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Morphological traits in nitrogen fixing heterocytous cyanobacteria: possible links between morphology and eco-physiology

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Abstract Heterocytous cyanobacteria are able to fix nitrogen (in heterocytes) and to produce dormant cells (akinetes). Heterocyte and akinete shape, size, and relative position have taxonomical relevance and possibly ecological value too. We collected—from literature and nature—and compared morphological data on vegetative cells, heterocytes, and akinetes across four genera taxonomically separated from *Anabaena*. In average, heterocyte size doubled that of vegetative cells—probably because of extra cell wall deposition. Heterocyte morphology was remarkably similar across genera, both in size and shape (spherical). The latter may decrease oxygen diffusion from adjoining vegetative cells. Akinetes were huge (one order of magnitude bigger) compared to vegetative cells, probably because of its massive genome replication, extra deposition of wall layers, allocation of storage and number of vegetative cells fused during akinete differentiation. Akinete shape was mostly cylindrical, or oval, but rarely spherical. In line with molecular data, we found morphological differences between *Anabaena* (non-aerotopated, soil or benthic) and *Dolichospermum* (aerotopated, planktonic), including vegetative cell size, and akinete size, shape, and relative position to the heterocyte. Differences may relate to adaptations to their contrasting environments (benthic versus planktic). Further research is needed to generalize our results to other heterocytous genera.

Keywords Traits · Heterocyte · Akinete · Shape · Size · Phytoplankton

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Introduction

Many heterocytous nitrogen fixing cyanobacteria cause blooms in water bodies around the globe occasioning severe ecological, economical, and social problems. This ecological group have a wide suite of

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traits that provide fitness in a range of environmental settings, including the ability to fix nitrogen (in heterocytes) and to produce dormant cells (akinetes). Both heterocytes and akinetes differentiate from vegetative cells—in response to environmental triggers—and undergo major morphological and functional changes. The shape and size of akinetes, as well as the relative position of heterocytes and akinetes have taxonomical relevance (Komárek, 2013) and we believe that they have an ecological value too.

Exploring the morphological traits in heterocytous nitrogen fixing cyanobacteria will allow us to explore its trait distributions and trait–trait relations (e.g., positive relations, trade-offs). It also sets the basis to assess the links between morphological and physiological traits (e.g., how cell size relates to growth rate or light acquisition traits). Indeed, ecological traits are used in phytoplankton studies to explain and predict species distribution along environmental gradients (Litchman et al., 2010; Schwaderer et al., 2011; Salmaso et al., 2014). Both size and shape are important traits to study phytoplankton distribution (Reynolds et al., 2002; Salmaso & Padisák, 2007; Padisák et al., 2009; Kruk et al., 2010). Morphological traits impact on phytoplankton reproduction, resource acquisition, and grazer resistance capabilities (Litchman & Klausmeier, 2008) and are relatively simple to measure (Arnold, 1983; Hodgson, 1999).

The heterocyte is a good example of how a morphological trait relates to a function, in this case the fixation of atmospheric nitrogen. The nitrogenase enzyme, responsible of the reduction of N_2 to NH_3, NH_4 or any organic nitrogen compound (Jaffe, 1992), has an extreme sensitivity to oxygen (Gallon, 1992). Within the heterocyte, oxygen concentration is extremely low (e.g., 0.6 µm in *Anabaena*, Tomitani et al., 2006), hence ensuring the functioning of the nitrogenase. When a vegetative cell differentiates into a heterocyte, major morphological (and physiological) changes occur, which ensure low oxygen availability within mature heterocytes, including the loss of photosystem II (hence lacking the ability to perform oxygenic photosynthesis and fixing carbon dioxide), the addition of several wall layers (which decrease oxygen diffusion from the environment), increased oxidase activity (Wolk et al., 1994), and the development of nodule poles (rich in cyanophycin) in the points of contact with vegetative cells (which decrease molecular flux, Mullineaux et al., 2008). These changes are reflected in heterocyte morphology: they are pale colored (as they lack one photosystem), have thick walls and polar nodule, hence it is very easy to identify heterocytes in the microscope.

Moreover the timing of the evolution of the heterocyte as a trait innovation—about 2.5 billion years ago—coincided with the oxygenation of the planet Earth (Tomitani et al., 2006) when high oxygen levels hindered nitrogen fixation. The capability to differentiate heterocytes allowed Nostocales to be able to fix nitrogen at daytime, in aerobic environments. This monophyletic clade (e.g., Rajaniemi et al., 2005a, b) has dramatically diversified compared to other non-heterocytous nitrogen fixing cyanobacteria, underscoring the fitness provided by the trait of fixing nitrogen within the heterocyte. Nevertheless, we lack knowledge on whether morphological traits such as shape and size vary across heterocytous cyanobacteria, or if these traits may relate to heterocyte functioning.

The akinete allows the survival of populations through dormancy in periods which hinder growth or cause mortality (Kaplan-Levy et al., 2010). These resting cells appeared in the evolution after heterocytes; the most ancient akinetes date from 2.1 to 1.5 billion years ago (Tomitani et al., 2006) and only a subgroup of Nostocales have this trait. These specialized cells may develop from one vegetative cell (Clark & Jensen, 1969; Cmiech et al., 1984) or from the fusion of several neighboring cells (Komárek, 1975; Hindák, 1999, 2008). Akinete differentiation, such as heterocyte differentiation, involves major morphological and physiological modifications. These include: the formation of a multilayered cell wall, the loss of gas vesicles (Komárek, 2013), the allocation of storage in the form of granules of cyanophycin and glycogen (Simon, 1987), massive multiplication of genome (Sukenik et al., 2011), and loss of inorganic polyphosphate bodies (Sukenik et al., 2009). Akinetes can have different shapes (mostly cylindrical, spherical, or oval) and relative positions to the heterocyte (adjacent or distant). These traits are species-specific and are used in taxonomical classification (Komárek, 2013). There is lack of a quantification of the frequency distribution of akinete morphological traits—shape, size, and relative position toward the heterocyte- and of the possible causes leading to morphological differences in this dormant cell.

The genus *Anabaena* Bory 1822, considered a consistent genus for a long time, has recently been divided into several new genera—planktic *Dolichospermum*, *Sphaerospermopsis*, and *Chrysosporum,* and non-aerotopated *Anabaena* (Komárek & Zapomělová, 2007, 2008; Wacklin et al., 2009; Zapomělová et al., 2009, 2012)—based primarily on the 16S rRNA gene sequencing (Iteman et al., 2002; Gugger et al., 2002; Rajaniemi et al., 2005a, b; Hoffman et al., 2005). It would be interesting to test if differences in morphological traits across genera support the molecular findings.

In this investigation, we aim to build a solid knowledge on morphological traits within heterocytous cyanobacteria. For this we collected, synthesized and compared morphological data of different cell types (vegetative cells, heterocytes, and akinetes) in a wide range of heterocytous species within the recently separated genera *Anabaena*, using information from literature and from natural populations. We assessed trait distributions as well as trait–trait relationships and tried to find links between morphology and function within this relevant ecological group.

Materials and methods

Data collected from the literature

We collected, from the third volume of "Cyanoprokaryotes" (Komárek, 2013), data on length, width, and shape of vegetative cells, heterocytes, and akinetes for all species within the non-aerotopated genus *Anabaena*, and the planktic genera *Dolichospermum*, *Sphaerospermopsis,* and *Chrysosporum*. As mentioned in the Introduction, these four genera used to belong to the traditional genus *Anabaena* but were separated according to modern phenotypic and molecular criteria (Iteman et al., 2002; Gugger et al., 2002; Rajaniemi et al., 2005, b; Hoffman et al., 2005; Komárek & Zapomělová, 2007, 2008; Wacklin et al., 2009; Zapomělová et al., 2009, 2012, 2013).

For each species and cell type, we computed the average between the minimum and maximum length and width, assuming a normal distribution of these traits, based on the argument of Kerkhoff & Enquist (2009) that most traits have a normal distribution. We validated this approach by assessing the same cell dimensions in natural samples and testing if these followed a normal distribution (see Data collected from natural populations).

Next, we calculated cell size (volume) following Hillebrand et al. (1999). We also collected information about the relative position of the akinete to the heterocyte (e.g., distant or adjacent) from Komárek (2013).

Data collected from natural populations

We collected data on length and width of vegetative and specialized cells from natural samples collected in the Czech Republic and Argentina (Table 1). For each species we: (a) took microphotographs of at least 30 trichomes under 400× magnification using a digital camera (Olympus DP70 and AxioCam 5S Carl Zeiss) attached to a light microscope (Olympus BX51 and Nikon TS100 Eclipse) and (b) measured five vegetative cells per trichome in 30 trichomes, and as much akinetes and heterocytes as possible. Only mature akinetes were measured (those which possessed fully developed thickened cell wall) as recommended by Komárek (1996). We used this information to test whether cell length and width followed a normal distribution (Jarque–Bera test) and to compare results among data sets (literature and nature).

Statistical analyses

For the data collected from literature we:

(i) assessed the following trait–trait relationships: length to width (of each cell type), vegetative to akinete size, and vegetative to heterocyte size. In each case, we tested which curve fitted best the data (linear, logarithm, power, or exponential) based on the significance of ANOVA test and the coefficient of determination (R^2).

(ii) compared length to width relationships between cell types (vegetative cell versus heterocyte, vegetative cell versus akinete, and heterocyte versus akinete) using ANCOVA test after meeting the assumptions. Also, for each cell type (vegetative, heterocyte, akinete) we compared the length to width relationship across data sets (e.g., vegetative cell length to width relationship from literature data versus those obtained

Table 1 Geographical position and environment where the natural samples were obtained

Species	Geographical position and environment
Anabaena sp.	Sokolov area, Czech Republic
Anabaena sp.	50.1503689 N, 12.4016761E, Soos Natural Reserve, (spring), Františkovy Lázně, Czech Republic
Anabaena sp.	50.1500939 N, 12.4044656E, Soos Natural Reserve, (mud surface), Františkovy Lázně, Czech Republic
Anabaena sp.	50.2384222 N, 12.7046586E, (pool after coil mining), Vřesová, Czech Republic
Anabaena sp.	50.2384222 N, 12.7046586E, (temporary puddle), Vřesová, Czech Republic
Anabaena sp.	50.2384222 N, 12.7046586E, (pool after coil mining), Vřesová, Czech Republic
Anabaena sp.	50.2714039 N, 12.7563286E, Smolnická (mine disposal site), Chodov, Czech Republic
Anabaena sp.	50.1484439 N, 12.4030494E, Soos Natural Reserve, Císařský (spring), Františkovy Lázně, Czech Republic
Anabaena sp.	50.1484439 N, 12.4030494E, Soos Natural Reserve, Císařský (spring) Františkovy Lázně, Czech Republic
Anabaena sp.	50.1500939 N, 12.4044656E, Soos Natural Reserve, (mud surface), Františkovy Lázně, Czech Republic
Anabaena sp.	50.1503689 N, 12.4016761E, Soos Natural Reserve, (spring), Františkovy Lázně, Czech Republic
Anabaena sp.	50.1503689 N, 12.4016761E, Soos Natural Reserve, (spring), Františkovy Lázně, Czech Republic
Dolichospermum lemmermannii	48.9989158 N, 14.7704314E, Svět (fishpond), Třeboň, Czech Republic
Dolichospermum flos-aquae	49.0313686 N, 14.4398547E, Naděje (fishpond), Bavorovice, České Budějovice, Czech Republic
Dolichospermum sigmoideum	50.1975339 N, 12.8572225E, Březová (reservoir), Karlovy Vary, Czech Republic
Dolichospermum sigmoideum	49.0039836 N, 14.4347478E, Černiš (fishpond), České Budějovice, Czech Republic
Dolichospermum lemmermannii	49.0382619 N, 13.9928483E, Husinec (reservoir), Prachatice, Czech Republic
Dolichospermum sigmoideum	49.0707172 N, 14.7009086E, Koclířov (fishpond), Lomnice nad Lužnicí, Czech Republic
Dolichospermum sigmoideum	49.0105144 N, 14.3056583E, Dehtář (fishpond), Dehtáře, Czech Republic
Dolichospermum lemmermannii	49.7845892 N, 13.3931064E, Senecký (fishpond), Plzeň, Czech Republic
Dolichospermum sp.	Czech Republic
Dolichospermum tenericaule	50.5766417 N, 14.6476078E, Máchovo (fishpond), Doksy, Czech Republic
Sphaerospermopsis aphanizomenoides	48.9989158 N, 14.7704314E, Svět (fishpond), Třeboň, Czech Republic
Sphaerospermopsis reniformis	48.7819850 N, 16.7836761E, Hlohovecký (fishpond), Lednice, Czech Republic
Sphaerospermopsis aphanizomenoides	34°10′-34°17′S—58°48-58°53′W (shallow lake) Grande, Buenos Aires, Argentina
Sphaerospermopsis torques reginae	34°10′-34°17′S—58°48-58°53′W (shallow lake) Grande, Buenos Aires, Argentina
Sphaerospermopsis aphanizomenoides	34°21′11.44″S—62°13′2.50″W (shallow lake) La Picasa, Santa Fe, Argentina
Sphaerospermopsis aphanizomenoides	34°21′11.44″S—62°13′2.50″W (shallow lake) La Picasa, Santa Fe, Argentina
Sphaerospermopsis reniformis	49.1246686 N, 14.7404336E, Pěšák (fishpond), Lomnice nad Lužnicí, Czech Republic
Sphaerospermopsis aphanizomenoides	50.0428369 N, 14.5311786E, Hostivař (reservoir), Prague, Czech Republic
Chrysosporum bergii	Očko sandpit, Czech Republic

from nature). Finally, we compared size trait relationships (vegetative cell to heterocyte, and vegetative cell to akinete) between literature and natural data sets.

(iii) tested, for each cell type (vegetative cell, heterocyte, and akinete), if there existed significant differences in size among *Anabaena*, *Dolichospermum*, *Sphaerospermopsis,* and *Chrysosporum*. For this, we run one-way ANOVA tests (all variables were transformed to logarithm to meet the test assumptions). When significant differences were found, we used Hochberg GT2 post hoc test, which is suitable for comparing unbalanced treatments.

(iv) Examined the frequency distributions of akinete relative position to the heterocyte in the genera with high number of species (*Anabaena* and *Dolichospermum*) running a Chi square test.

All analyses were performed with SPSS 17 Software, and Past 3 Software (Hammer et al., 2001).

Results

The four genera analyzed in the literature dataset encompassed 106 species. Most of these belonged to the non-aerotopated genus *Anabaena* (59 species), while the remaining (47 species) were planktic (aerotopated, allowing buoyancy regulation). In the latter group, most species belonged to *Dolichospermum* (38) while few belonged to *Sphaerospermopsis* (5) and *Chrysosporum* (4).

For natural populations, we measured cell dimensions in 866 individuals across 31 species (12 of *Anabaena*, 10 of *Dolichospermum*, 8 of *Sphaerospermopsis,* and 1 of *Chrysosporum*, Table 1). The total number of normality tests run was 186: 2 cell dimensions (length and width) *3 cell types (vegetative, heterocyte, and akinete)*31 species. The majority of traits studied (92%) had a normal distribution ($P > 0.05$) on linear scale. This suggests that the approach used in this study (calculation of average cell length and width from the minimum and maximum values informed in the literature) is adequate for estimating—and comparing—cell size across different genera and cell types.

We found significant ($P < 0.05$, $n = 106$) and positive relationship between cell width and length, for each cell type; in all cases the power curve was the best fit for the data (Fig. 1a–c). The coefficient of determination (R^2) and the slope of the regression models were much higher in vegetative cells and heterocytes than in akinetes (Fig. 1a–c). In line with the previous information, the vegetative cell and the heterocyte showed a similar length to width ratio (close to the unit and with low standard deviation: 1.1 ± 0.3 in vegetative cell and 1.2 ± 0.4 in heterocyte, $n = 106$ for each cell type). For the akinete, however, its length more than doubled its width, and showed higher variability (2.5 ± 1.4, $n = 106$). We found similar slopes (ANCOVA, $P > 0.05$, $n = 106$) for the length to width relationships between cell types (vegetative to heterocyte, vegetative to akinete, and heterocyte to akinete). For each cell type (vegetative, heterocyte, and akinete) the length to width relationship was similar (ANCOVA $P > 0.05$) between literature ($n = 106$) and nature ($n = 31$) data sets (e.g., akinete length to width relationship obtained from literature data compared to that obtained in natural data).

Vegetative cell size ranged from 2.5 to 1022.1 μm^3 (mean: 160.5 μm^3); it was significantly smaller in *Anabaena* than in *Dolichospermum* ($P < 0.001$) (Fig. 2a). Vegetative cell shape was mostly cylindrical (71.4% in *Anabaena*, 39.5% in *Dolichospermum*, 60% in *Sphaerospermopsis* and 100% in *Chrysosporum*) or spherical (25.4% in *Anabaena*, 47.4% in *Dolichospermum* and 40% in *Sphaerospermopsis*); very few were oval (3.4% in *Anabaena* and 13.2% in *Dolichospermum*). Heterocyte size was similar across genera ($P > 0.05$) (Fig. 2b) and ranged between 8.2 to 950.3 μm^3 (mean: 228 μm^3); the most frequent shape was spherical (68%). Akinete size ranged from 54 to 12477 μm^3 (mean: 1823 μm^3) and was significantly smaller in *Anabaena* than in *Dolichospermum* ($P = 0.032$) (Fig. 2c). In most species, akinetes were cylindrical (50%) or oval (39%); few species had akinetes with spherical shape (11%). Within *Sphaerospermopsis,* all species have spherical akinetes and within *Chrysosporum* all species have oval shape. In *Dolichospermum* and *Anabaena* species have akinetes with cylindrical, oval, or spherical shape.

Regarding the relative position of the akinete to the heterocyte, most species showed either distant (60%)

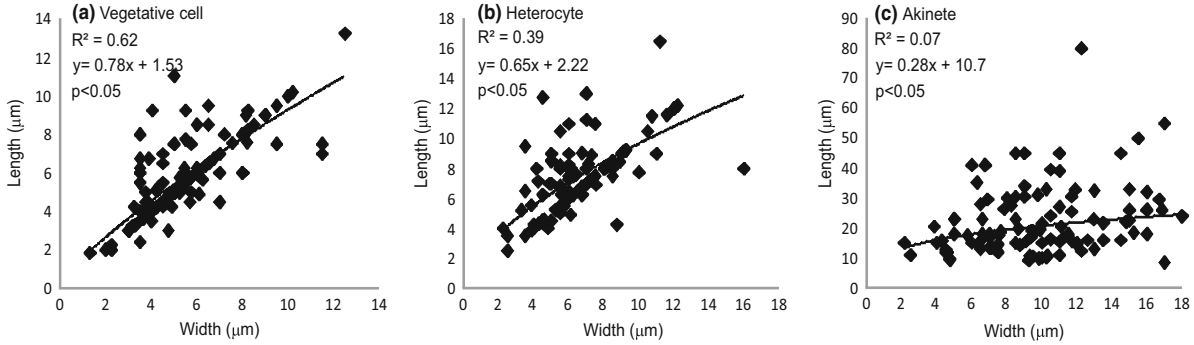

Fig. 1 Length to width relationship in **a** vegetative cell, **b** heterocyte and **c** akinete, $n = 106$ species. The coefficient of determination (R^2) slope, constant, and significance of each curve fit are included in the graphs

or adjacent (30%) position; very few species (9%) differentiate akinetes in both positions (Fig. 3, $n = 106$). In *Anabaena,* there was a similar frequency of species with akinetes distant (50%) or adjacent (37%) to the heterocyte, while in *Dolichospermum* most species (81%) formed akinetes in a distant position to the heterocyte (Fig. 3). The difference in the frequency distribution of akinete position between *Anabaena* and *Dolichospermum* was statistically significant ($\lambda_1 = 8.08$, $P = 0.04$). In all species within *Sphaerospermopsis* ($n = 5$), the akinete develops adjacent to the heterocyte. The opposite happened in *Chrysosporum*, where the akinete develops distant from the heterocyte in 3 out of all 4 species. In *C. ovalisporum* akinetes usually develop distant to the heterocyte, but sometimes may occur in an adjacent position.

Vegetative cell size was significantly and positively related to heterocyte size; the best curve fit was linear ($P < 0.001$, $n = 106$) (Fig. 4a). This pattern was similar (ANCOVA $P > 0.05$) between the literature and the natural population data sets. The ratio of heterocyte to vegetative cell size, in average was two (mean: 2.1, range 0.3–10.6, $n = 106$).

The relationship between vegetative cell and akinete size showed a saturating trend around 4000 μm^3; the best curve fit was power ($P < 0.001$, $R^2 = 0.49$, $n = 106$) (Fig. 4b). This pattern differed from the natural populations (ANCOVA, $P = 0.026$). Both data sets showed positive relationships; however data obtained from nature showed a more limited distribution compared to the range of values obtained in the literature data. This is not surprising as the number of species surveyed from the literature was much higher than those compiled from nature. The ratio of the akinete to the vegetative cell size was in average 9.4 (range 1–55, $n = 106$).

Discussion

We found striking differences in morphological traits among the heterocytous cyanobacteria studied here. Across the four genera analyzed, we found remarkable morphological similarity for the heterocyte, both in terms of size and shape. Conversely, vegetative cell size, as well as akinete size, shape, and relative position to the heterocyte differed between genera.

The trait–trait analysis show that heterocyte size increased proportionally to vegetative cell size. Because the heterocyte develops from a single vegetative cell (Komárek, 2013), the linear relationship found between these cell types seems related to the fact that heterocytes differentiate from one vegetative cell. Heterocytes were about two times bigger than vegetative cells. We hypothesize that this may be related to the extra cell wall deposition that develops during heterocyte differentiation. As heterocytes differentiate from vegetative cells, we were expecting heterocytes to be smaller in *Anabaena* than in *Dolichospermum*; however, we found that heterocyte size was similar (around 200 μm^3, in average) across genera. We also found that the majority of heterocytes were spherical. Though we still lack evidence to explain the causes leading to the striking similar morphology in heterocytes across the genera analyzed here, we speculate that it might be linked to surface to volume relationships minimizing oxygen diffusion

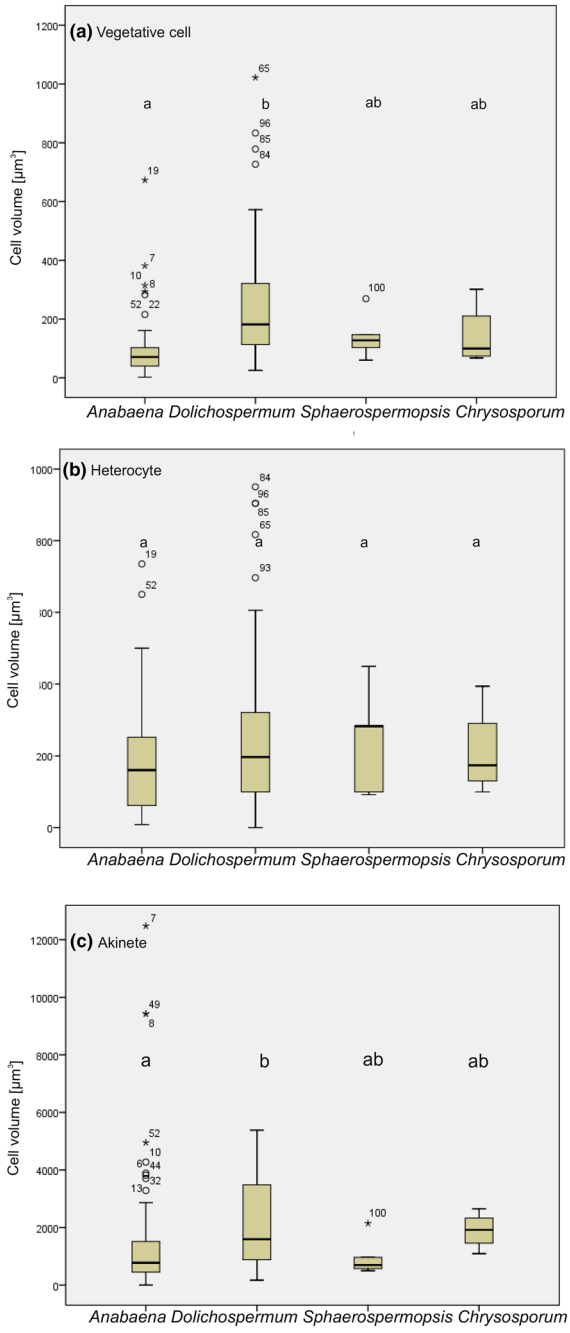

Fig. 2 Comparison of cell size (volume) across the 4 genera analyzed (*Anabaena*, *Dolichospermum*, *Sphaerospermopsis,* and *Chrysosporum*) for the different cell types across 106 species: **a** vegetative cell, **b** heterocyte, and **c** akinete. *Lowercase letters* "*a*" and "*b*" indicate significant differences ($P < 0.05$); *letters* "*ab*" indicate similarities ($P > 0.05$) among genera

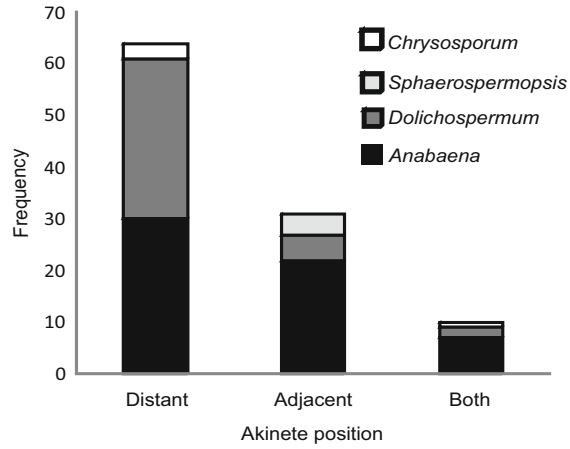

Fig. 3 Frequency distribution of akinete position (*adjacent*, *distant*, or *both*) in each of the 4 genera analyzed ($n = 106$)

(hence favoring nitrogen fixation). Indeed, several authors suggest that the narrow "neck" at the heterocyte-vegetative cell interface reduces the area of contact among cells (Lang & Fay, 1971, Flores et al., 2006; Walsby, 2007). In average, there are about 50 microplasmodesmata at the heterocyte-vegetative cell interface compared to the 200–300 at vegetative–vegetative cell interfaces (Giddings & Staehelin, 1981).

Fay (1969) and others later (Nichols & Adams, 1982; Herdman, 1987, 1988; Sukenik et al., 2013) acknowledged that akinetes are much bigger than the vegetative cells. In line with this evidence, we found that average akinete size was one order of magnitude bigger than average vegetative cell size. The huge size of mature akinetes may be linked to the massive genome replication (Sukenik et al., 2011), extra deposition of wall layers and allocation of storage (Komárek, 2013). We further propose that akinete size may be also linked to the number of vegetative cells fused to form an akinete. For example, akinete differentiation in *Anabaena augstumalis* occurs after the fusion of several neighbor cells (Hindák, 1999). However, our understanding of the number of vegetative cells involved in forming an akinete is very poor, and we still do not know if it occurs in a species-specific way or if it responds similarly within a genus. Finally, the trait–trait relationship between vegetative cell and akinete size showed a saturating trend,

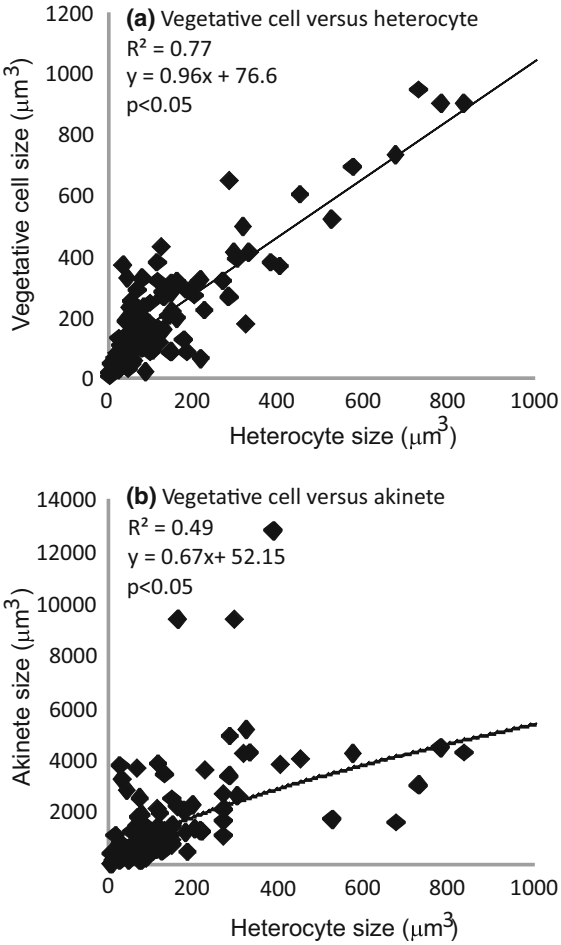

Fig. 4 Trait-trait relationship between cell types: *a* vegetative cell and heterocyte and *b* vegetative cell and akinete ($n = 106$). The coefficient of determination (R^2) slope and constant of each curve fit are included in the graphs

indicating that akinete size increases with vegetative cell size up to a threshold after which akinetes seem to reach its maximum size. Conversely, heterocyte size (as mentioned before) increases proportionally with vegetative cell size.

Most akinetes in the species that we studied were either cylindrical or oval. This may reflect akinete evolutionary history; most ancient akinetes were cylindrical (Tomitani et al., 2006, p. 5444, Fig. 2). Only 12 species (5 benthic and 7 planktonic) out of the 106 species here analyzed had spherical akinetes, including 5 species belonging to *Sphaerospermopsis* (in this genus akinetes exclusively develop adjacent to the heterocyte). We are still unable to explain why differences in akinete shape exist, but we believe it must have an ecological meaning (if shape was driven by chance, we would expect similar proportions of all three akinete shapes). Plausible explanations are that the shape is related to akinete formation strategies, that is, if akinetes differentiate from one or from several vegetative cells. Moreover, though there is consensus that akinete function is related to dormancy and reproduction, current germination experiments that we are undertaking (Paula de Tezanos Pinto Daniela Gangi, Andreja Kust & Eliška Kozlíková-Zapomělová, unpublished data) suggest that shape may be linked to different germination strategies: either if the endospore divides within the akinete cell wall or after breaking the akinete wall. We believe that this may result in different needs of external nutrient supply at germination, though more studies are needed.

We found that most species have akinetes developing distant from the heterocyte, particularly in planktonic species. We speculate that akinete position toward the heterocyte may result in different internal nitrogen stores in the akinete. This, in combination with external nutrient availability at germination, and akinete shape (proxy of germination strategies?), may affect population recruitment success (to form blooms or not). Future studies will allow testing our ideas.

Morphological trait differences among recently taxonomically separated genera

We found several morphological differences between the genera *Anabaena* (non-aerotopated) and *Dolichospermum* (planktonic, aerotopated). These groups have recently been taxonomically separated based on molecular criteria (Iteman et al., 2002; Gugger et al., 2002; Rajaniemi et al., 2005a, b; Hoffman et al., 2005; Komárek & Zapomělová, 2007, 2008; Wacklin et al., 2009; Zapomělová et al., 2009, 2012, 2013). The morphological differences that we identified include: (a) smaller size of vegetative cells and akinetes in *Anabaena* compared to *Dolichospermum*, (b) prevalence of cylindrical akinete shape in *Anabaena* compared to dominance of oval akinete shape in *Dolichospermum*, and (c) similar proportion of species which develop akinetes distant and adjacent to the heterocyte in *Anabaena*, while most species within *Dolichospermum* develop akinetes in a distant position from the heterocyte. We suppose that these morphological differences may be linked to the contrasting

habitats where these genera thrive (benthic or soil in *Anabaena* and planktic in *Dolichospermum*). For example, *Anabaena* species tolerate extreme temperatures and irradiances (Kust et al., 2015), probably because they inhabit sites prone to desiccation. We think that their smaller vegetative cell size—compared to planktonic species—might be an adaptation to tolerate the extreme conditions of temporary habitats. We are still unable to hypothesize why akinete shape and position differ among these groups.

For the other two planktonic genera analysed in this study—*Sphaerospermopsis* and *Chrysosporum*—cell morphology (vegetative, heterocyte, and akinete) were similar to both planktonic *Dolichospermum* and benthic *Anabaena*. This finding seems controversial, as we would expect only to find similarities among planktonic species. Nevertheless because in *Sphaerospermopsis* and *Chrysosporum* diversity is low (≤ 5 species) compared to *Dolichospermum* and *Anabaena* (38 and 59, respectively), results are probably affected by the power of the test, i.e., the ability of the statistical test to find differences when these exist (even if the one-way ANOVA test and the comparisons used are robust to compare means in treatments with unbalanced data).

Concluding remarks

We found remarkable morphological differences among the three cell types and genera analyzed. Though we tried to find mechanistic explanations for the observed morphological differences, we need more research to test if the patterns found across four genera hold for other heterocytous cyanobacteria species, particularly within genera which recurrently form harmful algal bloos (e.g., *Aphanizomenon*, *Anabaenopsis,* and *Cylindrospermopsis*).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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