FEATURE ARTICLE

Larger offspring associated with lower temperatures across species of *Microporella*, a widespread colonial invertebrate

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ABSTRACT: Among life history traits, offspring size has one of the most direct impacts on fitness, influencing growth, recruitment and survival of the individual, therefore affecting population, and ultimately macroevolutionary outcomes. Despite its ecological and evolutionary importance, little is known about how offspring size varies in lineages over macroevolutionary timescales, especially for colonial organisms. Here, we use the cheilostome bryozoan genus *Microporella* to investigate variation in offspring size over the history of the lineage. The genus *Microporella* is species-rich, cosmopolitan, has a calcified skeleton (and is hence readily preserved in the fossil record), and is a brooder. The brood structure (ovicell) reliably reflects the space occupied by a larva and hence ovicell size is a good proxy for offspring size. Using a suite of biotic and abiotic factors, we ask what best explains offspring/larval size variation in contemporary and fossil species of *Microporella*, and how offspring/larval size changes through the millions of years of the history of the lineage. We find that offspring size is affected by a combination of module size and water temperature (or latitude when fossil species are included), while fecundity and levels of nutrients have a weak to no effect on this life history trait. Among *Microporella* species, descendant species are statistically more likely to have larger offspring than their putative ancestors, with the size difference between species pairs little explained by temporal latitudinal shifts. Our results suggest that both contemporary ecological controls and historical considerations are important in understanding life history trait evolution.

KEY WORDS: Bryozoa · Larval size · Fecundity · Latitude · Sea surface temperature · Bergmann's rule · Atkinson's rule · Cope's rule

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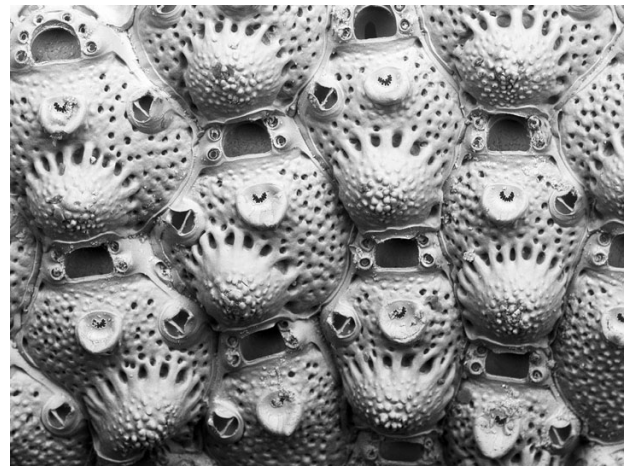
Scanning electron micrograph of fertile zooids of a contemporary species of the cheilostome bryozoan genus *Microporella* from New Zealand (magnification: 50×).

Photo: Emanuela Di Martino

1. INTRODUCTION

Life history traits have direct impact on both the fitness of the individuals involved and the growth and decline of the populations to which they belong (Stearns 1992, Marshall & Keough 2008). As such, life history traits, including reproductive strategies (e.g. sexual versus asexual reproduction, sexual dimorphism and investment in offspring), can also influence macroevolutionary outcomes, such as extinction probability (O'Dea et al. 2007, Martins et al. 2018). In solitary organisms, offspring size often trades off with fecundity, i.e. the number of offspring produced (e.g. Ramirez Llodra 2002, Marshall et al. 2018a) and

is modulated by the ecological challenges present in the environment and the constraints imposed by the biology of the organism in question (e.g. Strathmann & Strathmann 1982, Strathmann et al. 1984). Our understanding of the historical covariance of marine offspring and adult size is largely informed by work on solitary organisms, including mollusks (e.g. Jablonski & Lutz 1983) and echinoderms (e.g. Jeffery & Emlet 2003), where the size (and hence feeding capabilities) of larvae have evolutionary implications. However, colonial metazoans, which represent a major component of marine global biodiversity (Jackson 1977), often do not fit well into the ecological and evolutionary models developed for unitary organisms (e.g. Jackson 1985, Hiebert et al. 2020), hence the necessity of performing quantitative studies and/or testing model predictions directly on colonial organisms. The paucity of studies on offspring size of colonial invertebrates (e.g. cnidarians, bryozoans, ascidians, and sponges) over macroevolutionary timescales is partly due to the extremely low preservation potential of marine larvae in the fossil record (Raff et al. 2006), i.e. we have to rely on inferences made from extant organisms.

Here, we overcome this obstacle by using the size of the skeletal brooding structures (ovicells), a reliable proxy for offspring/larval size (Herrera & Jackson 1996), to investigate offspring/larval size evolution in the species-rich, cosmopolitan cheilostome bryozoan genus, *Microporella* Hincks, 1877. *Microporella* includes, to date, 149 described species (Bock 2020), 33 of which are exclusively found as fossils. Like all cheilostome bryozoans, *Microporella* is marine, with the great majority of species having encrusting colonies of indeterminate, extensive, sheet-like growth, and has a calcified skeleton where ovicells in mature colonies brood 1 larva at a time (see Fig. 1). Its larvae, like that for the great majority of cheilostomes, are non-feeding and thought to settle within hours to a couple of days at most (e.g. d'Hondt 1977). The morphology of its feeding modules (i.e. autozooids, henceforth simply 'zooids'), with the characteristic combination of porous frontal surface, semielliptical orifices, ascopore and often lateral adventitious avicularia (see Fig. 1), makes *Microporella* one of the most distinctive bryozoan genera. *Microporella* spans latitudes from the equator to 80° (Taylor & Mawatari 2005) in both the northern and southern hemispheres and can be found living in the intertidal zone (e.g. Dick et al. 2005) down to the deep waters of the continental slope (e.g. Figuerola et al. 2018). In *Microporella*, as in many cheilostomes, large zooid size has shown to be advantageous in competition for living space (Liow et al. 2017, 2019), and zooid size evolution seems to conform to Cope's rule, normally applicable to the body size of solitary organisms (e.g. Heim et al. 2015), where descendant species frequently have larger zooid size than ancestral species (Liow & Taylor 2019). Likewise, a study based on a few species within several different genera (including *Microporella*) suggests that zooid size in cheilostomes may conform to Bergmann's rule, with contemporary species from higher latitudes showing larger zooid size than congeneric species from lower latitudes (Kukliński & Taylor 2008). However, a relationship between zooid size and ambient water temperature at the time the zooids are built (i.e. Atkinson's rule), with larger zooids being produced in colder waters and smaller zooids being produced in warmer waters, empirically valid for a few different cheilostome bryozoan species (e.g. O'Dea & Okamura 1999, 2000), has not been tested on *Microporella*.

Because ovicells are polymorphs thought to be structurally related to zooids, their size is hypothesized to be genetically constrained by zooid size in the same colony (Jackson & Herrera Cubilla 2000). How much of ovicell and hence larval size is controlled by the average intra-colony zooid size; how much of it is influenced by external factors (e.g. physical conditions of the habitat); or what its relationship with fecundity is (usually a trade-off in solitary organisms), are, however, currently unknown. Short-term experiments on cheilostome larval size (e.g. Marshall & Keough 2004, Marshall & Monro 2013) have focused on the drivers of selection for size and the effect of size on post-metamorphic performance, using invasive species commonly fouling piers (i.e. *Bugula neritina*, *Watersipora subtorquata*, *Celleporaria* sp.). For instance, an experimental reduction in maternal colony size resulted in smaller larvae (Marshall & Keough 2004), while an increase in the number of biotic interactions is associated with larger ones (Allen et al. 2008, Marshall & Keough 2009). Larger larvae can survive a longer period before settling, hence likely have a higher dispersal potential (Marshall & Keough 2003). They also tend to develop into larger, more fecund colonies (Marshall & Keough 2007, Muniz Dias & Marshall 2010).

Previous investigations on cheilostome larval size variation were undertaken experimentally for single species (e.g. Marshall & Keough 2004, 2007, 2009, Allen et al. 2008, Muniz Dias & Marshall 2010, Marshall & Monro 2013, Di Martino & Liow 2021) or summarized from within entire communities (Jackson & Herrera Cubilla 2000), but little is known of what, if

Fig. 1. Scanning electron micrograph of a group of zooids of *Microporella hyadesi* (Jullien, 1888) (specimen ID: Natural History Museum London [NHMUK] 1990.10.10.25, contemporary, *Discovery* Expedition Stn WS 249, Falkland Islands) from our database. Outlined in black is the perimeter of a zooid (number 16) and outlined in white is the perimeter of an ovicell (number 4) from which the areas of these polymorphs were estimated. The numbers indicate the zooids (black) and the ovicells (white) haphazardly selected and measured in this work. White asterisk: a deformed ovicell (i.e. teratology). Obvious malformations were avoided from measurements (see Section 2.1)
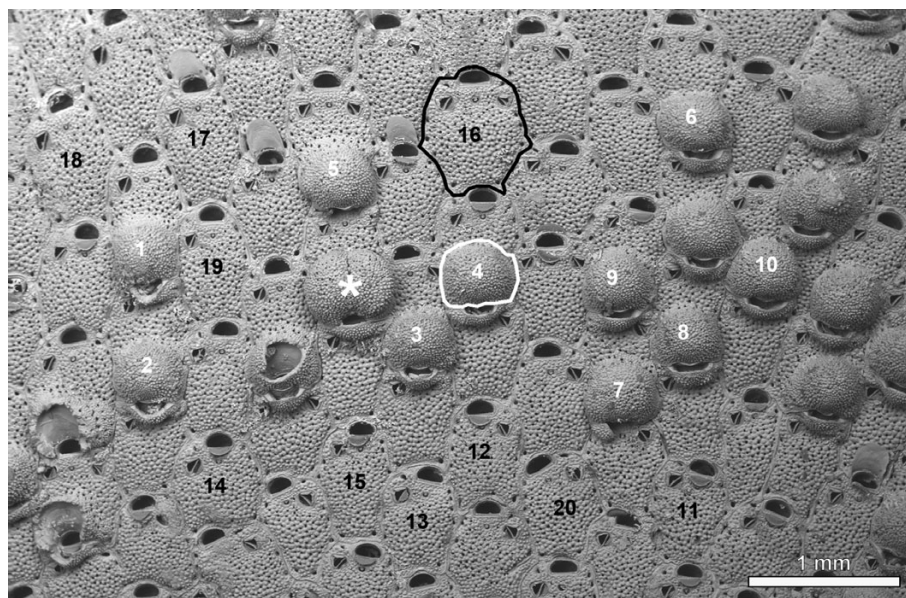
any constraints, might exist within lineages. Here we focus on a lineage, using multiple species of the same genus, *Microporella*, to ask the following questions: (1) Does zooid size explain most of the variation in larval size in contemporary species, or do trade-offs with density of ovicells (termed fecundity hereinafter for simplicity; see Section 2.1 for details and Section 4 for caveats) and/or environmental variation (temperature and/or nutrient levels) increase our ability to explain interspecific offspring/larval size variation? (2) We ask if the inferences we make for contemporary species hold for fossil species, using paleolatitude as a proxy for temperature. (3) Capitalizing on data from both fossil and contemporary species, we ask if both zooid and offspring/larval size increase over the millions of years of evolutionary history of this genus and if latitudinal shifts (a proxy for temperature changes), rather than Cope's rule, might explain evolutionary increases in size traits (i.e. an out-of-the-tropics scenario).

2. MATERIALS AND METHODS

2.1. Data collection

Our raw data consist of measurements of ovicell and zooid sizes (i.e. outline of areas as shown in Fig. 1) for all of the *Microporella* species (contemporary and fossil, described and undescribed) for which we could gather reliable measurements directly from scanning electron micrographs (SEMs). We use ovicell size as a proxy for offspring/larval size, following

Herrera & Jackson (1996). Those authors demonstrated using contemporary populations of 2 species of ascophoran-grade cheilostome bryozoans that the size of ovicells is positively correlated with the size of brooded larvae (Kendall's coefficient of rank correlation tau = 0.52−0.89, p = 0.0051−0.0004).

We aimed to measure as many distinct species as possible to capture interspecific variation. For this study, we measured 58 contemporary and 27 fossil species (of which 13 are awaiting formal description) from SEMs (Fig. 1) using ImageJ (https://imagej.nih.gov/ij/). We measured 10 ovicells and 10 autozooids from a single colony, from 1 or multiple images where possible. In a few instances, measurements were taken from 2 or 3 colonies, with the constraint that they were from the same sampling site collected at the same time. Ovicells and autozooids were measured only if they were well defined, undamaged, free from distortion due to the substrate or teratology, and astogenetically mature (the first generation of zooids budded directly from the metamorphosed larva [i.e. ancestrula] are usually much smaller than later zooids). Fecundity was estimated as the ratio of ovicells to the total number of zooids (with and without ovicells) on the SEM (Fig. 1). Note that the SEMs from which we measured/counted the traits were taken previously for other diverse purposes and can be considered random with respect to the traits measured. For our main analyses, we use species means of zooid and ovicell area as a proxy for size (analyses involving length and width are shown in Figs. S1 & S2 in Supplement 1 at www.int-res.com/articles/suppl/m662p001_suppl1.pdf). We assume that

measurement error is negligible for area, as the precision of our repeated measurements is high (see Liow et al. 2017, Di Martino & Liow 2021).

SEMs were supplemented with taxonomic identifications, geographical coordinates (latitude and longitude) and, for fossil species, geological age (see Datasets S1–S4 in Supplement 2 at www.int-res.com/articles/suppl/m662p001_suppl2.xlsx for specific information). The source of the geological age range in million years, if not explicitly expressed in the SEMs' metadata (see Supplement 2 for details of the original data sources), was taken from the International Chronostratigraphic Chart v. 2020/01 (https://stratigraphy.org/chart). The latitude of the fossil sampling sites was converted to paleolatitude using the Paleolatitude Calculator (www.paleolatitude.org/) with the default setting (van Hinsbergen et al. 2015).

Data for sea surface temperature (SST) and chlorophyll *a* (chl *a*, a proxy for ocean productivity or nutrient level for phytoplankton-feeders, like bryozoans) for the years 2002–2012 were downloaded from the NASA Earth Observations (NEO) website (MODIS-Aqua Data 2020a,b). These data serve as a compromise between data availability for a relative temperature proxy we require and the fact that the majority of the colonies in our database were collected prior to 2002 (hence we did not use available SST and chl *a* data from later years). We matched the location of the monthly measured, 10 yr average of SST and chl *a* with the 1 × 1° coordinates of the location where a given colony was found as an estimate of the (relative) environmental conditions it experienced.

Data on zooid size for 46 species from Liow & Taylor (2019) were merged with the new data, adding 8 contemporary and 19 fossil species data from this study. Note that only 3 zooids per colony per species were measured in the 2019 study. For those species added from Liow & Taylor (2019), we also measured ovicell size and fecundity where possible using the same SEMs or from different SEMs but part of the same specimen/colony. Fig. 2 shows the zooid and ovicell area variation of measured species singly and grouped by geological time and (paleo)latitude. We also show the variation averaged from 20 different colonies of *M. agonistes* spread over ca. 2 million yr measured in Liow et al. (2017) to suggest that much
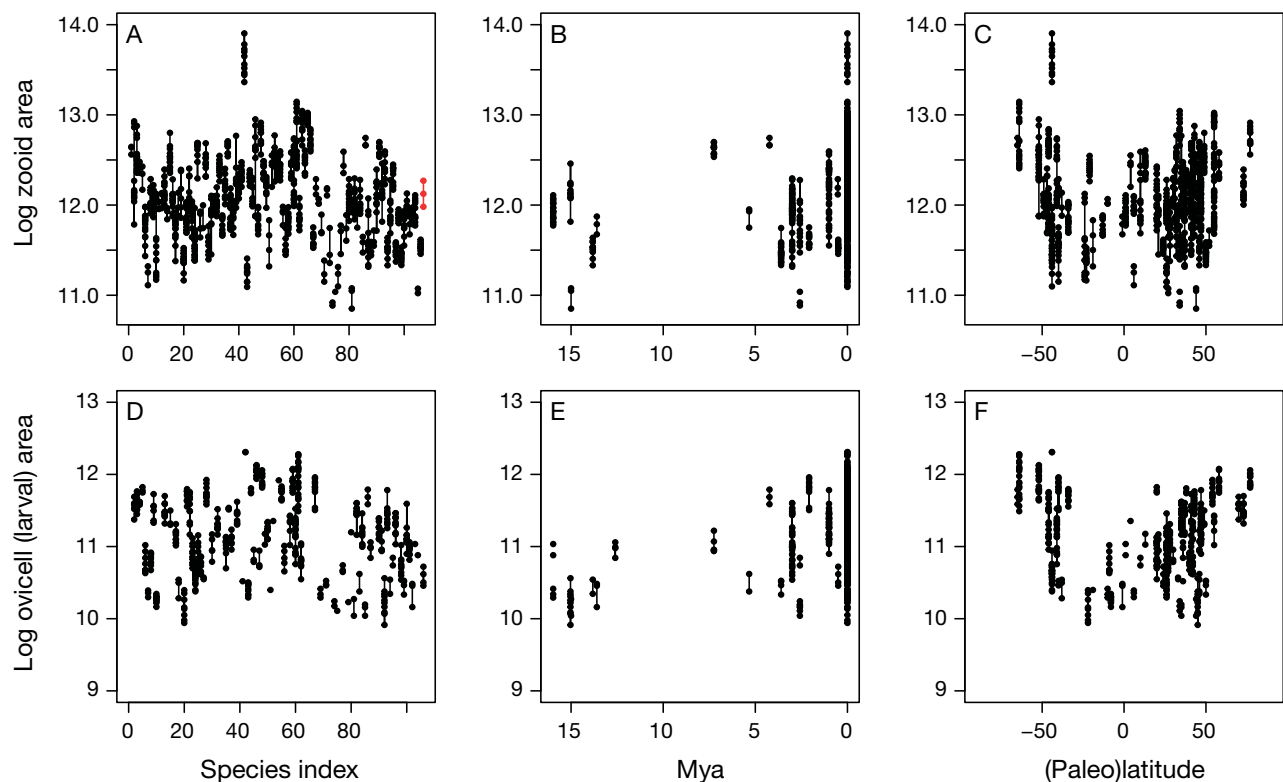

Fig. 2. Variation in zooid and larval size in *Microporella.* (A–C) The measured natural logged zooid areas (originally measured in µm²) for each species: (A) individual zooids measured in each species joined by vertical lines; red dots: mean and 95% CI distribution of 20 colonies of *M. agonistes* measured over 2 million yr from Liow et al. (2017); (B) species grouped by stratigraphic age (in millions of years ago, Mya); and (C) species grouped by (paleo)latitude in degrees. (D–F) The equivalent for natural logged ovicell (i.e. larval) areas: (D) the individual species; (E) grouped by age; and (F) grouped by (paleo)latitude

of the within-species variation is likely captured with only 1 to a few colonies per species.

2.2. Data analysis

As the natural log of the ovicell area across our contemporary species is normally distributed (Shapiro test, p = 0.39), we used linear models for studying this trait. Our full model is

$$O = b_0 + b_z\, Z + b_{fec}\; fec + b_{SST}\; SST + b_{chl}\; chl \quad (1)$$

where O is mean natural log ovicell area, Z is mean natural log autozooid area, fec is the mean ratio of ovicells to the total number of zooids for species, while SST and chl are the 10-yr averages of SST and chl a (see Section 2.1) where the specimens measured were collected. b_0 is the intercept for the model and b_{fec}, b_{SST} and b_{chl} are the slope for their respective terms. We compare 15 models (see Table 1) where we dropped or additively combined the 4 explanatory variables from Eq. (1). We are interested in which of these 15 models with their specific combination of explanatory variables will give the highest Akaike information criterion (AIC) weight (Burnham & Anderson 2002) but are also interested in how much (additional) information each explanatory variable will give. The latter will be done by examining the standardized slopes and comparing the adjusted R^2 values of the best model. We will also apply this general approach while using (paleo)latitude data and fossil species (see next paragraph). We also note that the variance inflation factors (VIFs) for all our analyses (see Code S1 in Supplement 1) are <2 and hence assume that there are no issues of multi-collinearity among our included parameters (Zuur et al. 2010).

We do not have geographic location-specific estimates for SST for fossil colonies, hence we explore the suitability of latitude as a substitute for SST. Note that this serves as a rough approximation, as many additional factors (e.g. ocean currents, upwelling, stream confluence, turbidity) can influence the relationship between SST and latitude. We find that for the locations where our contemporary taxa are found, absolute latitude explains SST quite well ($R^2 = 0.76$, see Fig. S3 in Supplement 1), where a 0.43°C increase corresponds to 1° latitude move towards the equator in our model. Natural log ovicell area across our fossil species is also normally distributed (Shapiro test, p = 0.20), hence we compare 7 linear models, where we used the potential explanatory variables log zooid area, fecundity, and absolute latitude (as a

substitute for SST) in different additive combinations for the fossil colonies (see Table 2). We also analyzed fossil and contemporary species together (see Table 3), and separately, to quantify any differences in log zooid area, fecundity and latitude as explanatory variables for ovicell size given the 2 different classes of data (i.e. fossil and contemporary).

In addition to genetic constraints and environmental controls, evolutionary time may also point to how larval size may have evolved. As can be seen from Fig. 2, both larval and zooid size seem to have increased through geological time. We ask if the species within *Microporella* may follow Cope's rule, where descendant species have a higher chance of having larger zooids (for both zooids and ovicells) than their putative ancestors, following the 2 sampling procedures laid out in Liow & Taylor (2019), in the absence of an independent phylogenetic hypothesis of the relationships among *Microporella* species. In the first procedure, any stratigraphically older species can give rise to any and multiple younger species, and the binomial probability of having a larger-sized descendant is calculated. In the second, a stratigraphically older species can give rise only to a single randomly selected descendant species. However, because there are many potential descendants to be picked for any older species, we repeat the analysis 1000 times to supply a distribution of the binomial probability of having a larger-sized descendant and compare this distribution to one where the size differences of pairs of species, regardless of their chronological age, are calculated (for more details, see Liow & Taylor 2019). To ask if size differences are further explained by latitudinal shifts where there might be an over-representation of younger species that move north or southward from the equator (i.e. 'out-of-the-tropics' hypothesis) (Hunt & Roy 2006, Jablonski et al. 2006), we also model zooid and ovicell size differences among putative ancestor–descendant pairs given the changes in geological time and latitudinal shifts. As the descendants must be randomly selected for each older species, we sample, without replacement, 1000 times from our data to create data subsets. These are then subject to 4 binomial models of size differences where a descendant is either larger (1) or not (0), with latitudinal shifts and time differences in different additive and multiplicative combinations (see Table 4). For the best model, we show the distribution (from the 1000 iterations) of the effect size and the distribution of the p-value for the variable in order to examine if the variable has any explanatory power.

All analyses were run in R v.3.6.1 (R Core Team 2019). Our new data, the data from Liow & Taylor

(2019) supplemented with new measurements, and the *M. agonistes* data from Liow et al. (2017) used in Fig. 2 are available in Supplement 2, and the R code for running all the above described analyses (and reproducing our plots and results) is available as Code S1 in Supplement 1.

3. RESULTS

3.1. Ovicell/larval size in contemporary species

Using the data subset of all the contemporary species for which we were able to measure ovicell area (N = 30, each represented by at least 3 zooids, but with a median of 13 and maximum of 26), the best AIC-ranked linear model includes size and SST (Table 1). Here, an increase in 0.79 natural log units of zooid size gives a corresponding increase in 1 natural log unit of ovicell size, as does a decrease in SST by −0.02°C (Table 1, Fig. 3). Upon rescaling the variables, so they are mean-centered and scaled by 2 SD, the slope for zooid size is 0.84 (SE = 0.14) and SST is −0.02 (SE = 0.1) for the best model, suggesting that zooid size is more important than SST. The higher AIC-ranked models all include autozooid size; SST,

where included, always contributes significantly; and all models that include autozooid size have adjusted R^2 values that are at least 0.6. On the other hand, models that include fecundity and/or chl *a* are much less preferable based on AICc (Table 1). By including 14 more species with all the variables in the best model measured (N = 44, Model i), the estimates and the adjusted R^2 value are very close to that estimated using N = 30 (Model 1). Substituting SST with latitude also yields similar estimates (Table 1). In summary, zooid size explains most of the variation in larval size in contemporary species as indicated by its slope estimates, but trade-off with the density of ovicells have shallow slopes and this variable does not even appear in the top AIC-ranked model. However, SST (but not chl *a*) increases our ability to explain interspecific ovicell variation, improving the adjusted R^2 somewhat (Table 1, compare Models 1 and 3).

3.2. Ovicell/larval size and zooid size in fossil species and all data combined

Using the data subset with only fossil species and all covariates available (N = 27), and absolute latitude as

Table 1. Models and estimates for ovicell (larval) area for contemporary species. Fifteen different linear additive models (N = 30) combining log area of zooids (*Z*) and ovicells (*O*), sea surface temperature (SST) (or (paleo)latitude, in the last row), fecundity (fec) and chlorophyll *a* (chl) are compared, using corrected Akaike information criterion (AICc), and their degrees of freedom (df), model weights and adjusted R^2 are shown, together with the estimated size effect in the corresponding columns. Model i shows estimates for Model 1 when more data are used (N = 44) and Model ii where absolute (paleo)latitude (lat) is substituted for SST. **Bold**: estimates that are significant at p < 0.05. Models i and ii are not compared with the models above (since the data used are different) and hence the df, AICc and weight columns are labeled NA (not applicable)

| Model no.	Model	Intercept	*Z*	fec	SST[a]	chl	df	AICc	Weight	Adj. R^2
1	~*Z* + SST	1.172	**0.845**		**−0.016**		4	21.135	0.433	0.687
2	~*Z* + fec + SST	2.172	**0.771**	−0.219	**−0.018**		5	22.888	0.180	0.687
3	~*Z*	−0.880	**0.989**				3	23.348	0.143	0.645
4	*O*~*Z* + SST + chl	1.165	**0.845**		**−0.016**	0.003	5	24.033	0.102	0.675
5	~*Z* + chl	−0.849	**0.983**			0.050	4	25.720	0.044	0.635
6	~*Z* + fec	−0.450	**0.957**	−0.117			4	25.740	0.043	0.635
7	~*Z* + fec + SST + chl	2.221	**0.765**	−0.244	**−0.017**	0.034	6	25.888	0.040	0.676
8	~*Z* + fec + chl	−0.176	**0.931**	−0.178		0.075	5	28.025	0.014	0.629
9	~fec + SST	**12.062**		**−0.695**	**−0.035**		4	39.018	0.000	0.432
10	~fec + SST + chl	**11.989**		**−0.746**	**−0.034**	0.082	5	41.456	0.000	0.419
11	~SST	**11.848**			**−0.036**		3	43.357	0.000	0.308
12	~SST + chl	**11.869**			**−0.037**	−0.020	4	46.011	0.000	0.282
13	~fec	**11.433**		**−0.729**			3	50.432	0.000	0.123
14	~fec + chl	**11.318**		**−0.855**		0.209	4	51.022	0.000	0.152
15	~chl	**11.117**				0.103	3	54.982	0.000	0.020
i	~*Z* + SST (N = 44)	1.915	**0.793**		**−0.020**		NA	NA	NA	0.668
ii	~*Z* + lat (N = 44)	1.152	**0.802**		**0.008**		NA	NA	NA	0.652

[a]In the last row, lat is substituted for SST
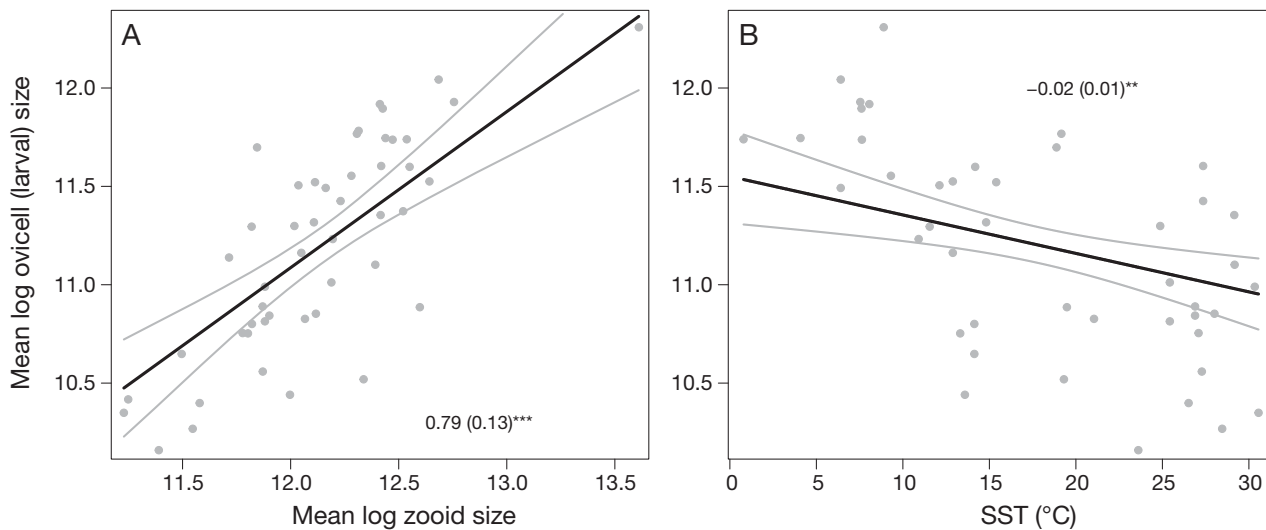
Fig. 3. Best model for ovicell (larval) size. Plots show species averages (grey dots) for ovicell and zooid sizes (areas, natural logged, originally measured in μm²) for contemporary species. Sea surface temperature (SST) values are the 10-yr means from the closest geographic coordinates (see Section 2.1). Black solid lines: model fits for each of the explanatory variables; grey lines: 95% CI (see Table 1). Numbers within each plot: effect size and standard error (in parentheses) for the dependent variable; **p < 0.01; ***p < 0.001

a proxy for SST, the best AIC-ranked linear multivariate model includes only natural log zooid size (Table 2), where an increase in 0.75 natural log units of zooid size gives a corresponding increase in 1 natural log unit of ovicell size. Upon rescaling the variables, so they are mean-centered and scaled by 2 SD, the slope for zooid size is 0.73 (SE = 0.12) and latitude is 0.20 (SE = 1.2) for the best model, suggesting that zooid size is more important than latitude. In the lower ranked models, fecundity and latitude had little to no explanatory power for ovicell size (Table 2). By including 7 more species with all the variables in the

best model measured (N = 34), the estimates and the R² are close to that estimated using N = 27 (Table 2). Combining fossil and contemporary species and fitting the same 7 models we applied to the fossil-only dataset, we find that the model with log zooid size and latitude still ranks best (Table 3), with fecundity supplying little additional information. By fitting the model separately to fossil, contemporary and all data, we see that the slope of the relationship between latitude and size is slightly steeper for the relationship between ovicell size and latitude (Fig. 4), but note that without including zooid area as an explanatory vari-

Table 2. Models and estimates for ovicell (larval) area for fossil species. Seven different linear additive models (N = 27) combining log zooid area (Z), absolute paleolatitude (lat) and fecundity (fec) are compared, using corrected Akaike information criterion (AICc), and their degrees of freedom (df), model weights and adjusted R² are shown, together with the estimated effect size in the corresponding columns. Model i shows estimates for Model 1 when more data are used (N = 34). As Model i is not compared with the models above, the df, AICc and weight columns are labeled NA (not applicable). **Bold**: estimates that are significant at p < 0.05

Model no.	Model	Intercept	Z	fec	lat	df	AICc	Weight	Adj. R²
1	~Z + lat	2.205	**0.706**		0.005	4	−4.917	0.404	0.630
2	~Z	1.736	**0.763**			4	−6.328	0.395	0.606
3	~Z + fec + lat	2.513	**0.682**	−0.130	0.006	5	−4.779	0.102	0.618
4	~Z + fec	1.732	**0.763**	0.002		5	−6.328	0.099	0.589
5	~fec + lat	**10.538**		−0.642	**0.013**	4	−15.184	0.000	0.209
6	~lat	**10.390**			**0.011**	3	−16.908	0.000	0.137
7	~fec	**10.949**		−0.468		3	−18.666	0.000	0.017
i	~Z + lat (N = 34)	2.488	**0.688**		0.006	NA	NA	NA	0.494

Table 3. Models and estimates for ovicell (larval) area for fossil and contemporary species combined. Seven different linear additive models (N = 63) combining log zooid area (Z), absolute (paleo)latitude (lat) and fecundity (fec) are compared, using corrected Akaike information criterion (AICc), and their degrees of freedom (df), model weights and adjusted R^2 are shown, together with the estimated effect size in the corresponding columns. Model i shows estimates for Model 1 when more data are used (N = 78). As Model i is not compared with the models above, the df, AICc and weight columns are labeled NA (not applicable). **Bold**: estimates that are significant at p < 0.05

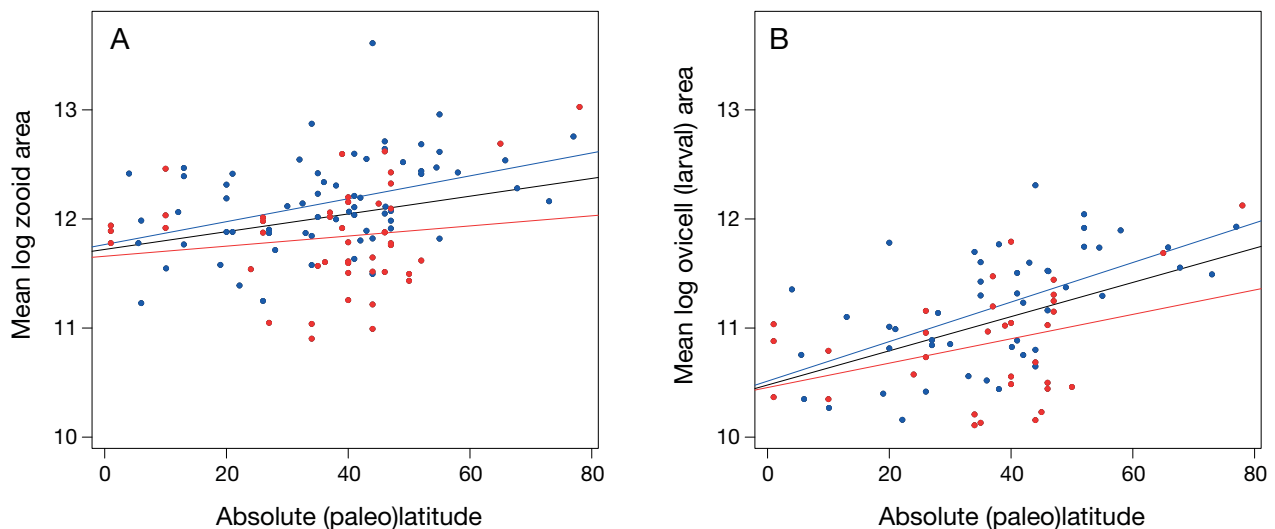| Model no.	Model	Intercept	Z	fec	lat	df	AICc	Weight	Adj. R^2
1	~Z + lat	0.825	**0.828**		**0.006**	4	39.401	0.603	0.680
2	~Z + fec + lat	1.166	**0.802**	−0.144	**0.007**	5	40.833	0.295	0.679
3	~Z	0.144	**0.904**			3	43.550	0.076	0.651
4	~Z + fec	0.286	**0.894**	−0.070	NA	4	45.631	0.027	0.646
5	~fec + lat	**10.638**	NA	**−0.564**	**0.016**	4	92.188	0.000	0.260
6	~lat	**10.482**	NA	NA	**0.015**	3	96.322	0.000	0.193
7	~fec	**11.188**	NA	−0.494	NA	3	107.064	0.000	0.044
i	~Z + lat (N = 78)	**1.265**	**0.792**		**0.007**	3	NA	NA	0.621

Fig. 4. Relationship between size and absolute (paleo)latitude. (A) Data and fit for a simple univariate linear model for zooid area and absolute (paleo)latitude; (B) the same but for ovicell area (both originally measured in µm^2). Red points: data from the fossil record (regardless of age); blue points: from contemporary material. Black lines: fits for all the data; blue lines: for contemporary; red lines: for fossil only. Zooid area is positively related to latitude for contemporary species (slope = 0.01, SE = 0.003, p = 0.0008) but not for fossil ones (0.004, 0.005, 0.3). Ovicell area is positively related to latitude for both contemporary species (0.02, 0.0034, 1.62 × 10^{-5}) and fossil ones (0.01, 0.005, 0.0325)

able, the amount of variation explained is low (see Table 3). In summary, the qualitative inferences we make for contemporary species hold for fossil species using paleolatitude as a proxy for temperature.

3.3. Ovicell/larval size and zooid size through time (Cope's rule and 'out-of-the-tropics' hypothesis)

The binomial probability of a descendant having larger zooids is 0.67 (95% CI 0.66–0.69, putative ancestral–descendant pairs: N = 3714), while that for ovicells is 0.66 (95% CI 0.64–0.68, putative ancestral–descendant pairs: N = 2164), using our first approach for tackling unknown ancestral–descendant relationships (Fig. 5A,B). Likewise, in the second approach, the binomial probability of the descendant having both larger zooids and ovicells is clearly larger than randomized pairs of species (Fig. 5C,D). Fitting 4 models (Table 4) with randomly selected ancestral–descendant pairs, we find that the model best describing size differences is one that involves only latitudinal shifts, i.e. the amount of time that has passed does not matter. The distribution of the effect of latitudinal
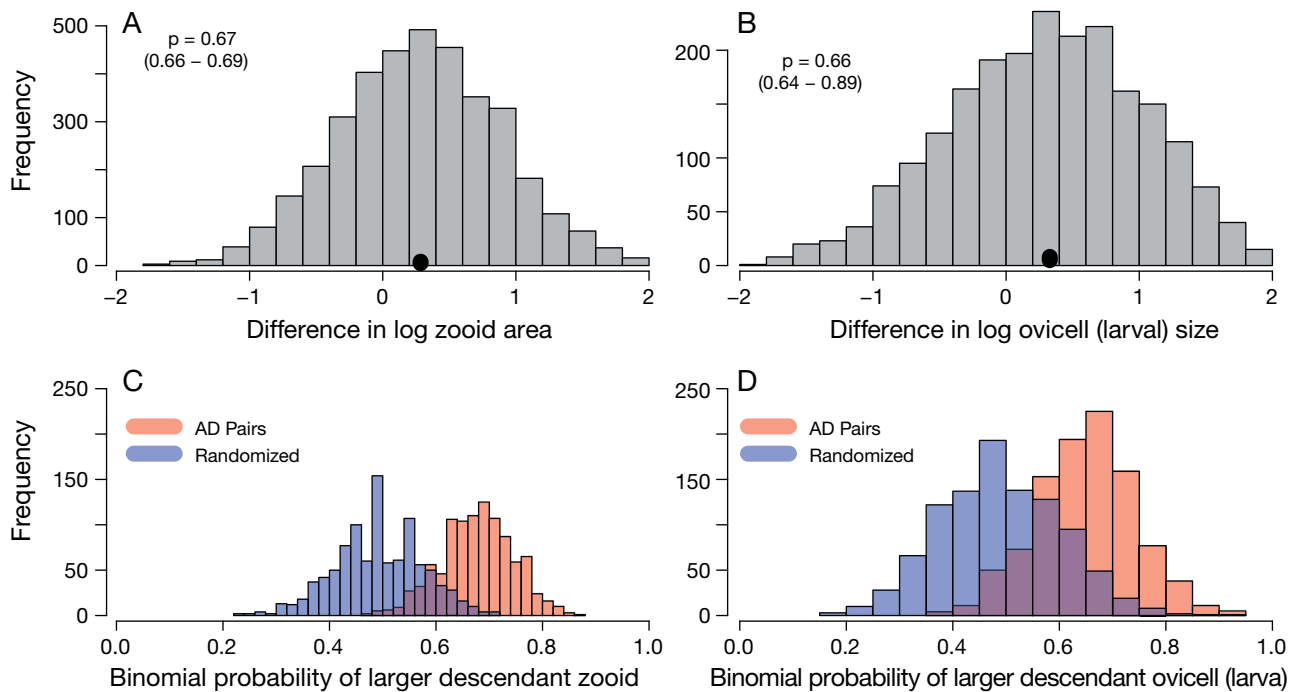
Fig. 5. Cope's rule for zooid and ovicell area in *Microporella*. (A,B) Distribution of the differences between all plausible ancestor−descendant pairs for (A) log zooid size and (B) log ovicell size. The binomial probability of descendants being larger is shown on the left of each plot, with 95% CI in parentheses. Black solid circles indicate the mean of the distributions, both of which are greater than 0. (C,D) Distribution of binomial probabilities of descendant being larger in ancestor−descendant pairs (AD pairs) which are repeatedly drawn (red), compared with species pairs repeatedly drawn, but disregarding temporal order (blue), for (C) zooid and (D) ovicell

Table 4. Models for latitudinal shifts in ancestor−descendant pairs. Four models were run 100 times (since ancestor−descendant pairs must be selected), using the magnitude of latitudinal differences (shift) and the time that has passed (time.diff). No. times: how many times of the 1000 runs the model was ranked top; model weight: the median model weight when that model is selected as top

Model no.	Models	No. times	Model weight
1	~shift	594	0.473
2	~shift+time.diff	18	0.437
3	~time.diff	357	0.443
4	~shift×time.diff	76	0.517

shifts is close to zero (Fig. 6A) in repeated runs of the best model (in Table 4), and the distribution of its p-value clearly much larger than 0.05 (Fig. 6B), indicating that latitudinal shifts do not explain much beyond what is already described by categorical, putative ancestral−descendant pairs (i.e. the dependent variable in these models). In summary, Cope's rule seems sufficient for explaining the changes in ancestor−descendent size difference, i.e. there is no need to invoke an 'out-of-the-tropics' scenario.

4. DISCUSSION

Little is currently known about how offspring size varies within lineages and over macroevolutionary timescales, much less what constrains or drives the evolution of larval size in marine invertebrates (e.g. Marshall et al. 2018a). Our study system offers unique insights into the evolution of offspring size in deep time, as it is possible to measure offspring size in multiple contemporary and fossil congeneric species within a well-defined evolutionary lineage.

As already hinted upon in Jackson & Herrera Cubilla (2000) and quantified here for *Microporella*, it is clear that ovicell, and hence larval size, is constrained by zooid size, although the underlying mechanisms of this allometric relationship remain to be investigated. We surprisingly did not find a trade-off between offspring size and number. This could be because our data are interspecific rather than intraspecific, suggesting that the differences in offspring size and number among species might be regulated by different factors than energy balance (e.g. Marshall et al. 2018b). The patterns we found using SST and latitude give support to previous less extensive studies on zooid size variation, where larger zooids are thought
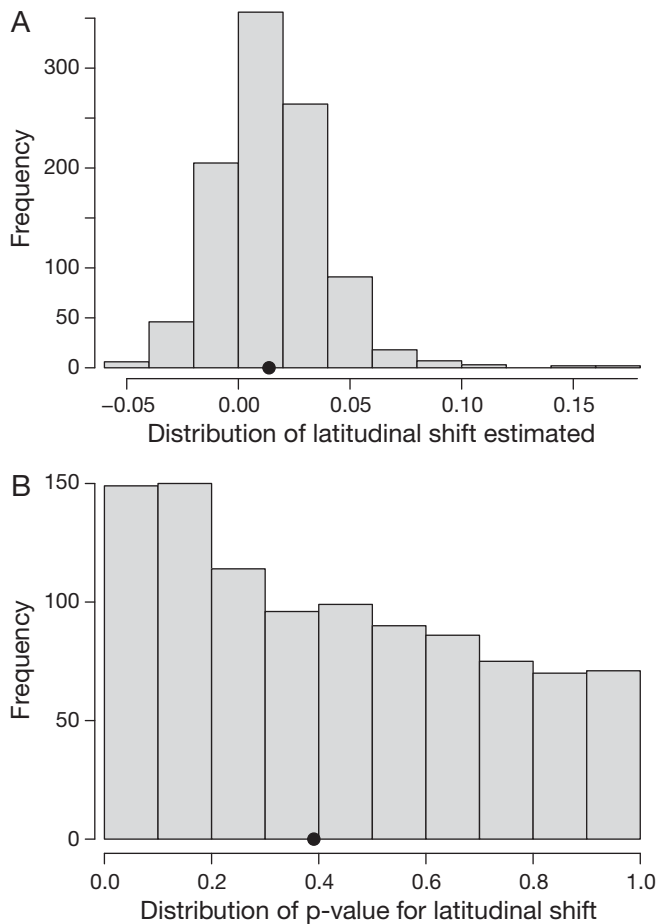
Fig. 6. Testing the out-of-the-tropics hypothesis. (A) Distribution of the estimated effect size of latitudinal shift in repeatedly sampled putative ancestor–descendant pairs from the first (~shift) model in Table 4. (B) Distribution of the p-value of these effect sizes. Black dots in each panel are means

to be associated with higher latitudes (Kukliński & Taylor 2008) and colder waters (e.g. Ryland 1963, Menon 1972, Okamura & Bishop 1988, Hunter & Hughes 1994, O'Dea & Okamura 1999). Our results also confirm body size-codified generalizations, i.e. Bergmann's rule (e.g. Meiri & Dayan 2003) — the tendency for organisms living in higher latitudes to be larger than their counterparts in lower latitudes — and Atkinson's temperature–size rule (Atkinson 1994) — the favoring of larger body sizes in cooler environments — for ovicell/larval size in addition to zooid size for a colonial organism whose colony size is indeterminate. That said, it is likely that the mechanisms for larger size in colder waters in such colonial organisms are likely different from those of solitary organisms, often couched as a consequence of the product of growth rate and development rate (e.g. Forster & Hirst 2012). Bryozoan temperature–size re-

sponse may be explained with oxygen limitation, where lower temperatures can hold higher concentrations of oxygen, all things being equal (e.g. O'Dea & Okamura 1999, O'Dea 2005).

In a handful of cheilostomes (*Flustra*, *Conopeum*, *Celleporella*), zooid size is found to be strongly influenced by ambient water temperature but independent of food levels (e.g. Hunter & Hughes 1994, O'Dea & Okamura 1999, 2000, O'Dea 2005) as we also show here, with chl *a* level as a proxy for nutrient levels. In *Electra pilosa*, there is an optimal food concentration for zooid size (Hageman et al. 2009). In contrast, Jackson & Herrera Cubilla (2000), studying the differences in zooid and ovicell size variation at community level between the 2 sides of the Isthmus of Panama, suggest that larger zooids and ovicells are generated in oligotrophic but stable environments. In other words, size has a complex relationship with nutrient levels and among species, as in our study, the effect of varying nutrient levels may be masked by genetic constraints, which are more dominant than phenotypic responses to ecological effects.

Our results contradict those found in marine invertebrates with non-feeding larvae but mostly based on non-bryozoan taxa, in which larger offspring are associated with lower level of nutrients (Marshall et al. 2012). It remains possible that temperature effects swamp any nutrient signals in our data (see also Pettersen et al. 2019, Álvarez-Noriega et al. 2020, Marshall et al. 2020). In addition, we do not detect any differences in offspring size between southern and northern hemispheres (*t*-test for contemporary species, Northern hemisphere: N = 45, Southern: N = 21, $t = -0.14$, 95 % CI: -0.63 to 0.57, p = 0.82), as is apparent for other marine invertebrates (from the phyla Annelida, Echinodermata, Mollusca) with non-feeding larvae (Marshall et al. 2012).

Microporella module size, regardless of whether zooids or larvae (ovicells) are measured, conform to Cope's rule (e.g. Heim et al. 2015) — the increase of the body size of evolutionary lineages through geological time — as previously observed for zooids across different cheilostome bryozoan species (Liow & Taylor 2019). The probability of a descendant having larger ovicells or zooids than its putative ancestor is higher than a null hypothesis of no size difference, with this size difference little explained by a latitudinal shift happening over time, casting doubt on an out-of-the-tropics scenario when interpreted in the light of a latitudinal relationship of module size distribution (Figs. 2 & 4). It is somewhat paradoxical that there is a clear trend in zooid size increase which is simultaneously controlled by environmental condi-

tions (temperature, Fig. 2), yet whose effect is unclear over time (Fig. 4). The evolutionary push and pull between time (i.e. survival and speciation in time, given biotic interactions and genetic constraints) and physical conditions hence call for further study.

A number of caveats need to be considered. Our module size and fecundity dataset, although being the most comprehensive in relation to the number of *Microporella* species measured, includes two-thirds of the species known to date. The species missing are usually described in early publications before SEM imaging was available, rarely collected thereafter, and with their type specimens currently unavailable to us. However, we believe that the subset of species included in this study are randomly distributed with regards to our traits of interest, which are ovicell and zooid sizes. Measurements error may account for variation in size traits, but we are confident that the repeatability of our zooid measurements is highly accurate, as demonstrated earlier (see Liow et al. 2017, Di Martino & Liow 2021). We used the number of ovicells as an estimate for fecundity, as it is common practice in studies based on cheilostome bryozoans (see Yagunova & Ostrovsky 2010 and references therein); however, this is an apparent, instantaneous fecundity which can either be an over- or an underestimation of the (relative) life-time fecundity of the colony. Not all ovicells will produce successful larvae that are recruited to the next generation, and perhaps some ovicells can be re-used, although this has never been observed for *Microporella*. Lastly, pending an independent and robust *Microporella* phylogeny that includes both fossil and contemporary species (see Orr et al. 2018), the relationship between ancestral and descendant species could only be speculated using chronological age as information.

5. CONCLUSIONS

Offspring/larval size is likely genetically constrained by the characteristic size of non-reproductive modules but also a consequence of the external environment a species experiences. However, the imprint of evolution, where ancestral species tend to give rise to larger module-size descendants, cannot simply be explained by a tendency for younger species to have moved to higher latitudes. Offspring investment (in terms of offspring size) of these modular organisms does not seem to trade off with offspring number, but is instead a consequence of genetic constraints and environmental temperatures.

Our study illustrates how capitalizing on among species variation in both contemporary organisms and their fossil counterpart can give insights that are otherwise difficult to obtain, but also how using model systems at different scales (i.e. single species, communities, species within a lineage as we have done here) can reveal different aspects and the complexity of the variation.

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