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Assessing patterns of morphological and physiological trait variations across heterocytous cyanobacteria at cellular and population levels

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Abstract Heterocytous Cyanobacteria show high trait variation at the cellular, organismal, and population levels. Members of this group can produce specialized cells such as akinetes and heterocytes that influence their ecology, including bloom development and population survival. This study characterizes patterns of variation in the traits of these species, including the traits of specialized cells, to expand our ecological knowledge and predictive capacity for this group. We compiled and synthesized morphological and physiological traits of planktic heterocytous Cyanobacteria from the published literature and experiments, and assessed trait distributions, trait relationships, and their similarities among species. Although the volumes of akinetes and heterocytes were positively related to that of vegetative cells, the shape of cells differed in ways that may reflect their function, and the position of heterocytes within filaments may relate to growth rate. Maximum growth rates differed significantly among genera, yet surprisingly did not correlate with cell volume. Also, despite the high energetic cost of N fixation in low N conditions, our results suggest that growth rate seems unrelated to nitrogen availability. The degree of trait variation within heterocytous Cyanobacteria, which suggests the existence of three functionally distinct subgroups, may offer new insights into which taxa dominate bloom assemblages under different conditions.

Keywords Size and shape · Heterocyte · Akinete · Growth rate

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Introduction

Cyanobacteria blooms negatively impact aquatic ecosystems around the globe; many are caused by a monophyletic group of species comprising the Family

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Nostocaceae (heterocytous Cyanobacteria hereafter). This group has highly diverse cell and filament morphology, and most species have the ability to fix nitrogen (N_2) in a specialized cell called the heterocyte, a trait that provides competitive advantages in N-limited situations. All planktic members can also produce a dormant specialized cell, the akinete, considered a life history trait that allows population survival in the sediments under adverse conditions. The ability to produce such specialized cells through its life cycle is facultative, and its presence can markedly influence the survival and performance of the population (Hense & Beckmann, 2006).

Despite sharing the ability to fix nitrogen and produce dormant cells, heterocytous Cyanobacteria show high trait variability at the cellular (size, shape), organismal (relative position of specialized cells, filament morphology), and population levels (growth rate). Most studies using traits in phytoplankton ecology (Reynolds et al., 2002; Padisák et al., 2009; Kruk et al., 2010, 2017) and in phylogeny (Uyeda et al., 2016) focus on the presence of specialized cells and on organismal morphology. Although this approach is adequate to differentiate heterocytous Cyanobacteria from other phytoplankton (both taxonomically and ecologically), the inclusion of trait variation is also needed to differentiate among species of this group, as specialized cells integrate key resource acquisition and life history traits.

Trait variation may affect the heterocytous Cyanobacteria species' distributions along environmental gradients; indeed, Dolman et al. (2012) highlighted that this group should not be treated as a unit when considering the potential effects of changes in nutrient loading. Also, Reynolds et al. (2002) and Padisák et al. (2009) acknowledged three functional groups for heterocytous Cyanobacteria—SN, H1, and H2, mostly based on their differential tolerances and sensitivities to light and nutrient availability. Thus, predictions considering this group homogeneous might fail to anticipate the diversity of conditions where blooms can actually form.

The scenarios of global climate change forecast increased frequency, magnitude, and duration of cyanobacteria blooms (Paerl & Huisman, 2008); hence, there is a need to increase our ability to explain and predict which heterocytous Cyanobacteria traits or species are expected to occur along different light and nutrient gradients. Understanding the key traits responsible for bloom development in each species and identifying their environmental sensitivities might provide management actions for controlling freshwater systems (Mantzouki et al., 2016). In phytoplankton, morphological traits are good predictors of organism physiology and function (Kruk et al., 2010); often species that have similar morphologies share similar functions and exhibit similar responses to environmental variables, regardless of their phylogenetic relatedness (Reynolds et al., 2002).

In heterocytous Cyanobacteria, heterocytes and akinetes develop from vegetative cells, and hence, it could be expected that both specialized cells retain the size and shape of the vegetative cells that they are derived from. Indeed, our previous studies assessing morphological traits of the different cell types in roughly one-third of the heterocytous Cyanobacteria genera (*Dolichospermum*, *Chrysosporum,* and *Sphaerospermopsis*) showed a positive relationship between the size of vegetative and specialized cells (de Tezanos Pinto et al., 2016). Nevertheless, regardless of the shape of the vegetative cell, the heterocytes were mostly spherical, while akinetes were mostly prolate spheroid in shape or cylindrical. This suggests that the shapes of specialized cells differ consistently from those of vegetative cells, probably due to the different functions of each cell type. For the heterocyte, we proposed that the consistency of its shape (and size) reflects adaptations to hinder O_2 diffusion into the cell, which would inhibit the enzyme responsible for N-fixation. For the akinete, we proposed that the nonrandom distribution of shape could be attributed to different patterns of akinete formation and germination (de Tezanos Pinto et al., 2016). However, other heterocytous Cyanobacteria genera that were not considered in our first survey (e.g., *Cylindrospermopsis*, *Aphanizomenon*, *Cuspidothrix*. *Raphidiopsis*, and *Nodularia*) seem to have different cell morphologies (both vegetative and specialized). Hence, the emerging morphological patterns observed for a subset of the heterocytous Cyanobacteria need to be validated across the entire group. If previously identified patterns prove to be general, they could support hypotheses linking morphology to function in the different cell types.

Heterocytes and akinetes are located in species-specific positions in filaments of vegetative cells. Heterocyte position (intercalary or terminal) may have ecological implications; indeed Yema et al. (2016)

showed that species with different positions had different responses along nutrient gradients in terms of filament length and biomass. The differentiation of vegetative cells into heterocytes implies an energetic cost and results in loss of the cell´s asexual reproduction ability. Hence, heterocyte position in the filament may also affect growth rates as the number of heterocytes is of a maximum of two per filament in species with terminal heterocytes, but can reach many per filament in species with intercalary positions. Moreover, the akinete can develop either adjacent or distant to the heterocytes, and we believe this may affect the N content of the dormant cell, which could in turn affect germination and recruitment success. Assessing the distribution of the position of specialized cells in the filament could enable new inferences about possible physiological links between specialized cells, and which traits are favored in particular environments.

At the organismal level, filament morphology (straight or coiled) is not a trait used in taxonomical identification, as it can be altered to a certain degree in response to environment conditions (Komárek, 2013). It is unclear if the shape of the filament is driven by other morphological traits, as cell morphology. Nevertheless, the organism´s architecture has an important role in light acquisition (Kirk, 1994; Reynolds, 2006) and predator avoidance (Litchman & Klausmeier, 2008), and consequently may influence when and where species blooms occur.

Maximum growth rate is a physiological trait integrating reproduction and resource acquisition (Reynolds, 2006; Litchman & Klausmeier, 2008). Although higher growth rates have been found for smaller cells in phytoplankton (Reynolds, 2006 and cites there in; Kremer et al., 2017a), it is unclear if such tradeoff between growth rate and cell size also holds in heterocytous Cyanobacteria. It is also unknown if growth rate is conserved among taxonomically related species, and if so, at what level. Several pieces of evidence indicate that growth rates decline during N-fixation (Zevenboom et al., 1981; Kenesi et al., 2009), yet it remains unclear whether this is a species-specific tradeoff or it generalizes to the whole heterocytous Cyanobacteria group. Exploring this possible physiological constraint may help explain species distributions along nitrogen gradients.

The aim of this study was to characterize relevant trait variation in heterocytous Cyanobacteria at cellular and organismal scales, to increase our understanding and predictive capabilities for this important ecological group. For this, we exhaustively compiled and synthesized the following critical traits: six morphological and one physiological trait from the published literature and from our own experiments. We then assessed trait variation, trait relationships, and trait similarities among species. Finally, we make the assembled database available to the scientific community for future studies.

Materials and methods

Data collection

Morphological traits for heterocytous Cyanobacteria were extracted from the updated literature (Komárek, 2013). These traits included dimensions (length and width) and shape of the different cell types (vegetative cell, heterocyte, and akinete), the position of the heterocyte in the filament, the relative position of the akinete to the heterocyte, and filament morphology. For each cell type, the average length and width were calculated, assuming normal distribution of the traits, based on the argument of Kerkhoff & Enquist (2009) and tested in de Tezanos Pinto et al. (2016). The shape of each cell type was classified into three categories: cylindrical, prolate spheroid, and spherical, based on the description and figures in Komárek (2013). Cell volume based on measures of size for each cell type was computed, using the average length and width and shape data following Hillebrand et al. (1999). Maximum growth rate data were extracted from published laboratory experiments performed in conditions that favor the growth of most heterocytous Cyanobacteria, including: phosphorus (P) sufficiency, nitrogen (N) sufficiency (N-uptake situations) or deficiency (N-fixing situations with high P availability), temperatures within 20–25°C (except for *Cylindrospermosis* that generally requires higher growth temperatures of 25–27°C), neutral pH, no salinity, and growth-saturating light ($\geq$ 50 µmol photons m^{-2} s^{-1} but preferably about 100 µmol photons m^{-2} s^{-1}). In N-uptake situations, whenever several nitrogen sources were used, data from growth on NO_3^- was selected, as this chemical form is the one most commonly used in laboratory experiments. Information regarding the growth medium and photoperiod were recorded. To

avoid confounding factors because of the different photoperiods reported in the literature, maximum growth rates were standardized to a photoperiod of 12-h light:12-h darkness, whenever necessary, as done by Bruggeman (2011). The method used to measure growth rates (optical density, chlorophyll, or cellular density) was also recorded. In addition, the type of culture (batch, continuous, semi-continuous) was annotated to consider mortality rates when necessary. In the cases where the results were expressed as doublings (G), the specific growth rates (μ) were calculated from $G = \ln 2/\mu$ (Fogg & Thake, 1987; Reynolds, 2006), where 1 doubling yields $\mu = 0.69\ d^{-1}$.

Growth rates were also obtained from laboratory experiments (de Tezanos Pinto) for *Dolichospermum flos-aquae* (Brébisson ex Bornet et Flahault) Wacklin et al. 2009, and *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju 1972, under scenarios of N-fixation and N-uptake. Monocultures were grown in a semi-continuous regime (daily dilutions of 0.2 d^{-1}) for 28 days at constant photoperiod (14-h light:10-h dark), irradiance of 100 µmol photons m^{-2} s^{-1} and temperature of 25°C. For obtaining the different N scenarios two contrasting N concentrations were used (N = 0 and N = 1000 µM) at phosphorus sufficiency (P = 20 µM). For each species and treatment, three replicates were used. Samples were taken weekly and filament density was estimated using a compound light microscope with a Palmer cell counting chamber. The maximum growth rate was calculated by fitting a linear regression to the linear portion of the natural logarithm (filament density) to time relationship, including at least 3–4 points. The dilution rate was subtracted from the slope to obtain the maximum growth rate. Then the value obtained was standardized to a photoperiod of 12-h light:12-h darkness, as described above.

Data analysis

Trait distributions

Morphological traits To determine whether cells of a particular type—vegetative, heterocyte, and akinete—tend to have similar volumes a one-way ANOVA test was run (across all heterocytous Cyanobacteria species). When significant differences were found in this or all subsequent ANOVAs, post hoc Tukey HSD tests were performed. To test whether cell volumes differ among genera, generalized least squares regressions (GLS, 'varIdent' function in the nlme package in [R], Pinheiro et al., 2018) were run for each different cell type (vegetative, heterocyte, and akinetes). This approach accounts for heteroscedasticity and deviations from normality.

Potential relationships between the volumes of vegetative cells and heterocytes or akinetes were examined. Specifically, we tested to determine which of the four functional forms (linear, logarithm, power, or exponential) best captured these relationships, based on comparing their significance (favoring lower P-values) and coefficients of determination (R^2).

To determine whether cell types tend to exhibit different shapes (across all species of heterocytous Cyanobacteria species) a Chi-square test of independence was performed. We then consider whether the shapes and position of specialized cells differ among genera within cell types, using descriptive statistics. To explore if the association between cell shapes and filament morphology was random, a Chi-square test of independence was performed.

Maximum growth rates To test for possible differences in maximum growth rates, under N-uptake conditions and across genera, a univariate general linear model (GLM) was used: ANOVA with type III sums of squares for unbalanced models. Subsequently, to examine how N availability (N absence or N sufficiency) and the position of heterocytes within filaments (intercalary or terminal) might jointly affect maximum growth rate, a 2-way ANOVA was run. Growth rate data used for this analysis included only strains that were exposed to both N absent and N sufficient conditions. The genus *Anabaenopsis* was excluded from this analysis, as members of this genus can present heterocytes both at an intercalary or terminal position in the filament, throughout their life cycle. Finally, the relationship between maximum growth rate and vegetative cell volume was also examined. The function that best fit the data (linear, logarithm, power, or exponential) was selected based on comparing their significance (favoring lower P-values) and the coefficient of determination (R^2).

In all cases, when normality and homoscedasticity assumptions were not met, the volume data were logtransformed. Analyses were conducted using R

3.3.3 (R Development Core Team 2017) and SPSS 20 Software.

Taxonomic signals of trait variation

Sufficient data on the maximum growth rates of multiple strains of each species were available in the literature to support an analysis of how variation in growth rate is partitioned among species within genera versus across genera. To conduct this analysis, a standard mixed effects model was run using the lmer() function in R's lme4 package (Bates et al., 2015). This model contained random effects for genus and species (nested within genus). The significance of the random effects was estimated using parametric bootstrapping, via the PBmodcomp function in the pbkrtest package (Halekoh & Højsgaard, 2014). Unfortunately, similar analyses were not possible for the cell volume data, due to lack of multiple estimates of cell sizes within individual species. Also, higher-level taxonomic groupings were ignored, as all genera in this study fall within the family Nostocaceae.

Trait similarities among species

Neighbor-Joining clustering was used to assess the similarity of the cell volumes of the vegetative cell, heterocyte, and akinete (quantitative variable) across the diversity of heterocytous Cyanobacteria. The volume was chosen as this trait integrates length, width, and shape. Species were then categorized by the morphology of their filaments (straight, coiled, or both, qualitative variable). The analysis was performed using PAST 3.1 software (Huang et al., 2013).

Results

Morphological traits

Table 1 summarizes the morphological traits collected and analyzed for each of the 86 planktic morphospecies of heterocytous Cyanobacteria across 10 genera, and the complete database is presented in Table S1.

Cell volume

Cell volume ($n = 86$ for each cell type) differed significantly across cell types (ANOVA $P < 0.001$, df = 2, $F = 88.48$) (ranges can be observed in Table 1). The vegetative cell volume (mean: 156.7 ± 152.1 μm^3) was similar to the heterocyte volume (mean: 226.2 ± 228.7 μm^3) (Tukey $P = 0.107$). Vegetative cells, however, were nearly an order of magnitude smaller than the akinetes (mean 1428.5 ± 1369.2 μm^3) (Tukey $P < 0.001$).

Vegetative cell volume significantly differed among genera: it was the smallest in *Cylindrospermopsis,* intermediate in *Nodularia* and *Aphanizomenon,* and the largest in *Dolichospermum* (Fig. 1a). Likewise, heterocyte volume significantly differed among genera: it was small in *Cylindrospermopsis,* and similarly large in *Dolichospermum, Chrysosporum*, *Sphaerospermopsis*, *Anabaenopsis*, and *Aphanizomenon* (Fig. 1b). Finally, akinete volume also significantly differed among genera; the smallest akinetes occurred in *Raphidiopsis* and *Cylindrospermopsis,* and the largest in *Dolichospermum* and *Chrysosporum* (Fig. 1c).

We found a significant positive relationship between the volume of vegetative cells and heterocytes (Fig. 2a). The best curve fit was a power relationship (log–log) ($P < 0.0001$; log heterocyte volume = $0.47 + 0.95 \times$ log vegetative volume; $R^2 = 0.758$). For particular genera, when considering the ratio of vegetative cell volume:heterocyte volume (Table S1), this pattern holds but with some variations. In some genera the volume of heterocyte in proportion to vegetative cell is larger than the media (*Chrysosporum, Sphaerospermopsis, Aphanizomenon*), and *Anabaena* constitute an exception as most species of this genera have vegetative cells larger than their heterocytes. We also found a significant positive relationship between the volumes of vegetative cells and akinetes (Fig. 2b). Again, the best curve fit was a power relationship ($P < 0.0001$; log akinete volume = $1.62 + 1.08 \times$ log vegetative volume; $R^2 = 0.62$). When observing the ratio of vegetative cell volume: akinete volume for particular genera (Table S1), the same pattern of positive relationship is recognized, although the enlargement of the akinete could be slightly reduced in some genera (e.g., *Raphidiopsis* and *Anabaenopsis*). Besides, akinetes

Table 1 List of traits of heterocytous Cyanobacteria compiled and analyzed

Trait	Type of trait	Range and categories	N
Morphological traits			
Length[a,b] (μm)			
Vegetative cell	Continuos	3–22	78
Heterocyte	Continuos	3.5–14	65
Akinete	Continuos	6–61.4	84
Width[a] (μm)			
Vegetative cell	Continuos	1.55–13	86
Heterocyte	Continuos	2–13.3	80
Akinete	Continuos	2.5–21.5	86
Volume[c] (μm^3)			
Vegetative cell	Continuos	14.7–833	86
Heterocyte	Continuos	10.9–1231.2	80
Akinete	Continuos	45.4–5384.3	86
Shape (vegetative cell, heterocyte, and akinete)	Categorical	Cylinder, prolate spheroid or sphere	86 (81 for heterocyte)
Filament morphology	Categorical	Straight or coiled	86
Akinete position relative to heterocyte	Categorical	Adjacent or distant	79
Heterocyte position	Categorical	Intercalary, primary terminal, secondary terminal, and absent	86
Physiological traits			
Maximum growth rate	Continuos	0.100–1.410 (12-h light, N-uptake)	74
		0.312–0.890 (12-h light, N-fixing)	17

N represents the number of species with data available

[a]Mean values of minimum and maximum data obtained from the literature

[b]When cells were spherical, only width was considered

[c]Volume was calculated using the mean length and width, and shape obtained from the literature

of *Chrysosporum* are larger in proportion to vegetative cell than the rest of genera.

Cell shape

The cell shape distributions were associated to the cell types ($\chi^2 = 70.80$, df = 4, $P < 0.0001$). Vegetative cells were mostly cylindrical ($\sim +2.5$ Pearson residuals) and to a lesser extent were spherical ($\sim +0.5$ Pearson residuals), but rarely prolate spheroid shaped (~ -2.5 Pearson residuals) (Fig. 3). Heterocytes were predominantly spherical ($\sim +4.5$ Pearson residuals, 58 out of 86 species), including most species in *Chrysosporum*, *Sphaerospermopsis*, *Anabaenopsis,* and *Dolichospermum* (Fig. 3). Least frequently, heterocytes were cylindrical (~ -3 Pearson residuals); in *Cylindrospermopsis*, *Nodularia, Cuspidothrix*, and *Aphanizomenon* heterocytes were mostly cylindrical and to a lesser extent prolate spheroid (Fig. 3). Akinetes were mostly prolate spheroid ($\sim +3$ Pearson residuals or cylindrical ($\sim +0.5$ Pearson residuals), but rarely were spherical (~ -4 Pearson residuals), except for *Sphaerospermopsis,* as all five species had spherical akinetes, and a few species of *Dolichospermum* and *Anabaena* (Fig. 3).

The shape of the vegetative cell was associated with filament morphology ($\chi^2 = 11.16$, df = 2, $P < 0.01$): cylindrical vegetative cells were associated with straight filaments ($\sim +1$ Pearson residuals), and spherical vegetative cells with coiled filament morphology ($\sim +2$ Pearson residuals).

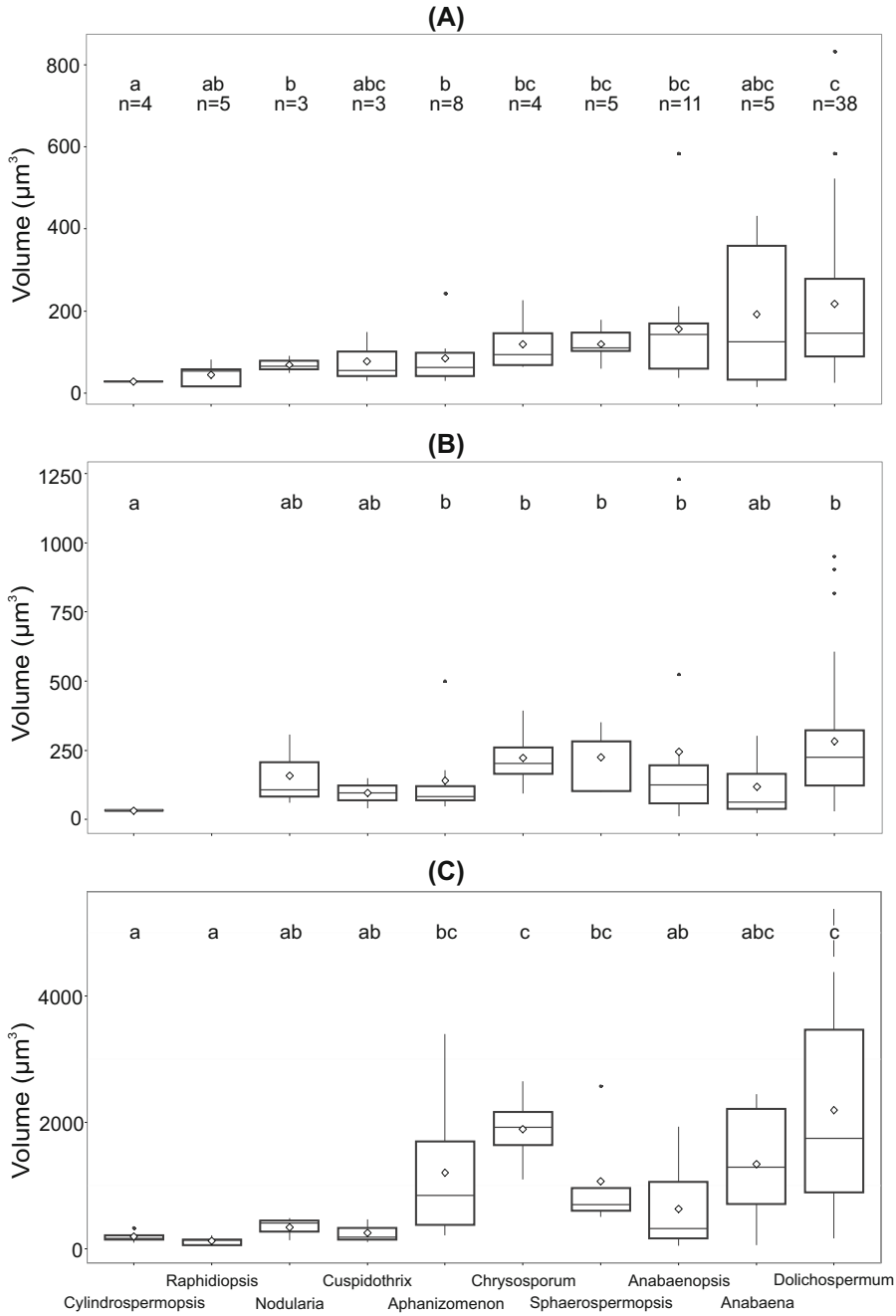

Fig. 1 Volume distributions of vegetative cells (**A**), heterocytes (**B**), and akinetes (**C**) of all 86 species of Nostocales, sorted by genus. Groups that are significantly different ($P < 0.05$) possess different letters. Points are outliers, diamonds are mean values

Specialized cell's position in the filament

Most species develop heterocytes in an intercalary position in the filament (66 out of 81 species across nine genera), while only the four species of *Cylindrospermopsis* develop heterocytes in a terminal position. All 11 species of *Anabaenopsis* have secondary terminal heterocytes (which initially have an intercalary position, but become terminal after filaments fragment), and the five species of *Raphidiopsis* lack heterocytes entirely.

Akinetes are five times more likely to develop in a distant (64 species) than in an adjacent (12 species) position to the heterocyte. The few species which

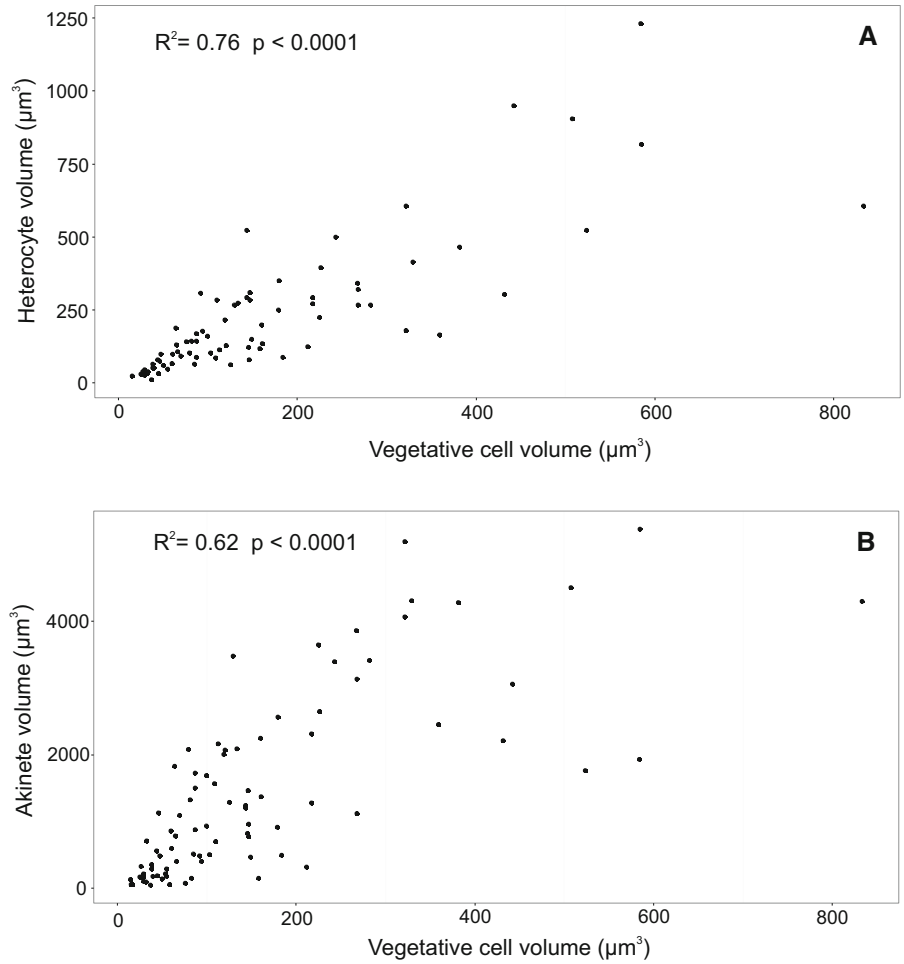

Fig. 2 Trait relationship between the volumes of **A** the vegetative cell and the heterocyte (n = 78 as all species within *Raphidiopsis* lack heterocytes), and **B** the vegetative cell and the akinete (n = 86)

develop akinetes adjacent to the heterocyte are limited to three genera: all species within *Sphaerospermopsis* and a few species within *Dolichospermum* and *Cuspidothrix*. Finally, very few species are able to develop akinetes in both positions (distant and adjacent): two species in *Dolichospermum* and two species in *Cylindrospermopsis*.

Maximum growth rates

We obtained 91 values for the trait of maximum growth rate across 12 species across 7 genera (Table S2). About two-thirds of the data collected belonged to three bloom-forming species: *Cylindrospermopsis raciborskii* (31), *Dolichospermum flos-aquae* (18), and *Aphanizomenon flos-aquae* (Ralfs ex Bornet & Flahault, 1888) (13) (Table S2). Most of the maximum growth rates collected (ca. 75%) were measured under N-uptake conditions (sufficient dissolved inorganic N in the medium) and less frequently in N-fixing situations (the absence of supplied N in the medium) (Table 1).

Under N-uptake conditions, maximum growth rates were on average 0.48 day^{-1} with a range of 0.1–1.41 day^{-1}. These rates significantly differed among genera (F = 6.004, df = 6, $P < 0.0001$). Growth was sthe lowest in *Nodularia*, and the fastest in *Cylindrospermopsis* and *Sphaerospermopsis* (Fig. 4). We found 15 cases where maximum growth rate was measured for the same strain under both N-uptake and N-fixing situations, including strains of species with heterocytes located in intercalary (n = 6)

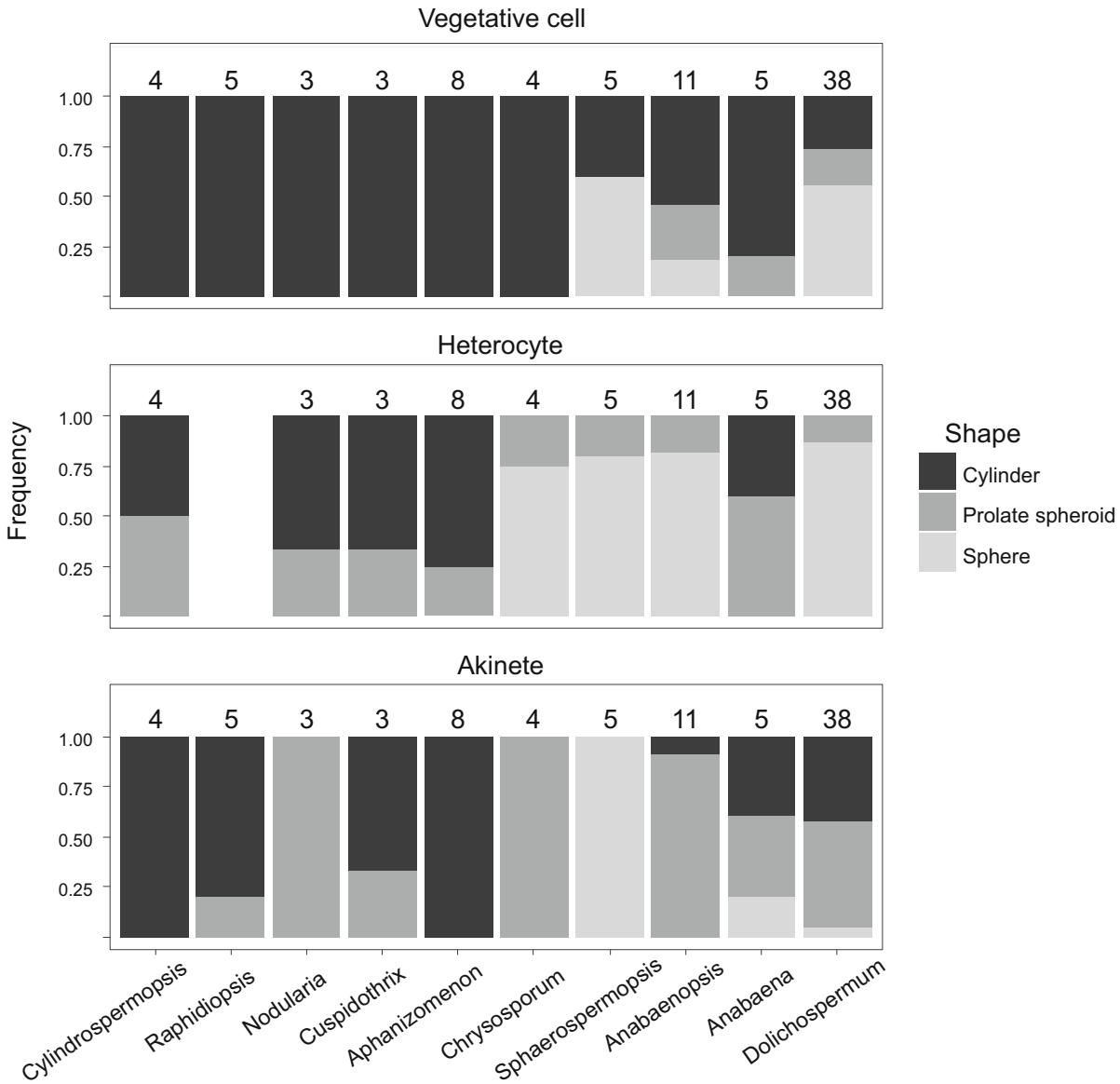

Fig. 3 Cell shape frequencies within each cell type and across genera. Numbers above the bars indicate how many species in each genus contributed to data

and in terminal ($n = 8$) position in the filament (Table S2). These taxa had similar mean maximum growth rates under N-fixing and N-uptake situations (0.58 and 0.68 day^{-1}, respectively). However, heterocyte position significantly affected maximum growth rates ($F = 6.041$, df = 1, $P = 0.02$). Species with terminal heterocytes (i.e., *Cylindrospermopsis*) grew faster than species with intercalary heterocytes (e.g., *Aphanizomenon*, *Dolichospermum,* and *Chrysosporum*), at 0.75 day^{-1} versus 0.5 day^{-1}, respectively. The interaction between N supply and heterocyte position was not significant ($F = 0.01$, df = 1, $P = 0.92$). We also found no significant relationship between maximum growth rate in the N-uptake situation and vegetative cell volume ($P = 0.488$; maximum growth rate = 0.463–2.517 $\times$ 10^4 $\times$ Volume; $R^2 = 0.049$; $n = 12$).

Finally, our analysis of taxonomic variation in maximum growth rate shows that this trait is more variable across genera (accounting for 21.5% of total

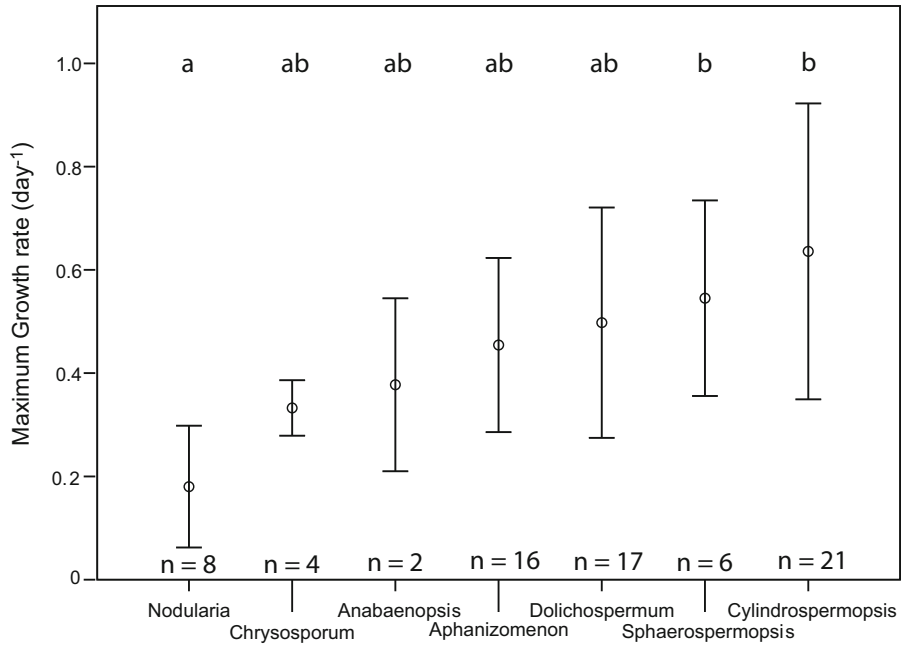

Fig. 4 Mean maximum growth rate, sorted by genus. Bars denote standard deviation. Groups that are significantly different ($P < 0.05$) possess different letters; n = number of strains in each genus

variation) than among species within genera ($\sim$ 6% of total variation) (Table S3).

Trait similarity among species

The cluster analysis rendered two main groups, A and B (Fig. 5). Cluster A was composed of 35 species with large cell volumes (average volume of vegetative cells: 250 μm^3, heterocytes: 338 μm^3 and akinetes: 2727 μm^3); these species volumes were about three times greater than those in cluster B. Cluster A encompassed most of the species within *Dolichospermum* (66%), *Chrysosporum* (75%), and *Anabaena* (60%). Cluster B could be further separated in two, B1 with 25 species with intermediate volume cells (average volumes of vegetative cells: 93 μm^3, heterocytes: 144 μm^3 and akinetes: 710 μm^3) and B2 that gathered 21 species with very small cell volumes (average vegetative cells: 66 μm^3, heterocytes: 61 μm^3 and akinetes: 177 μm^3). B1 encompassed most species within *Sphaerospermopsis* and *Nodularia*, and to a lesser extent *Dolichospermum*, *Aphanizomenon*, and *Anabaenopsis*, while cluster B2 encompassed all representatives of *Cylindrospermopsis*, *Raphidiopsis,* and *Cuspidothrix*. In each group (A, B1, and B2), we identified both types of filament morphology (coiled and straight) (Fig. 5). Likewise, within each genus, we found both filament morphologies, albeit for *Cuspidothrix*, *Aphanizomenon*, and *Anabaena* with exclusively straight filaments.

Discussion

The quantitative characterization of morphological trait variation in heterocytous Cyanobacteria confirmed for the whole diversity of this planktic group what we previously observed for a subset of genera (de Tezanos Pinto et al., 2016). There is a positive relationship between the volumes of vegetative and specialized cells; hence, in general, larger vegetative cells produce larger N-fixing and dormant cells. Vegetative cells and heterocytes share similar volumes, probably because one heterocyte differentiates from one vegetative cell. Akinetes volumes, however, show a weaker correlation with the volume of vegetative cells and are about one order of magnitude larger, as others authors have also acknowledged (e.g., Nichols & Adams, 1982; Sukenik et al., 2013). This volume difference is probably associated to akinetes' high content of stored material, extra wall deposition (Komárek, 2013) and massive genome replication

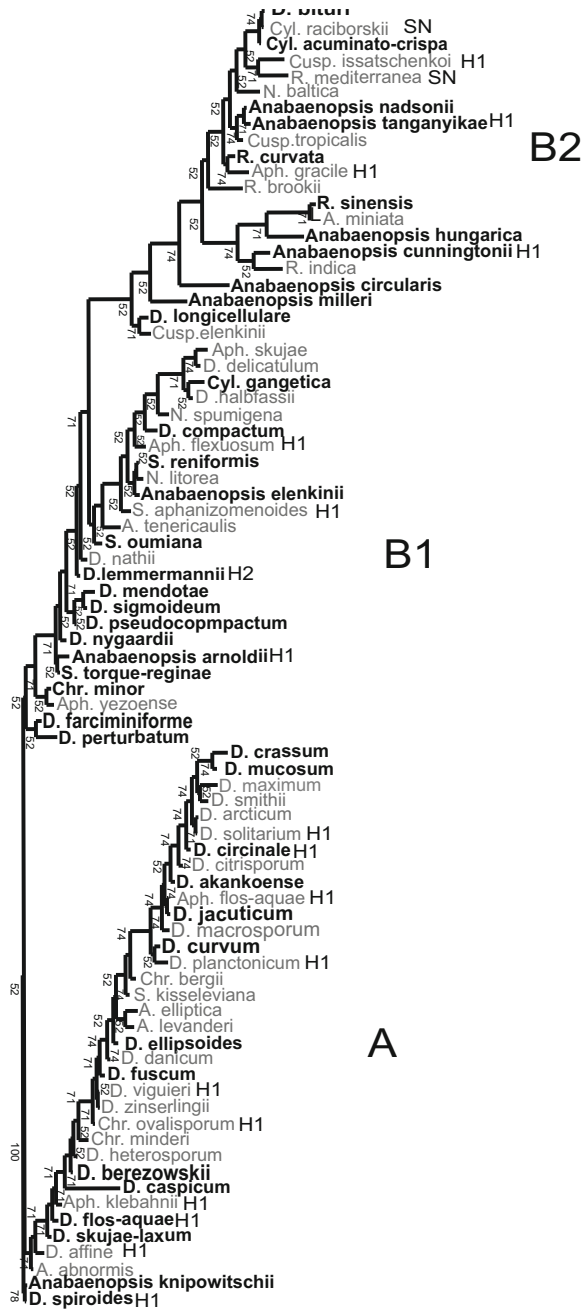

Fig. 5 Neighbor-Joining Cluster of the diversity of heterocytous Cyanobacteria calculated using the following traits: vegetative cell, heterocyte, and akinete volume, and filament morphology. Species with coiled filaments are in black and those straight filaments are in gray. For species names, note that: D = *Dolichospermum*, S = *Sphaerospermopsis*, N = *Nodularia*, R = *Raphidiopsis*, A = *Anabaena*, Aph = *Aphanizomenon*, Cyl = *Cylindrospermopsis*, Cusp = *Cuspidothrix*, Chr = *Chrysosporum*. H1, H2, SN = functional groups sensu Reynolds et al. (2002) and Padisák et al. (2009)

(Sukenik et al., 2011). Also, its large volume may be related to how akinetes differentiate; some authors have hypothesized that several vegetative cells fuse during akinete differentiation (Komárek, 1975; Hindák, 1999, 2008). However, it is still largely unclear if in fact such fusion occurs and how many vegetative cells are involved, and if this occurs in a species-specific way or it is related to particular genera. Exploring this may provide insights into the life cycle of heterocytous Cyanobacteria.

Shape significantly differs among cells types: the vegetative cell is mostly cylindrical, the heterocyte is mostly spherical, and the akinete is mostly prolate spheroid (and to a lesser extent cylindrical). This shape distribution is nonrandom, and may reflect particular functions for each cell type (photosynthesis, nitrogen fixation, dormancy), as well as the major morphological and physiological changes that cells undergo during differentiation. For example, the cylindrical shape—characteristic of most vegetative cells—has the highest surface area-to-volume ratio, compared to spherical and prolate spheroid shapes, and could favor light capture for photosynthesis in vegetative cells. We found that cylindrical vegetative cells were associated with straight filaments; this trait combination could be favored in low-light situations due to high surface area-to-volume ratio, both at the cell and the filament levels. Reynolds (1997) stated that species morphologically attenuated (reduced in some dimension) grow best under subideal conditions. Indeed, O'Farrell et al. (2007) found that the trait of straight filaments prevailed in low-light scenarios. On the other hand, spherical vegetative cells were associated with coiled filaments, and this trait combination has low surface area-to-volume ratio both at the cellular and the filament level.

The prevalence of spherical shape in heterocytes may be one of the many adaptations that N-fixing Cyanobacteria have to hinder oxygen diffusion. Spherical shapes have not only the lowest surface area-to-volume ratios but also the lowest contact areas between heterocytes and neighboring vegetative cells (Lang & Fay, 1971; Walsby, 2007). It is plausible that other heterocyte shapes would be less efficient in limiting oxygen diffusion, which could in turn affect nitrogen fixation rates. In that case, species with spherical heterocytes (e.g., *Dolichospermum*, *Sphaerospermopsis*, *Chrysosporum,* and *Anabaenopsis*) would achieve higher nitrogen fixing rates than species with cylindrical shape (e.g., *Cuspidothrix*, *Aphanizomenon*, *Nodularia,* and *Cylindrospermopsis*), although this hypothesis needs to be tested.

The shape of primitive akinetes was cylindrical according to fossil evidence (Tomitani et al., 2006), but our analysis shows that contemporary akinetes in planktic specimens are mostly prolate spheroid, less frequently cylindrical, and rarely spherical. Based on our personal observations of natural samples, we speculate that the different akinete shapes may play a role in its life cycle, in terms of how akinetes are formed (the prolate spheroid and spherical shapes may be associated with the enlargement of one vegetative cell, whereas the cylindrical shape might result from the fusion of several vegetative cells) and how akinetes germinate (as one cell in cylindrical akinetes or as a short filament in oval and spherical akinetes). It is probable that these observations are species-specific (e.g., Braune, 1980; Moore et al., 2004; Legrand et al., 2017), and this ideas needs to be validated.

Concerning the position of specialized cells, the heterocytes were most frequently found to occur in an intercalary position within filaments, with akinetes located distantly. Adams & Duggan (1999) suggested that adjacency between heterocytes and akinetes could be advantageous because akinetes need to accumulate large amounts of cyanophycin (requiring N). However, as this is the least frequent arrangement that we observed, it could suggest that akinete's N requirements are usually not met directly from N supplied by heterocytes.

Regarding physiological traits, the mean maximum growth rate in heterocytous Cyanobacteria is comparable to that of cyanobacteria as a group, as well as diatoms and dinoflagellates, yet much lower than green algae (Schwaderer et al., 2011). Our results suggest that there is little variation in maximum growth rates among species within a genus. The high amount of unexplained variation found could have arisen from experimental uncertainty, methodological differences among studies, or substantial variation among strains of the same species. While studies acknowledge a tradeoff between the maximum growth rate and cell volume for phytoplankton (Reynolds, 2006 and citations therein) we found no such relationship for heterocytous Cyanobacteria; this could be because most of the species shared similar maximum growth rates across a wide cell volume range. Results may change if the whole organism's volume, instead of the volume of individual cells is considered, as filament length can markedly vary in response to environmental variation (O'Farrell et al., 2015; Sarthou Suárez, 2016; Yema et al., 2016).

The lack of a significant effect of N supply on the maximum growth rate observed here is counterintuitive, as N-fixation is an energetically expensive process (Wolk et al., 1994) and cells differentiated into heterocytes (about one in every 10-20 vegetative cells being in N-free situations) are lost for further

asexual reproduction (Wolk et al., 1994; Zhang et al., 2006; Kumar et al., 2010). Our results would indicate that the distribution of heterocytous Cyanobacteria is independent from nitrogen availability, although the field studies of Dolman et al. (2012) showed that its distribution varies along nutrient gradients. It is possible that the lack of significance could be explained by the modest number of cases found (15), which may be too small to detect a tradeoff, if it exists. The heterocyte position does appear to affect maximum growth rate, as *Cylindrospermopsis*—which can only develop heterocytes in a terminal position—showed significantly higher growth rates than species with intercalary heterocytes. This suggests that *Cylindrospermopsis* would outgrow other species; however, laboratory experiments show that the biomass of species of this genus is markedly lower when relying on N-fixation than on N-uptake, as other factors operate on control of total biomass (Kenesi et al., 2009; Burford et al., 2016; Yema et al., 2016). Likewise, field surveys also show that the distribution of *Cylindrospermopsis* is constrained to situations where total nitrogen supply is high (Dolman et al., 2012; Kokociński & Soininen, 2012), where N-fixation rarely occurs. Hence, traits other than growth rate (e.g., heterocyte shape and position in the filament, P uptake velocity, and light saturation constant) may determine the competitive outcome of *Cylindrospermopsis* in natural environments.

Among the diversity of planktic heterocytous Cyanobacteria we identified three groups which share similar morphology. Based on the argument that morphology captures function (Kruk et al., 2010), it could be hypothesized that species with overlapping morphologies would have higher probabilities to bloom together, being redundant in function, than species with contrasting morphology.

According to the functional groups sensu Reynolds et al. (2002) and Padisák et al. (2009), species in codon H1 (habitat template: eutrophic stratified and shallow lakes with low nitrogen) were mostly limited to the larger volume clusters, whereas species in codon SN (habitat template: warm mixed environments) were exclusively found in the cluster with the smaller volume species. Based on the compilation of Padisák et al. (2009) we observed that roughly one-fourth of the heterocytous Cyanobacteria species are currently sorted into functional groups. Our compilation may help as a baseline for the sorting of species into functional groups based on morphological information, and it may also question the current location of species in particular functional groups. For example, *Cuspidothrix issatschenkoi* (Usachev) Rajaniemi et al. 2005, and *Aphanizomenon gracile* (Lemmermann 1907) are currently sorted in codon H1, yet, because of their small cell volumes and straight filament morphologies, they could be located into codon SN (which currently is formed exclusively by the species *Cylindrospermopsis raciborskii* and *Raphidiopsis mediterranea* (Skuja 1937). Field evidence is needed to validate the latter idea. Finally, though differences in morphology can, at least partially, explain and predict which species could be found together and which species would rarely bloom together, other traits not included in this study (e.g., light acquisition traits, phosphorus uptake, salinity tolerance, and toxin production) may also affect the distribution of particular species along environmental gradients.

This study provides a glimpse into the rich and important body of prior research on heterocytous Cyanobacteria (references in Tables S1 and S2). The information gathered and synthesized is made accessible to the scientific community, contributing to the necessary expansion of available data (Kremer et al., 2017b). The compiled trait data can be used to assess trait–environmental relationships, ease taxonomic identification, biovolume calculation, model parameterization, total evidence approaches in phylogeny, and functional diversity assessment, among others. It also lays a foundation for further expanding the current database and including other relevant traits (light and nutrient acquisition, toxicity) not addressed in this study.

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