters have been systematically excluded from octocoral cladistic research by octocoral systematic researchers, who have considered such characters “unreliable” or “highly subject to homoplasy” (as one of our reviewers pointed out). As Goloboff *et al.* (2006) noted, “[...] dismissing characters just because of their continuous nature is unwarranted”. Moreover, considering continuous characters more prone to homoplasy is in conflict with Hennig’s auxiliary principle as homoplasy can only be assessed after cladistic reconstruction has been done.

Quantitative morphological variation in octocorals constitutes a valuable resource routinely used in the description and identification of octocoral species (Bayer 1951, 1953, 1961; Grasshof 1988, 1992; Williams 1992; Williams & Lindo 1997; Breedy 2001; Sánchez 2001, 2005; Breedy & Guzman 2002, 2003a, b, 2004, 2005, 2007; Williams & Breedy 2005). However, the role of such variation

in the deduction of phylogenetic hypotheses of the group remained obscure until this study. Herein we showed that continuous traits could also be a source of characters for the study of octocoral phylogenetic relationships. Based on this finding, we urge octocoral researchers to use an integrative approach in which all available evidence (i.e. morphological discrete and continuous characters, and molecular data) is used together, rather than simply being contrasted (Sánchez *et al.* 2003b; McFadden *et al.* 2006b) or ignored (Sánchez *et al.* 2003a; Wirshing *et al.* 2005; McFadden *et al.* 2006a; Aguilar & Sánchez 2007; Grajales *et al.* 2007), in the study of octocoral phylogenetic relationships. Such an integrative approach will certainly result in a deeper understanding of the role that morphological characters play in the systematics of the Octocorallia.

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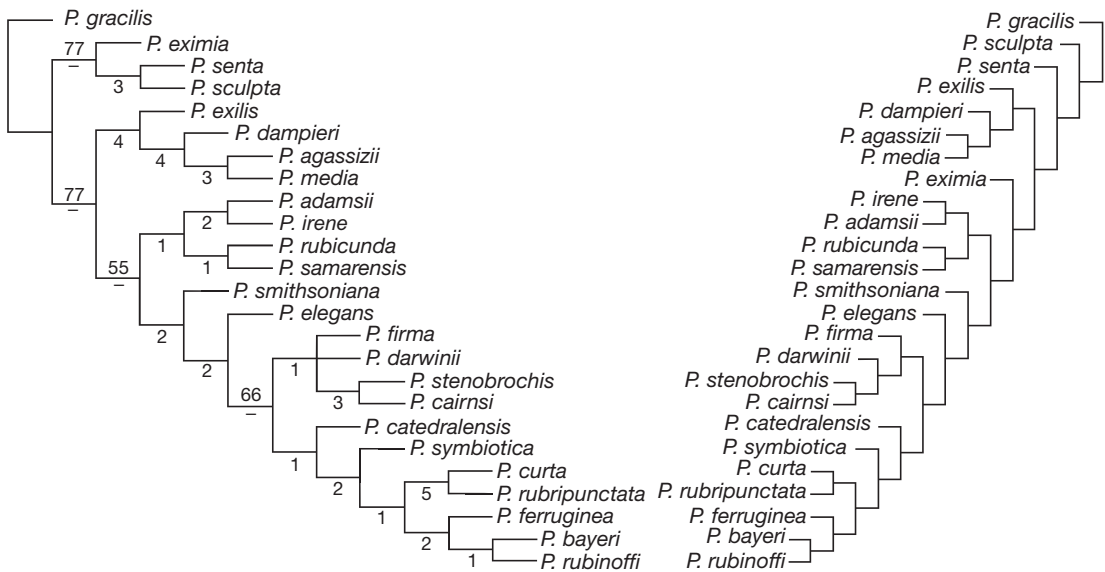


FIG. 4. — Unrooted tree of *Pacifigorgia* Bayer, 1951 using continuous characters as such. Left side: majority rule consensus tree of nine most parsimonious trees obtained using all characters; above branches: node frequency values, below branches: decay index (DI) values. Right side: most parsimonious tree obtained using discrete characters and continuous characters treated as such using the program TNT.

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APPENDIX 1

Final matrix used for the phylogenetic analysis of the genus *Pacifigorgia* Bayer, 1951.

| Species | Characters | | | | | | | | | | | | | | | | | |
|-----------------------------------|------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|-------|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| <i>Pacifigorgia adamsii</i> | 0 | 6 | 4 | 5 | 1 | 1 | 3 | 0 | 8 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1,2 |
| <i>Pacifigorgia agassizii</i> | 1 | 2 | 0 | 4 | 1 | 1 | 3 | 0 | ? | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1,2 |
| <i>Pacifigorgia bayeri</i> | 1 | 2 | ? | 3 | 0 | 1 | 2 | 0 | 2 | 1 | 1 | 4 | 1 | 0 | 0 | 0 | 0 | 2,3 |
| <i>Pacifigorgia cairnsi</i> | 9 | 5 | 4 | 4 | 5 | 4 | 2 | 2 | 2 | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 3 |
| <i>Pacifigorgia catedralensis</i> | 2 | 3 | 4 | 5 | 3 | 0 | 2 | 2 | 2 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0,3 |
| <i>Pacifigorgia curta</i> | 0 | 6 | 7 | 4 | 5 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 1 | 1 | 0 | 0 | 0 | 2,3 |
| <i>Pacifigorgia dampieri</i> | 0 | 9 | 4 | 5 | 4 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1,2 |
| <i>Pacifigorgia darwinii</i> | 3 | 3 | 2 | 4 | 4 | 3 | 3 | 3 | 2 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 3 |
| <i>Pacifigorgia elegans</i> | 1 | 5 | ? | 5 | 2 | 3 | 1 | 0 | ? | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 3 |
| <i>Pacifigorgia exilis</i> | 3 | 2 | ? | 4 | 5 | 0 | 2 | 0 | ? | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1,2 |
| <i>Pacifigorgia eximia</i> | 1 | 3 | 2 | 0 | 2 | 9 | 3 | 9 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0,1 |
| <i>Pacifigorgia ferruginea</i> | 0 | 3 | 4 | 3 | 1 | 2 | 2 | 0 | 2 | 1 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 3,4 |
| <i>Pacifigorgia firma</i> | 3 | 2 | ? | 3 | 4 | 4 | 1 | 1 | 3 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0,3 |
| <i>Pacifigorgia gracilis</i> | 0 | 4 | 2 | 3 | 2 | 3 | 1 | 2 | ? | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1,2 |
| <i>Pacifigorgia irene</i> | 0 | 7 | 2 | 5 | 3 | 0 | 2 | 0 | 9 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1,2 |
| <i>Pacifigorgia media</i> | 2 | 4 | 0 | 4 | ? | 1 | 4 | 0 | ? | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1,2 |
| <i>Pacifigorgia rubicunda</i> | 1 | 5 | 3 | 7 | 6 | 1 | 0 | 1 | 4 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0,1 |
| <i>Pacifigorgia rubinofii</i> | 2 | 0 | 2 | 1 | 0 | 2 | 2 | 1 | 3 | 1 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 4 |
| <i>Pacifigorgia rubripunctata</i> | 1 | 8 | 7 | 4 | 4 | 0 | 2 | 4 | 1 | 1 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0,3,4 |
| <i>Pacifigorgia samarensis</i> | 0 | 3 | 2 | 9 | 4 | 2 | 1 | 1 | 4 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0,1 |
| <i>Pacifigorgia sculpta</i> | 3 | 8 | 7 | 5 | 9 | 7 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0,1 |
| <i>Pacifigorgia senta</i> | 1 | 8 | 9 | 2 | 6 | 5 | 1 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0,1 |
| <i>Pacifigorgia smithsoniana</i> | 2 | 4 | 2 | 5 | 2 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 3,4 |
| <i>Pacifigorgia stenobrochis</i> | 6 | 5 | 1 | 2 | 1 | 9 | 0 | 8 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 3 |
| <i>Pacifigorgia symbiotica</i> | 3 | 3 | ? | 5 | ? | 2 | 9 | 1 | 2 | 1 | 1 | 2 | 1 | 0 | 0 | 0 | 1 | 2,3 |