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Reproductive and environmental traits explain the variation in egg size among Medusozoa (Cnidaria)

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Medusozoa (Cnidaria) are characterized by diverse life cycles, with different semaphoronts (medusa, medusoid, fixed gonophore, polyp) representing the sexual phase and carrying the gametes. Although egg size is often considered a proxy to understand reproductive and developmental traits of medusozoans, understanding of the processes influencing egg size variation in the group under an evolutionary context is still limited. We carried out a comprehensive review of the variation of egg size in Medusozoa to test whether this variation is related to biological/sexual or environmental traits. Egg size presents a strong phylogenetic signal ($\lambda = 0.79$, $K = 0.67$), explaining why closely related species with different reproductive strategies and different individual sizes have similar egg sizes. However, variation in egg size is influenced by the number of eggs, depth and temperature, with larger eggs frequently present in species with few eggs (1–15), in deep-sea species and in cold-water species. Conversely, the production of small eggs among cold-water species of Staurozoa might be associated with the development of a small benthic larvae in this group. Our study reinforces that egg sizes respond to reproductive and environmental traits, although egg size is highly conserved within medusa classes.

1. Introduction

Interspecific comparison of sexual traits helps to understand the diversity and evolution of reproductive patterns, for example, why species living in shallow and deep waters have different reproductive performances [1,2]. Some frequently studied sexual traits are gonadal morphology [3], fecundity (e.g. number of eggs) [4], gametogenesis [5], reproductive cycles (e.g. phenology) [6] and sexual strategies (e.g. predominance of asexual or sexual reproduction) [7]. Egg size has raised interest because it is considered a proxy to infer characteristics of the reproductive cycle of marine species [8]. Variation in egg size has been found to predict variation in larval and female size [9,10], developmental mode (lecithotrophic larval species have larger eggs in many taxa) [11], reproductive cycle (e.g. deep-sea echinoderms show egg size variation depending on their reproductive biology) [6] and fertilization success (e.g. larger eggs have a higher chance of fertilization in marine invertebrates with broadcast-spawning) [12]. Egg size was also demonstrated to respond to environmental conditions

[13,14], with larger eggs present in species living at low salinity (e.g. in estuarine amphipods [15]), in deep-sea habitats (e.g. in deep-sea fishes) [13], in low temperatures (with lipid-rich yolk stocks) [14] and in food-rich environments (e.g. in echinoderms) [16]. Similarly, a correlation between egg size and a given environmental factor may also depend on the species and latitude [14,17].

Sexual reproduction in Cnidaria (including Anthozoa, Endocnidozoa and Medusozoa [18]) is highly diverse [19]. Among the major cnidarian lineages, Medusozoa (a subphylum including the classes Hydrozoa, Scyphozoa, Cubozoa and Staurozoa) is particularly interesting because of its unique metagenetic life cycle [20]. The life cycle includes a swimming larva or planula, a sedentary polyp, and a free-living medusa [20], and the two main stages (polyp and medusa) may undertake sexual and asexual reproduction [21,22]. The medusa, when present, is the sexual adult phase carrying the gonads and gametes [22,23]. The development of the medusae is particularly plastic in the class Hydrozoa, where it may show several degrees of morphological reduction, even among species of the same genus [24]. Some species have medusoids (i.e. a short-lived reduced medusae [25,26]) that may remain attached to the gonotheca in a structure named a meconidium [27]. Other species have the medusa stage reduced to a fixed gonophore or sporosac [28], which is attached to the hydroid and considered to be the most reduced state of a medusa [29].

Medusozoan gonads are usually the focus in studies concerning sexual reproduction [30], and have been considered either as the regions where gametes are formed (therefore not true 'gonads', or not even an organ [31,32]), or true reproductive individualized organs with meiotic cells, sperm, and vitellogenic oocyte stages [33]. Gonadal development, location, and shape are also distinctive characters among medusozoans [34]. Scyphozoa, Staurozoa and Cubozoa are predominantly gonochoristic with gonads developing from the endodermal tissue of the bell [31,35,36]. Meanwhile Hydrozoa have gonads of ectodermic origin developing either in the region of the radial canals (e.g. in the leptothecate *Mitrocoma cellularia* [37]), around the manubrium (e.g. in the 'anthoathecate' *Sarsia lovenii* [38]) or spadix (in fixed gonophores such as in the 'anthoathecate' *Eudendrium* [39]). Gametes (egg and sperm) can be liberated directly into the water for external fertilization (broadcasting as in *Nemalecium lighti* [40]), which is the presumed ancestral state in marine invertebrates [41], or only the sperm is liberated and fertilizes the eggs inside the gastrovascular cavity (e.g. *Alatina alata* [42]), sometimes including brooding (e.g. *Eudendrium* [39]).

Some topics of the sexual reproduction in Medusozoa have been relatively well studied, such as gametogenesis [43] and fertilization [44]. However, there is little information concerning egg morphology and nutrient content in Medusozoa [45]. Egg size data are also scarce, and comparative studies focusing on its variation within the group have not yet been published, despite the importance and high variability of this reproductive trait among Medusozoa. For instance, the contribution of historical processes and environmental pressures to Medusozoa reproductive patterns is limited, with no estimates of the relative influence of phylogeny and environment in reproductive trait variation.

In this study we use comparative phylogenetic methods to understand the evolution of egg size in Medusozoa, considering the phylogenetic relationships within Medusozoa, and the relative contributions of reproductive (viz. egg number, individual size, reproductive strategies [polyp, medusa, medusoid], fertilization mode [internal, external]) and environmental (viz. depth range, water temperature) traits.

2. Material and methods

(a) Oocyte and egg

We highlight that the technical literature generally uses egg (or ovum, pl. ova) and oocyte (or ovocyte) as synonymous, referring to stages of differentiation of the female gametes from the primary oocyte during the oogenesis [46]. The egg is derived from the maturation of the ootid that resulted from the meiosis II of the secondary oocyte. Ovum is considered the mature female gamete after meiosis is completed, whereas the secondary oocyte is an immature egg cell produced by the meiosis I of the primary oocyte [47,48]. Therefore, strictly, the terms egg and oocyte are not synonymous. However, the use of the term egg to refer to a late vitellogenic oocyte stage (O_{iii}) is widespread, and we adopt the term herein. The largest oocyte in the gonad is considered to represent the late vitellogenic stage [49] and is therefore the one usually measured to obtain the egg size.

(b) Reproductive and ecological traits

Information on egg size was obtained for a total of 187 species of Medusozoa (136 Hydrozoa, 8 Staurozoa, 32 Scyphozoa and 11 Cubozoa), for which the taxonomy was standardized according to the World Register of Marine Species (WoRMS, 2021). Most of the species had their measurement surveyed in the literature, either by collecting data directly as provided in the paper or by measuring the eggs from photos or drawings available in species descriptions using ImageJ [50] (electronic supplementary material, table S1). We complemented the data by measuring eggs from histological preparations of gonads of hydrozoans (*Liriope tetraphylla*, *Orthopyxis crenata*, *Turritopsis nutricula*) and cubozoans (*Alatina alata*, *Carybdea marsupialis*, *Chiropsalmus quadrumanus*, *Copula sivickisi*, *Tamoya haplonema*) (electronic supplemental methods). When measuring eggs both from literature illustrations and histological preparations, we selected the largest eggs, considered to be in late vitellogenic stage (O_{iii}). For histological preparations, we measured up to 20 O_{iii} and used the average size value in the analyses. When egg size data available from the literature were given in ranges ($n = 90$), we used the average size value for subsequent analyses.

Information of reproductive traits was obtained for each species based on a literature review. We considered the following traits: (1) fertilization mode (external or internal), (2) number of eggs (1–15, 16–50, 51–100 and ≥101), (3) reproductive strategy (medusa, medusoid, fixed gonophore and polyp), and (4) individual size (see supplemental methods for further details). We also obtained information on the following environmental traits: (1) the mean sea surface temperature data for each species ($n = 185$; electronic supplementary material, table S1), and (2) the maximum depth of occurrence ($n = 187$; electronic supplemental material, methods).

(c) Phylogenetic signal

In order to evaluate if the evolution of egg size in Medusozoa is constrained by phylogeny, we tested for phylogenetic signal (statistical dependence among species' trait values due to their phylogenetic relatedness [51]) of egg size with Blomberg's K and Pagel's lambda (λ) [52], with the 'phylosig' function in the package 'phytools' in R [52]. Both use Brownian motion (BM) evolutionary process in their implementation but differ in their approach. Blomberg's K compares the variance of a trait with the variance from a BM model. Values of K can take higher than 1 representing more phylogenetic signal than expected (more variance between clades) and K lower than 1 representing less phylogenetic signal than expected (more variance within clades) [51]. Pagel's λ, on the other hand, illustrates the transformation of the phylogeny that fits a BM model so when λ equals 1, the phylogeny can explain changes in traits, indicating a high phylogenetic signal and when λ equals 0 the trait is evolving independently of the phylogeny [51]. We therefore use high indices (λ close to 1 and p-values significative) to assess that closely related species are similar in egg size [53]. In addition, we tested the phylogenetic signal for environmental traits which turned out significant in the analysis of model selection and phylogenetic ANCOVA. This was done in order to test the pattern of phylogenetic niche conservatism.

(d) Ancestral state reconstruction

To investigate the evolutionary history of egg size, egg number, temperature and depth we reconstructed ancestral states of these variables using the tree topology of 134 species (see supplemental methods for further details). Briefly, a composite phylogenetic tree including sequences of mitochondrial (16S and COI) and nuclear genes (18S and 28S) of 134 species was constructed based on the available published molecular phylogenies [54–59], and species for which we did not have egg size data were later removed from the tree. Bayesian estimates of divergence times were obtained using BEAST2 [53].

The ancestral state of egg size and temperature (continuous variable) was estimated using the 'fastAnc' function in the R package 'phytools' [53]. The model of evolution (Brownian motion, Ornstein–Uhlenbeck or early-burst) was selected using the R package 'geiger' [60] based on AIC scores. The ancestral states were mapped on the phylogeny using the 'contMap' function in R package 'phytools' [53]. We used maximum likelihood to compare models of traits evolution: Brownian motion (BM), Ornstein–Uhlenbeck (OU) and early burst (EB) models of trait evolution [61]. The ancestral state of egg number, and depth (categorical traits) were inferred by using stochastic character mapping implemented in R [62]. Transitions from one state to another can occur at different rates and in different directions in the phylogeny. For that, we first chose the best-fitting model of evolution for the reconstruction of this categorical trait among equal-rates (ER), symmetrical (SYM) and all-rates-different (ARD), identified by the lowest AICc score, calculated with the 'ace' function in the package 'APE' in R [63]. The uncertainty on the value of the trait at each ancestral node was described with the 'make.simmap' function (in 'phytools') after 1000 generations, resulting in a posterior probability distribution of character histories. The posterior distribution of character state history along each branch was summarized by using the 'describe.simmap' function [53].

(e) Model selection

We used PGLS to investigate the influence of phylogeny, reproductive (number of eggs, individual size, reproductive strategies), and ecological (temperature and depth) traits' on egg size. For this analysis, phylogenetic distances among species were obtained from our phylogenetic hypothesis based on molecular data (n = 134; see electronic supplementary material, methods), the same one used for the ancestral state reconstructions.

We assumed a Gaussian error distribution for the log transformed egg size variable, which seemed appropriated given the high number of species with data (134). Using the full model (constructing with all the variables), we compared different covariance structures corresponding to alternative models of evolution based on the lowest AIC value [64]. The tested covariance structures were BM, Martins (equivalent to OU model), Grafen (incorporates a calculation of branch lengths based on number of descendants), Blomberg (assumes that traits evolve under a BM model which rates either accelerate or slow through time) and Pagel (a modification of a BM model that incorporates phylogenetic signal). Between the plausible models we tested the multicollinearity using the 'vif' function in the R package 'car' [65].

We use the 'vif' function to remove the variable (greater than 3) to obtain our full model without multicollinearity. Then, we used the function 'model.sel' to identify which factors led to the lowest AIC. After building the full model considering additive effects among the predictive variables, we tested all possible combinations (between the variables number of eggs, individual size, reproductive strategies, class and temperature) using 'dredge' function from 'MuMIn' packages [66]. All models with delta AIC lower than 2 were considered equally plausible (see electronic supplementary material, table S3).

Model validation was performed with residual analyses (standardized residuals versus fitted values and Q-Q plots) (see the script in electronic supplementary material).

Finally, we tested the association of egg number and both reproductive strategy and fertilization mode fitting a phylogenetic generalized linear squares model (PGLS) [67]. Since evolutionary correlation of discrete characters is prone to reporting spurious significant associations [68], we transformed 'egg number' from a categorical to a continuous variable by using the mean number of each interval. Furthermore, has visual inspection of data is likely better to interpret these results [68,69], we mapped these traits using boxplots (see electronic supplementary material, figures S1 and S2, and script).

(f) Phylogenetic ANCOVA

In addition to model selection and in order to test the hypothesis that egg number, individual size, reproductive strategy, depth, or temperature affects egg size in Medusozoa, we implement an ANCOVA model using phylogenetic generalized least squares (PGLS). This was done because we have a combination of continuous and discrete traits as independent variables [70].

3. Results

(a) Phylogenetic signal

There is strong phylogenetic signal related to egg size across Medusozoa (Pagel's $\lambda = 0.79$, $p < 0.001$; $K = 0.67$, $p < 0.001$), considering that related species have similar egg sizes. The λ and K values (0.79 and 0.67 respectively) (close to 1) correspond to similar egg sizes between close taxa (figure 1), even though $K < 1$ shows that there is also moderate intra-clade variance. Likewise, it was also found that there is phylogenetic signal for temperature (Pagel's $\lambda = 0.80$, $p < 0.001$ and $K = 0.60$, $p < 0.001$) and depth ($p < 0.05$—rejects no signal model). This means that related species in the phylogeny have similar temperature values and depth ranges.

(b) Ancestral reconstructions of egg size and related reproductive/environmental traits

Reconstruction of the ancestral state of egg size in Medusozoa shows that intermediate egg sizes are plesiomorphic and highly conserved within the group (figure 1; best-fit was OU model, AIC = 1750.46). The smallest eggs occur in Staurozoa (18–72 µm), intermediate in Cubozoa and Hydrozoa, and some of the largest in Scyphozoa (figure 1).

A higher number of eggs ($n \geq 101$) is the ancestral condition in Medusozoa, conserved in Staurozoa, Scyphozoa, and Cubozoa (figure 2; best-fit was symmetrical model,

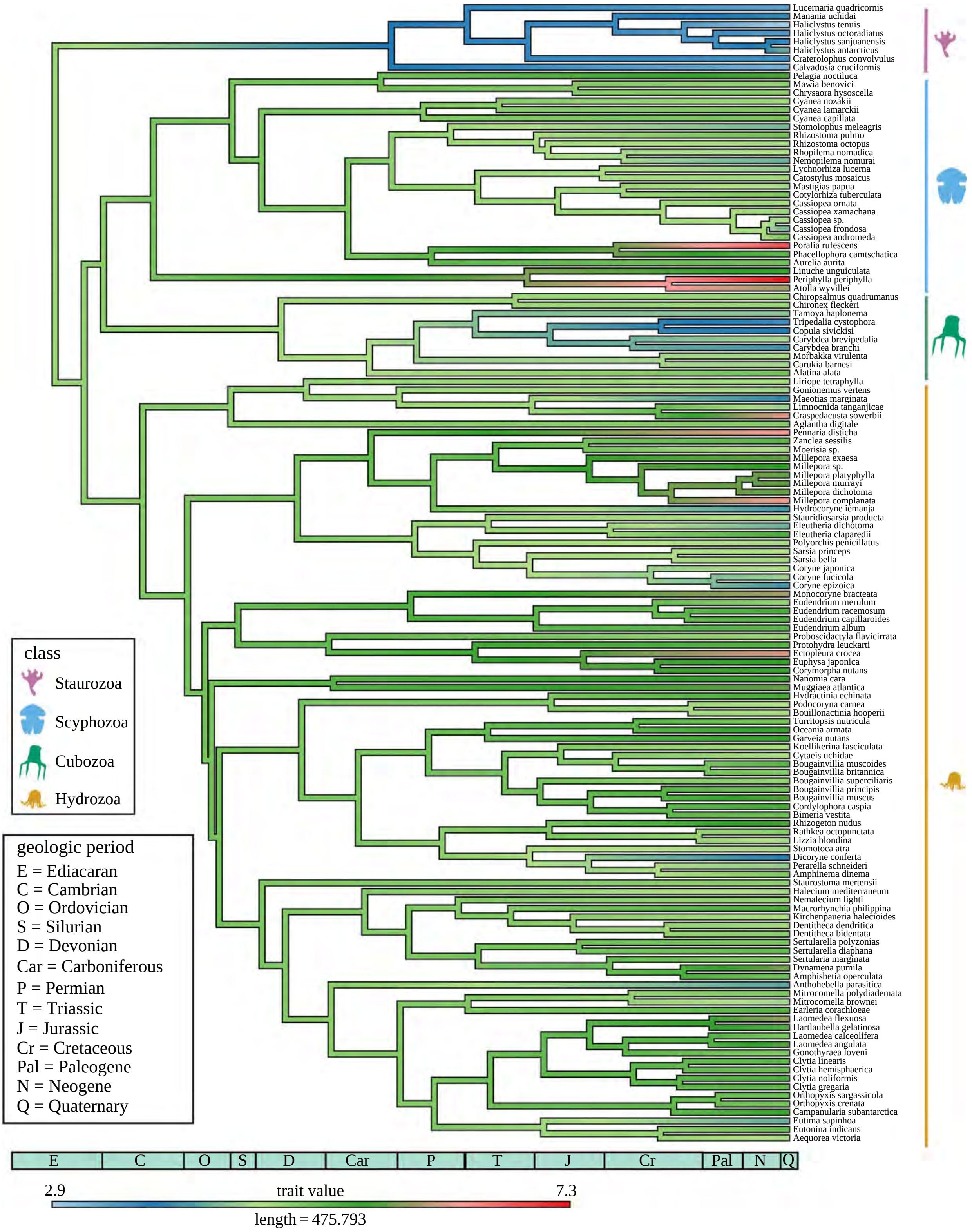

Figure 1. Reconstruction of ancestral egg size (log) in Medusozoa for the 134 species analysed in this study. Colour on branches indicates egg size along the topology. Trait value = min and max egg size (log) (2.9–7.3).

AIC = 135.43) (*n* = 103). The ancestral condition changed to fewer eggs (*n* = 1–15) in the origin of Hydroidolina. However, an intermediate number of eggs (*n* = 16–50 and 51–100) has evolved multiple times in less inclusive groups of Hydroidolina, such as Siphonophorae (e.g. *Muggiaea atlantica*, *Nanomia cara*), Capitata (e.g. *Coryne fucicola*, *Coryne epizoica*) and Macrocolonia (e.g. *Dentitheca dendritica*, *Sertularella polyzonias*). In addition, a reversal to a higher number of eggs (*n* ≥ 101) originated independently in Eirenida (e.g. *Aequorea victoria*), Campanulinida (e.g. *Earleria corachloeae*), Hebellidae (e.g. *Anthohebella parasitica*) and Bougainvilliidae (*Bougainvillia superciliaris*).

Reconstruction of the ancestral state of temperature (figure 3; best-fit was OU model, AIC = 885.96) shows that the Medusozoa ancestor (≈ 680 millions of years) probably lived in temperate waters (≈ 14°C). There was an increase in the average temperature (Devonian/Carboniferous period) occupied by the species throughout the evolution of Cubozoa and Scyphozoa (Kolpophorae) in tropical waters, as well as Hydrozoa, with some decreases to cold

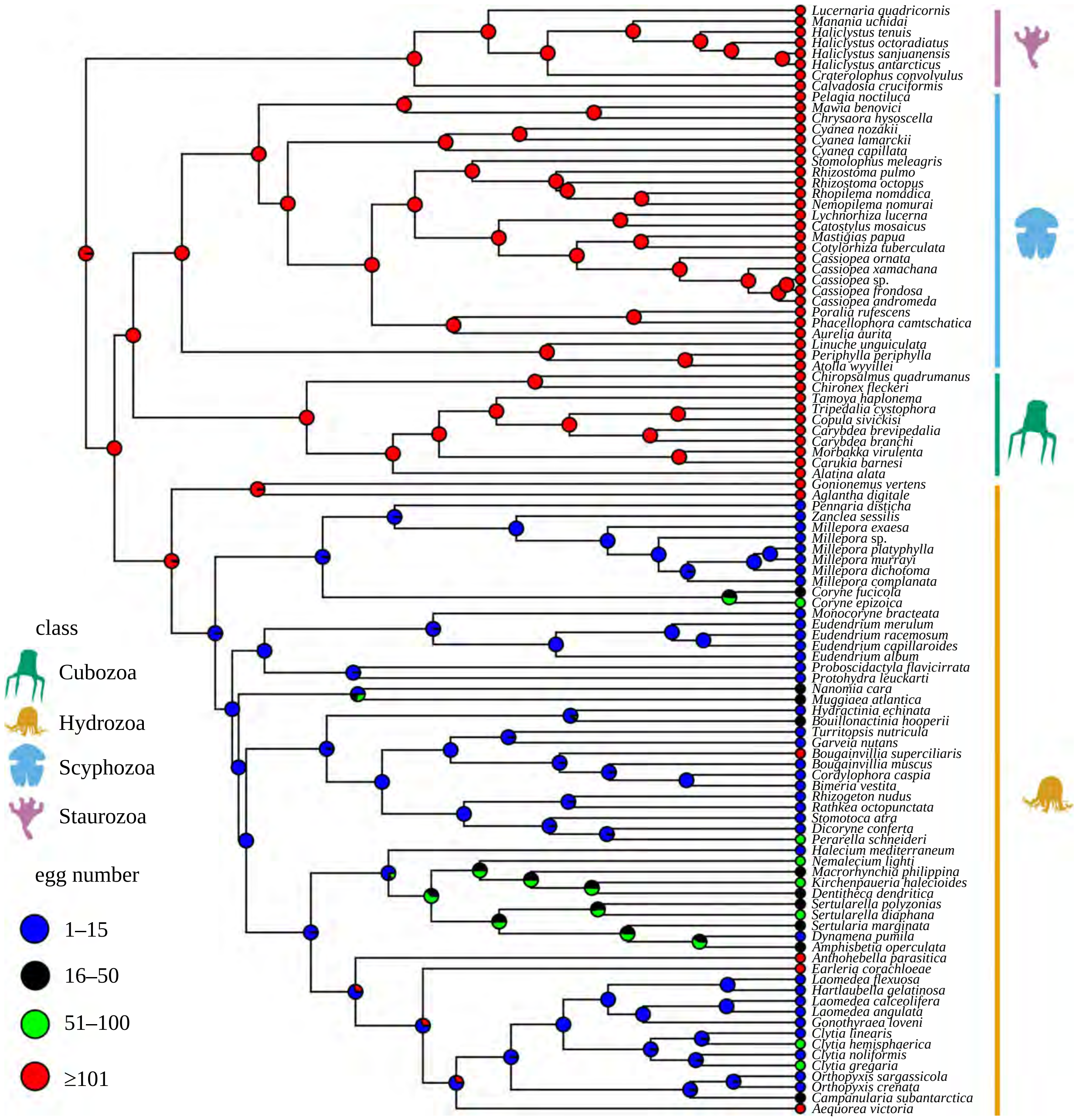

Figure 2. Inferred reconstruction of the number of eggs (1–15, 16–50, 51–100, ≥ 101) within Medusozoa, for 103 species included in the analysis. Colours inside the pie charts at the internal nodes represent the posterior probability of alternative categories of egg number.

temperatures in several clades (Campanulariida, Haleciida, Bougainvillia, Aplanulata). It is important to note that Staurozoa and a few Scyphozoa species currently occurring in cold waters present contrasting egg sizes (small eggs in Staurozoa and large eggs in deep sea Scyphozoa species, compare figure 1 and figure 3).

The ancestral state of the depth (best-fit was ER model, AIC = 118.8) shows that Medusozoa inhabited shallow waters (0–50 m), independently appearing in only a few species in deep waters (greater than 101 m) and intermediate waters (51–100 m) (electronic supplementary material, figure S3).

(c) Reproductive and environmental predictors of egg size

The evolutionary model based on AIC was for Grafen correlation (electronic supplementary material, table S2). Prior to model selection, multicollinearity was tested from the first model with six predictors (egg number, reproductive strategy, individual size, depth, and temperature). All predictors lower than 3, are showed on the final model. The best 5 models resulted under dredge function were compared using AIC (electronic supplementary material, table S3) and the best-fit model show that egg number, temperature and depth had an effect on egg size (AIC = 166.1, SE = 0.68, d.f. = 103) (figure 4 and electronic supplementary material, tables S3 and S4). The phylogenetic ANCOVA analyses show the same significant variables explaining the variation in egg size (depth, $p = 0.003$; egg number, $p = 0.037$; temperature, $p = 0.045$) as the results obtained with model selection.

Results from the phylogenetic generalized estimating equations analyses revealed a positive and significant correlation between the number of eggs and the 'medusa' reproductive strategy of Medusozoa (medusa: 1797.76, $t = 16.03$, $p < 0.001$) while not being significant for the other reproductive strategies (gonophore: 15.85, $t = 0.08$, $p = 0.93$;

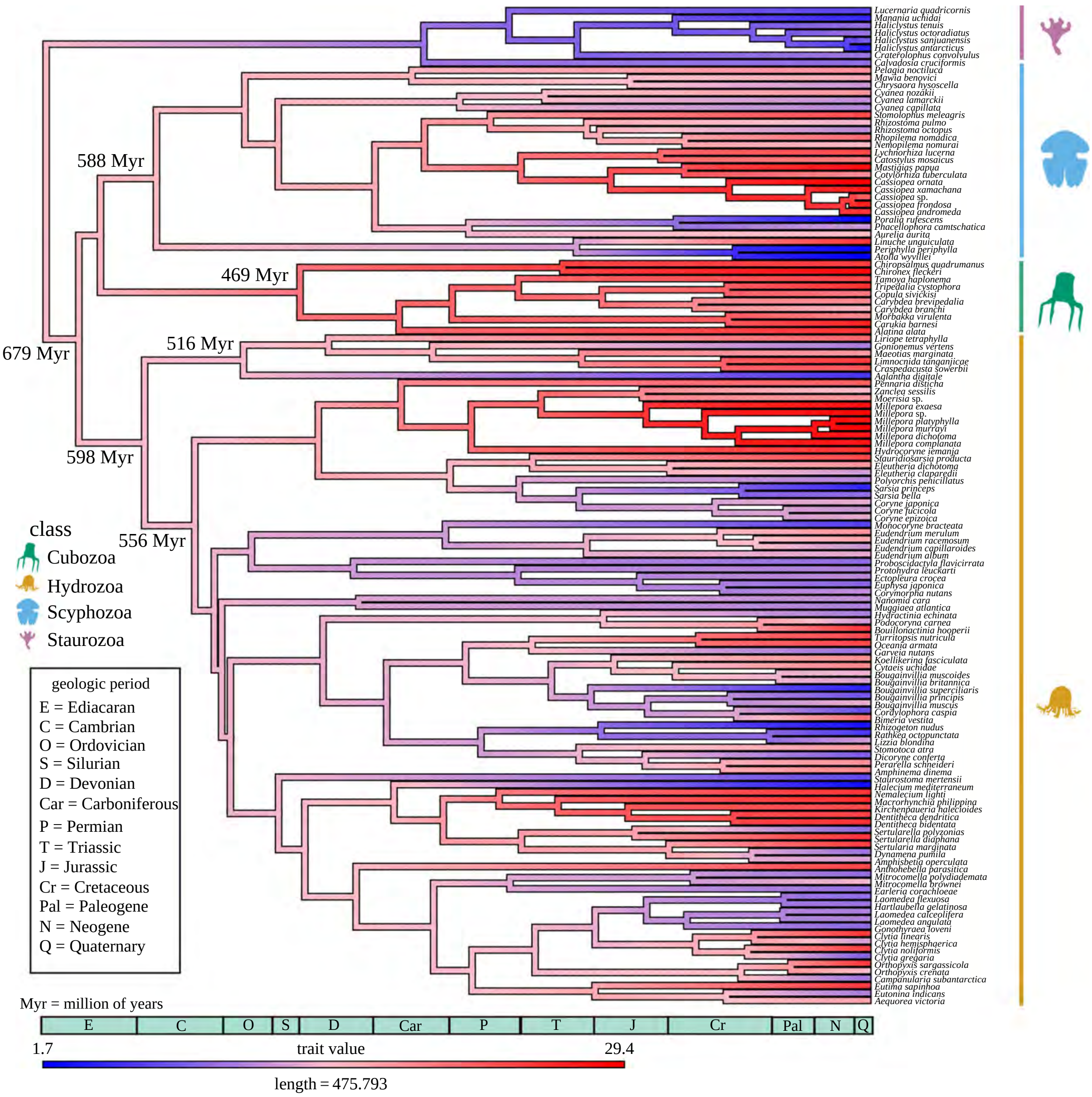

Figure 3. Ancestral reconstruction with time (in millions of years corresponding with geological periods) for temperature in Medusozoa. This graph assumes that the thermal niche of each lineage has not changed across the years. Colour figures: yellow = Hydrozoa, green = Cubozoa, blue = Scyphozoa, pink = Staurozoa. Trait value = min and max temperature (1.7–29.4°C).

medusoid: 242.44, $t = 0.91$, $p = 0.37$; polyp: 7.12, $t = 0.008$, $p = 0.99$) (electronic supplementary material, figure S1). Likewise, a significant correlation with the number of eggs was found for external fertilization (1626.54, $t = 8.71$, $p > 0.001$) but not for internal fertilization (–393.09, $t = -1.39$, $p = 0.174$) (electronic supplementary material, figure S2).

4. Discussion

(a) Evolution of egg size and its relationship with reproductive traits

Although small egg sizes are presumably a basal condition for animals [71], our analyses support that intermediate egg size is the ancestral condition in Medusozoa, being conserved along most of its phylogenetic history (figure 1). Among Medusozoa classes, however, Staurozoa and the cubozoan Tripedaliidae differ by having a relatively early transition (Carboniferous and Jurassic, respectively) to smaller eggs, while transitions to larger eggs in the scyphozoan Coronatae occurred since the Cretaceous (figure 1). Both genetic and environmental factors may explain egg size variation in marine invertebrates at different evolutionary levels, from individuals to populations, or even in species and more inclusive taxonomic groups [72]. Several medusozoan lineages have independent evolutionary shifts in egg size, but overall variation in egg size is correlated to the number of eggs related to different reproductive strategies (electronic supplementary material, figure S1). This suggests that egg size may be modulated by life-history tradeoffs and/or selective pressures unique to the evolutionary history of each taxa. Thus, for instance, species from different classes (e.g. Hydrozoa and Scyphozoa) living in similar habitats (e.g. deep sea species) are prone to have similar egg size.

Several models for the evolution of egg size in marine invertebrates have been suggested, often involving tradeoffs between egg size and egg number (e.g. [73]). For instance, larger eggs require a lower concentration of sperm for fertilization, and are related to reduced developmental time and

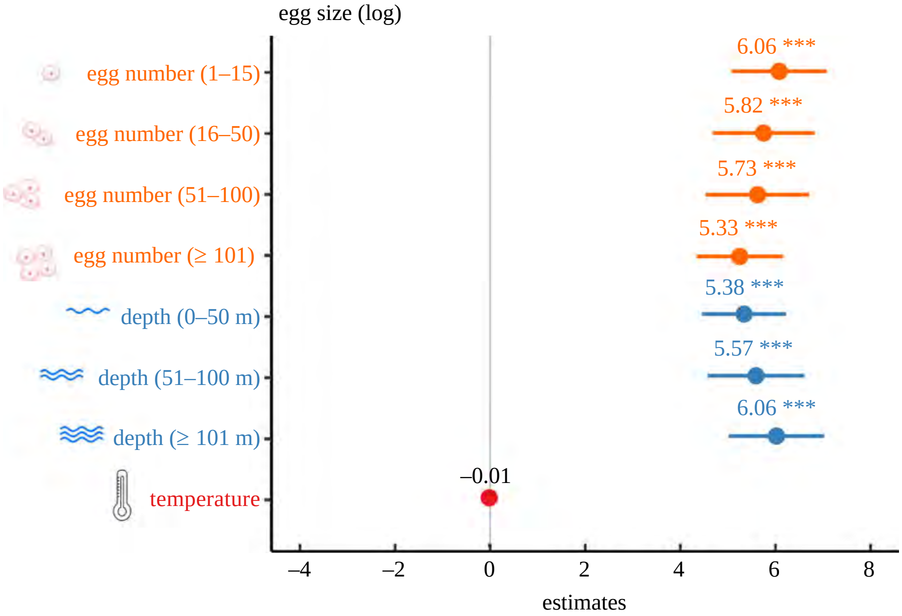

Figure 4. Forest plot of estimates of the best model with 95% confidence intervals. Variables that estimate overlap the line of null effect are not statistically significant while asterisks highlight significant variables (***$p > 0.001$). Values on the right side of the grey line represent positive estimates while values on the left side of the line (red) represent negative estimates.

offspring mortality, but have the disadvantage of an increase in resources allocated to each offspring, leading to a reduction in fecundity (or number of eggs) [12,73]. In Medusozoa, a higher number of eggs ($n \geq 101$) is the ancestral condition for the group (figure 2; see also [54]). In addition, a higher number of eggs is characteristic of species that show small to intermediate egg sizes (figure 4). A clear shift to a smaller number of eggs was observed in the appearance of Hydroidolina (Hydrozoa), which also presents a higher frequency of occurrence of fixed gonophores (electronic supplementary material, table S1). Indeed, our results show that species with medusa produce more eggs ($n \geq$ 101) (electronic supplementary material, figure S1) and also show a tendency to have smaller eggs than species with fixed gonophores ($n = 1$–15) (e.g. *Eudendrium bentart*, *Eudendrium klausi*, *Laomedea flexuosa*, *Synthecium flabellum*; figure 4; electronic supplementary material, table S1).

The relationship between egg size, egg number and reproductive strategy in Hydrozoa was proposed by Teissier [71], who suggested that the reduction of the medusa in some lineages of Hydrozoa could explain the fewer and larger eggs in species with fixed gonophores. This explanation was subsequently corroborated in differences concerning egg number and egg size among hydrozoans with different reproductive strategies (e.g. [26,74]). For instance, most species with a fixed gonophore strategy (in which eggs are produced by the polyp stage) are colonial hydroids that, as modular organisms, compartmentalize the functions in different modules [75] (polymorphs) [76,77] (see supplemental methods). This compartmentalization, allow the resources to be shared among the modules, with growth and reproduction often proceeding simultaneously [77,78]. Differently, the medusa, as a unique individual, has to balance growth and reproduction [79]. In modular colonies, however the eggs are produced in small numerous modules called gonozoids, which are distributed along the colony [78,80] and may also contribute to the existence of larger eggs. In fact, brooding in medusozoans is often associated with modular colonies, which present smaller and more sparsely distributed masses of eggs when compared to medusae. This is consistent with the view that the cost of brooding is associated with oxygen provision [81], as smaller and more spaced embryos facilitate oxygen supply (e.g. [82]), and in the case of modular colonies, could favour the occurrence of brooding [83].These different patterns would have consequences from basic physiology (e.g. energetic demand of the different reproductive system and trade off with other systems) to species interaction (e.g. competition for substrate or prey) and biogeography (e.g. advective events and range distribution).

Among species with a fixed gonophore (within Hydrozoa), the relationship of brooders producing fewer and larger eggs, as well as larger planulae, than non-brooders have also been suggested elsewhere [26]. Our results statistically corroborate and extend part of this hypothesis, showing that egg number could be related to fertilization mode in Medusozoa (electronic supplementary material, figure S2). Indeed, our study corroborates that species with extrernal fertilization more often produces a higher number of eggs [84], while we found no support for internal fertilization being associated with the production of fewer eggs, as suggested before [85]. This, however, may reflect insufficient data on egg size of species with internal fertilization, and highlights the need for additional studies on reproductive patterns in Medusozoa, especially considering that several species are thought to present internal fertilization, even though most inferences are based on indirect evidence [86,87]. In a scenario in which external fertilization is usually considered as a primitive character [41], Medusozoa presents a high diversity and complexity of reproductive traits when compared to other basal Metazoa, and, given the few studies, a high potential for the discovery of additional complex reproductive traits. For instance, it includes viviparous species, as *Crossota millsae* [34], as well as elaborate sexual behaviors, such as the courtship and sperm transfer via spermatophore in *Copula sivickisi* [88].

(b) Egg size and environmental conditions in Medusozoa

Environmental conditions are often correlated with the evolution of morphological characters (e.g. different egg morphologies depending on temperature or salinity in copepods [89]) and with reproductive traits in marine invertebrates [90]. Several environmental variables are suggested to trigger variation in the morphology of Medusozoa as a result of phenotypic plasticity [91]. For instance, species reared under laboratory conditions, such as *Millepora complanata* [92] and *Pennaria disticha* [93], produced larger eggs (both 600 µm) than wild collected specimens, suggesting that a greater investment of energy in reproduction under controlled environment with higher food supply increments the female gamete size [94].

Temperature plays an important role in reproduction, triggering mass reproductive events based on synchronized gametogenetic cycles [95], and affecting egg number [96] and egg size [97]. Many marine taxa (e.g. amphipods, echinoids, fishes) generally produce larger eggs in colder temperatures (e.g. [98]). Within Medusozoa, large eggs are known to be related to direct development, in which the developing medusa benefits from the high amount of energy stored in the yolk of these eggs [99–101]. Direct development mainly occurs in species adapted to colder temperatures in deep-sea species [3]. Indeed, we found that the largest eggs among medusozoans are in deep-sea species, *Atolla wyvillei* (403 µm), and *Periphylla periphylla* (1480 µm), that also present direct development [102] and are associated with lower temperatures [14].

Besides, temperature increases in ectothermic animals, leading to increased energetic costs of development which could explain for the smaller egg sizes [103]. However, the relationship between temperature and egg size may also reflect adaptive strategies shaped throughout the evolutionary history of the group [14,104], which could be the case of Staurozoa that mainly occurs in intertidal cold waters [105] and has small eggs.

According to the results obtained from the phylogenetic signal for temperature and depth, we recognize the possible existence of a pattern of phylogenetic niche conservatism (PNC) that may arise from multiple process including phylogenetic constraint where a common selective environment is shared in related species [106].

(c) Staurozoa, the class with smaller eggs

Staurozoa showed an early shift to smaller eggs in the ancestral state reconstruction, coinciding with their colonization of colder waters [105] (figure 1). In Cnidaria, the planula stage follows egg fertilization and embryonic development. The planula and egg in Medusozoa are similar in size, as described for eggs and larvae of other organisms (e.g. echinoderms [107]). It is possible that the conspicuous difference between the egg size of Staurozoa and other medusozoans is related to the staurozoan unique planula [108]. The diminutive eggs of Staurozoa (18–72 µm recorded in this study) are followed by diminutive (e.g. 100 µm long and 20 µm wide in *Haliclystus* sp.), non-swimming (benthic) planulae, with a constant number of 16 endodermal cells [109] and apparently able to feed when attached [110] (although staurozoan eggs were reported with yolk substances [108,109,111]). Moreover, these small planulae have limited movement ability, crawling on the substrate (i.e. vermiform movements of elongation and retraction) [108,109]. Indeed, staurozoan planulae may settle in groups, living side-by-side, apparently maximizing the efficiency in prey capture [112,113] and maybe in defence, and promoting genetically homogeneous populations [112]. Finally, their small eggs and planulae dimensions and habit would also be associated with lower metabolic demands, as suggested for other marine invertebrates [114].

5. Conclusion

This is the first comparative study addressing egg size in Medusozoa and we show the importance of evaluating this trait and its relationship with historical, sexual, and ecological traits. Egg size in Medusozoa has a strong phylogenetic signal, explaining why this group with many different reproductive strategies and different individual sizes tend to have eggs of similar dimensions. Indeed, egg size is considerably conserved, with intermediate egg sizes as the ancestral condition in the group. Egg size is also related to sexual traits, such as the number of eggs, with larger eggs frequently associated with species with few eggs (1–15); and to environmental traits as depth and temperature. On the other hand, Staurozoa has small eggs probably associated with their small benthic crawling planulae, which therefore would have presumably lower metabolic demands. After this study we can conclude that variation in egg size in Medusozoa is explained by biological/sexual and environmental factors, stressing the role of past ecological and genetic processes in Medusozoa evolution. Further information on deep sea species, as well as data on fertilization mode and egg size of more species of Medusozoa, shall contribute to provide a broader understanding of the evolutionary patterns of sexual reproduction in this group, as well as in other marine taxa.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material [115].

Authors' contributions. J.G.-R.: conceptualization, data curation, formal analysis, investigation, project administration, resources, software, supervision, validation, visualization, writing—original draft, writing—review and editing; A.F.C.: conceptualization, supervision, validation, writing—review and editing; A.M.-G.: data curation, formal analysis, investigation, methodology, resources, software, validation, visualization, writing—review and editing; A.G.-C.: data curation, formal analysis, methodology, resources, software, validation, writing—review and editing; A.C.G.: data curation, formal analysis, methodology, software, supervision, validation, writing—review and editing; L.S.M.: validation, writing—review and editing; F.C.S.: formal analysis, methodology, software, writing—review and editing; A.J.-B.: data curation, formal analysis, investigation, methodology, resources, software, validation, visualization, writing—review and editing; A.C.M.: supervision, validation, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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