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Null models for null hypotheses in taxonomy: a test using Scyphozoa

MICHAEL K. BROWN[1,*] and MARK J. GIBBONS[1]

[1]*Biodiversity and Conservation Biology, University of the Western Cape, Belville, Western Cape 7535, South Africa*

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Although molecular tools are becoming more important in the delineation of scyphozoan species there is, perforce, a need to substantiate new species definitions using morphological data. Access to type material is often difficult and detailed, raw morphometric data are rarely provided in older type descriptions, which makes comparisons of new with old challenging. Here, we use null models based on simple measures of central tendency to generate morphometric data sets for four species of *Aurelia*, three species of *Chrysaora* and two species of *Crambionella*. The results of PERMANOVA and CAP analyses indicate no significant differences between random and real data within species, but significant differences between congeneric species (null or real), suggesting that this multivariate approach may be a useful tool for defining species when comparative data are scant.

ADDITIONAL KEYWORDS: biodiversity – marine – morphology – Scyphozoa – statistics – taxonomy.

INTRODUCTION

Describing biodiversity is fundamentally important in the 21st century (Boero, 2001; Guerra García *et al.*, 2008), and while the use of molecules to delineate species is becoming increasingly widespread, there is still a need to substantiate these findings with morphological data (Dayrat, 2005). Such endeavours require access to comparative information and, in the case of new species descriptions, type and other specimens housed and curated in designated repositories. Unfortunately, especially in developing countries, access to such data and materials can become complicated by limited resources (Agosti, 2006), and this threatens the wider roll-out of taxonomic efforts (Wheeler & Valdecasas, 2005). Despite the efforts of, and pleas by, some institutions and bodies for wider access to raw data (e.g. Costello *et al.*, 2010), scientists may be forgiven for being hesitant to share what are in effect the fruits of their hard and costly labour (Agosti, 2006). Incidents such as the destruction of type specimens (Stokstad, 2017) heighten the apprehension of sharing type material. In such contexts, there is a clear benefit in developing methods to generate comparative data sets based on simple data from the accessible published literature.

Null models are frequently used in ecological studies to infer the effects of biological processes/interactions in observational data sets (Harvey *et al.*, 1983). Such models retain the key structure of the data set, but allow elements to vary stochastically in order to create new assemblage patterns that are then compared with the original (Gotelli & Ellison, 2004). We extend this line of thinking here to scyphozoan taxonomy, and generate null model data sets based on published measures of central tendency for representatives of three different families of the Discomedusae. These data are then compared with those of real conspecifics and congenerics using multivariate analyses in order to determine the utility of this approach in separating species where real data are scant.

MATERIAL AND METHODS

Multivariate data sets describing the morphology and meristics of three species of *Aurelia*, three species of *Chrysaora* and two species of *Crambionella* were

*Corresponding author. E-mail: 3650408@myuwc.ac.za

derived from Scorrano *et al.* (2017) and Brown *et al.* (2021), Ras *et al.* (2020) and Neethling *et al.* (2011), respectively. All specimens were of genetically confirmed species. Since the advent of integrative taxonomy in 2003 it has become conventional in scyphozoan taxonomy to standardize morphometric measures by dividing them by bell diameter (BD), as many features develop in an isometric way and can be readily compared across taxa. Others may vary in an allometric fashion, which complicates comparisons without a wide size range of material - that is often not available, and these features are ignored here. The full list of standardized measures and meristic counts used for each species is provided in the Supporting Information (Tables S1-S3).

Null model data sets were generated for each species from values of the mean and standard deviation derived from a randomly selected 50% of all specimens in the above listed sources and shown in the Supporting Information (Tables S1-S3) using LibreOffice Calc (2016). Because we are trying to establish the utility of the method in intra-specific comparisons (real vs. null), as a prelude to their use in inter-specific comparisons, we could not use the same individuals of the same species to derive mean (and standard deviation) measures. The RANDBETWEEN function used in the data generation draws from a uniform distribution. Because 99.4% of data lie between three standard deviations from the mean, we have selected random values for each measure within a range of three standard deviations above and below the mean (Eqn 1):

$$\text{RANDBETWEEN}\ (\bar{x} - 3\sigma, \bar{x} + 3\sigma) \quad \text{(Eqn 1)}$$

Where:

RANDBETWEEN generates a random integer between (min, max).

$\bar{x}$ is the mean standardized morphometric measure or meristic count for each species.

σ is the standard deviation for standardized morphometric measure or meristic count for each species.

Using the full list of isometric characters shown in the Supporting Information (Tables S1-S3), a total of 20 null individuals were created for each species.

The data set for each genus was imported into R and a similarity matrix (Euclidean distance) was generated between all individuals (null and real) following $\log(x + 1)$ transformation and normalization of the data. A PERmutational Multivariate ANAlysis Of VAriance (PERMANOVA) was then conducted to test for statistical differences between null and real data. PERMANOVA is a semiparametric method of geometric partitioning of multivariate variation based on a chosen dissimilarity matrix. The method allows tests and estimations of interactions, effects and hierarchical structures from classical partitioning, while allowing one to choose the dissimilarity measure and allowing non-normal data (Anderson, 2014).

A Canonical Analysis of Principle coordinates (CAP) analysis was also undertaken. Although it is not strictly necessary to do both a PERMANOVA and a CAP analysis if there are sufficient data, the latter method has the advantage in that provides a graphic output and can be used to calculate the likelihood of misclassification. CAP is an ordination method that displays a multivariate cloud based on any distance or dissimilarity measure chosen and allows testing of an a priori hypothesis. Fuller details of the method can be found in Anderson *et al.* (2008); however, in essence we have used "leave one out" diagnostics to determine the subset of subset of principle co-ordinate (PCO) axes used to provide the canonical eigenvalues and their associated eigenvectors, which were then used to generate a CAP plot. These CAP axes are linear combinations of a subset of orthonormal PCO axes, and were also used to cross validate specimen identity and determine misclassification errors. The CAP analyses were conducted in PRIMER7 (Clarke & Gorley, 2015).

RESULTS

All PERMANOVA analyses indicated that there were no significant differences between the null-model species and the real species, for any species in any of the genera (Table 1); however, there were significant differences between species for all genera (Table 1). Further, the results of all CAP analyses for all species in all genera indicate that null model specimens could be correctly assigned and differentiated from real congenerics (Figs 1–3). The CAP analyses for the *Aurelia* data sets (Fig. 1) show a separation of species along the first two CAP axes, with real and null model conspecifics grouping together. This pattern is also seen in the analyses for the species of *Chrysaora* (Fig. 2) and *Crambionella* (Fig. 3). Obviously, there were a large number of misclassification errors between real and null model conspecifics (Supporting Information, Table S5); however, with the exception of *Aurelia*, few between species. Indeed, when null model and real individuals were grouped as conspecific, inter-species misclassifications were few (20.25%, 0% and 3.17% for *Aurelia*, *Chrysaora* and *Crambionella*, respectively; Supporting Information, Table S6).

DISCUSSION

In both analyses, it was possible to distinguish between congenerics but not real or null individuals, which suggests that null models can be a useful tool

Table 1. Results of the PERMANOVA analyses testing for differences in the multivariate similarity between species, type (real or null) and the interaction between species and specimen, for the three genera examined here. Significant *P* values in bold

		Aurelia spp.					
	df	SS	MS	F. Model	P(perm)	Unique perms	Pr (MC)
Species	2	104.85	52.425	7.576	0.001	999	**0.001**
Type	1	9.3288	9.3288	1.3481	0.203	999	0.229
Species: Type	2	9.7895	4.8947	0.70735	0.788	999	0.769
Residuals	73	505.15	6.9198				
Total	78	702					
		Chrysaora spp.					
	df	SS	MS	F. Model	P(perm)	Unique perms	Pr (MC)
Species	2	321.62	160.81	43.824	0.001	998	**0.001**
Type	1	3.0749	3.0749	0.83798	0.532	999	0.521
Species: Type	2	10.208	5.1039	1.3909	0.174	998	0.147
Residuals	78	286.22	3.6695				
Total	83	747					
		Crambionella					
	df	SS	MS	F. Model	P(perm)	Unique perms	P(MC))
Species	1	53.867	53.867	6.0649	0.001	999	**0.001**
Type	1	15.679	15.679	1.7653	0.101	999	0.07
Species: Type	1	15.545	15.545	1.7502	0.105	998	0.084
Residuals	59	524.03	8.8818				
Total	62	682					

d.f.: Degrees of freedom; SS: Sum of Squares; MS: Mean Square; F. Model: Results of the Pseudo-F test; P(perm): P based on Permutations; Unique perms: Number of unique permutations; P(MC): Monte Carlo P values.

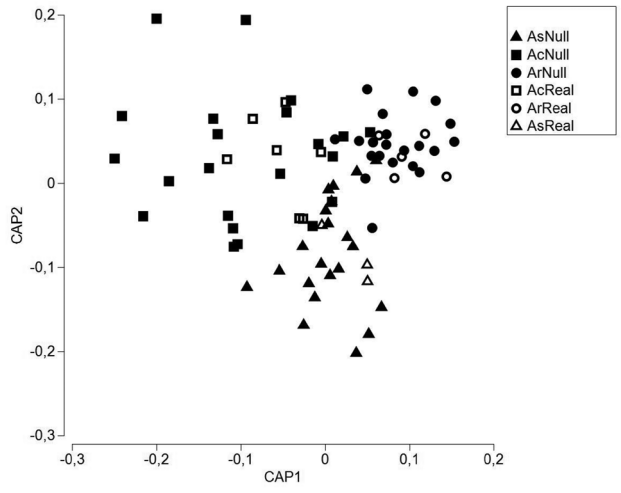

Figure 1. CAP analysis comparing null models of Aurelia spp. with actual measured individuals. Data from Scorrano et al (2017). Values for CAP1 and CAP2 were 0.81 and 0.71 respectively.

for scyphozoan taxonomists who do not have access to archived specimens or raw data for comparative studies. Such data are especially difficult to obtain from the older literature, and their gathering would require numerous visits to different institutions spread across the world, which at a time of travel and other restrictions is problematic.

In their description of *Drymonema larsoni*, Bayha & Dawson (2010) were faced with essentially similar problems. That is, they had quite a lot of new data for *D. larsoni*, but limited and literature-based data for the only two other species in the genus, *Drymonema dalmatinum* and *Drymonema gorgo*. In order to make quantitative comparisons, Bayha & Dawson (2010) first generated regression lines between individual measures and the bell diameter for all specimens of *D. larsoni*, with associated confidence limits. By placing the corresponding measures for *D. dalmatinum* and *D. gorgo* onto these plots, it immediately became clear which features differed among species (Bayha & Dawson, 2010: figs 10–11). This approach was possible because Bayha & Dawson (2010) effectively had access to the raw data for *D. dalmatinum* and *D. gorgo*: the same raw data that are generally not available in summarized descriptions.

Under these circumstances, our method should be seen as an additional tool that can be used to study jellyfish taxonomy. Our approach is integrative and

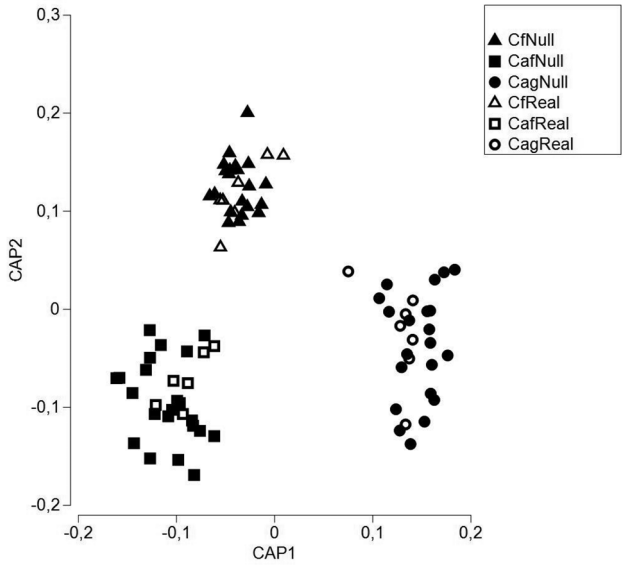

Figure 2. CAP analysis comparing null models of Chrysaora spp. with actual measured individuals. Data from Ras *et al*. (2020). Values for CAP1 and CAP2 were 0.97 and 0.91 respectively.

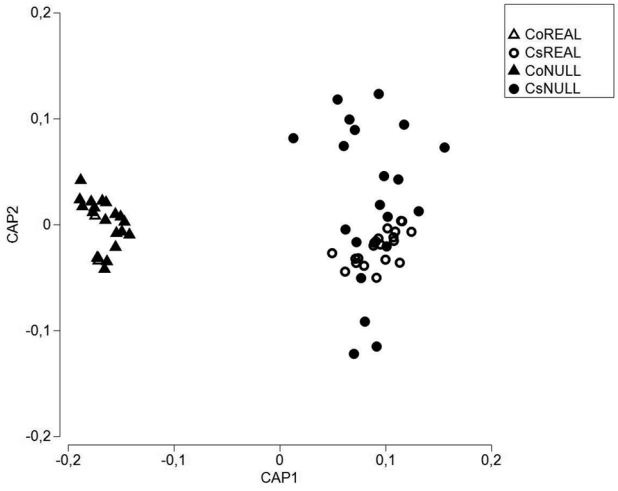

Figure 3. CAP analysis comparing null models of Crambionella stuhlmanni and Crambionella orsini with actual measured individuals. Data from Neethling *et al*. (2011). Values for CAP1 and CAP2 were 0.87 and 0.38 respectively.

uses a multivariate data set, whilst that of Bayha & Dawson (2010) employs a large number of separate parametric tests for separate measures, which of course comes with the issues linked to multiple testing. It is also not hard to imagine that with greater sampling, some of the differences between *D. gorgo* and *D. dalmatinum*, and *D. larsoni* shown in Bayha & Dawson (2010: figs 10–11) might disappear.

It could be argued that a true analogue of ecological null models would take the collection of real traits from all individuals, permute them among individuals (within species) and generate the distribution from that (as e.g., Lawlor, 1980). However, in his paper entitled 'Significance testing in ecological null models' Veech (2012) describes an indirect test of a null model thus: 'In essence, the indirect approach does not recognize the null distribution as a distribution of a test statistic but rather takes the null distribution as a set of simulated parameter estimates that is compared to the observed estimate(s) using chi square tests, *t* tests, regression or other parametric tests. Essentially, an indirect test takes an inherently non-parametric approach (i.e., data randomization to produce a null distribution) and turns it into a parametric test of significance' (Veech, 2012: 612). Thus, while parametric data (mean and standard deviation) were used here to derive 'individuals', as opposed to non-parametric data (median and inter-quartile range—which are rarely reported), they were analysed using non-parametric techniques.

Our null models have been generated using mean (and standard deviation) measures derived from individuals that were different from conspecifics against which they were tested. This was necessary to avoid circularity in our proof-of-concept, but in practise it would not be necessary to test null vs. real individuals, as interspecies comparisons would be made using all available data. It is important to note, as stated earlier, that this tool allows researchers to test or reject the null hypothesis of no difference between species. The fact that there is no significant difference does not mean that the individual can be assigned to the modelled species, but rather that when there is a significant difference, it is indicative that the individual is not the same as the modelled species. This tool may prove helpful when used in combination with molecular investigations, and provides a statistical methodology that can further strengthen integrative taxonomy when good comparative data are hard to obtain. Capacity development is one of the more pressing needs in modern science (Vanhove *et al.*, 2017) and taxonomy is not exempted from this. Despite perceptions that the number of taxonomists is high (Costello *et al.*, 2013), the truth is far from clear (Gomez-Daglio & Dawson 2019) because as taxonomy becomes progressively integrative, the skills needed to delineate species broaden and descriptions become appropriately multi-authored (Gomez-Daglio & Dawson, 2019). Unfortunately, it is often the regions that require the most taxonomic work that also have the greatest need for capacity development (Paknia, 2015), and which are likely the ones with the most

constrained budgets for libraries and travel. The approach posited here goes some way to providing a cost-effective remedy to alleviate some of these concerns, though we stress its use as cautioned above.

Clearly, the method cannot be used in the absence of data. Which is sometimes the case historically, when species were erected on the basis of a single specimen and no subsequent specimens have ever been collected [e.g. Cyanea annasethe (Haeckel, 1880)]. After all, no null individuals can be generated in the absence of any indices of dispersion around a mean measure. In order for the technique to be utilized fully, moving forward, it is important that authors report their published data in a manner that can be used by others. This study found it necessary to use data that had been standardized to BD, and to report those data using simple measures of central tendency (mean, standard deviation). It is important to stress the need for robust analyses of size-related changes in morphometric measures, because the technique will only work at this stage using isometric measures. Allometric data need to be highlighted and, where possible, the equations relating standardized morphometric measures to size need to be published so that future iterations of the null model approach can be refined.

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DATA AVAILABILITY

Standardized data of the 'real' individuals of *Chrysaora* and *Crambionella* are included in the Supporting Information (Table S4) (from Neethling *et al.*, 2011; Ras *et al.*, 2020) Corresponding data for *Aurelia* may be made available upon request from S. Scorrano (simoscorrano@gmail.com). Null individuals are not included in Supporting Information (Table S4) as they are represented by the measures of central tendency shown in Supporting Information (Tables S1-S3).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Mean (standard deviation) standardized values of the isometric features used in the generation of null model data of *Aurelia* spp. from Scorrano *et al.* (2017). All measures proportional to bell diameter.
Table S2. Mean (standard deviation) standardized values of the isometric features used in the generation of null model data of *Chrysaora* spp. from Ras *et al.* (2020). All measures proportional to bell diameter.
Table S3. Mean (standard deviation) standardized values of the isometric features used in the generation of null model data of *Crambionella* spp. from Neethling *et al.* (2011). All measures proportional to bell diameter.
Table S4. Real, standardized measures of *Crambionella* and *Chrysaora* individuals used in comparisons.
Table S5. Misclassification tests for all genera separating null modelled individuals and real individuals.
Table S6. Misclassification tests of all genera, grouping null modelled and real species into single species groups.