

PERSPECTIVE/HYPOTHESIS

Origin of Zygnematophyceae algae from mitotically dividing anydrophyte zygotes

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Abstract

It is now generally accepted that Zygnematophyceae evolved from the multicellular common ancestor with Embryophyta - Anydrophyta - by reduction to unicellular state. Here we propose and discuss possible scenarios how this reduction might have proceeded - either via stepwise evolutionary reduction or via an abrupt life-cycle reduction, establishing a single cell lineage and rendering a number of developmental genes redundant. Based on phylogenomic, developmental and physiological comparative analyses we argue that Zygnematophyceae are not product of a stepwise loss of multicellularity, but most probably evolved from multicellular into the single-cell organization by the evolutionary “quantum leap”- by mitotic division of Anydrophyta zygotes. We consider an alternative origin from mitotically dividing haploid cells as less probable. According to our hypothesis and in contrast to Embryophyta where anydrophytes dividing zygotes resulted in embryo/sporophyte evolution, these mitotically dividing zygotes have intermittently lost sexual reproduction and initiated a new stable, originally diploid stress-resistant single-cell lineage of Zygnematophyceae algae. Our hypothesis therefore proposes a common origin of sporophyte and Zygnematophyceae based on the propensity of Anydrophyta zygotes for mitotic divisions. This scenario is in concord with short phylogenetic branch leading to Zygnematophyceae indicating an abrupt evolutionary reduction, with multicellular genomic equipment of morphologically simple single-cell algae, with the loss of flagella, loss of oogamy and with *de novo* conjugative sex evolution. Diploid status of vegetative cells and peculiar life cycle ploidy changes - including reduction of ploidy before gametic cell division - observed during sexual reproduction of several zygnematophyte representatives might be also explained by zygotic diploid origin of Zygnematophyceae.

Introduction

The Coleochaete hypothesis on the origin of land plants (i.e. origin of sporophyte and alternation of generations), elaborated in 1908 by Bower on conceptual foundations of Čelakovský (1874) envisages mitotically dividing zygote attached to, and metabolically dependent on, haploid thallus of organisms similar to haplontic multicellular charophyte *Coleochaete* as a precursor of land plant embryo/sporophyte (antithetic or approximation hypothesis). An alternative homologous hypothesis, based on originally homomorphic and metabolically independent multicellular haploid gametophyte and diploid sporophyte, was proposed by Pringsheim (1876). As early as in 2002, based on the comparative analyses of plastidial rRNA subunits Turmel et al. concluded that from this perspective the class Zygnematophyceae is surprisingly the closest relative of land plants - not the multicellular charophytes. It took almost next 20 years before this conclusion was generally accepted - reports by Wodniok et al. (2011) and Timme et al. (2012) based on nuclear genes were milestones on this path. This led to the concept of “Anydrophyta” - a common ancestor of Embryophyta and Zygnematophyceae (Rensing 2020). This common ancestor was arguably multicellular, so the unicellular or filamentous organization and loss of oogamy and flagella in Zygnematophyceae are a result of reductive evolution (Cheng et al., 2019; Žárský et al., 2022).

Evolutionary reduction from multicellular ancestor to single-cell progeny in Zygnematophyceae - stepwise process or an abrupt macroevolutionary leap?

While embryophytes (or pre-embryophytes) preserved and further evolved original Anydrophyta multicellularity and oogamic sex with flagellated sperm cells, first zygnematophytes have lost both these features. We identified Cryogenian glaciations ecological context as a most probable stage of Anydrophyta establishment and Zygnematophyceae split and proposed that “cold” drought imposed by planetary continental fresh water to ice phase transition was a trigger of initial steps in plant terrestrialization (Žárský et al., 2022). Resulting first in the evolution of Anydrophyta followed by the split into embryophytes bound to water-saturated supra- and proglacial sediments providing a regular supply of fresh water from the thawing of glacial ice. These mineral-rich sediments and the predictability of water supply acted as a refuge for the multicellular life strategy and conservation of flagellated reproductive cells. Zygnematophytes adapted to the harsh conditions of the bare glacial ice and the surface of proglacial sediments, where their "microbial" life strategy enabled them to occupy much larger areas of the aquatic realm during the Sturtian and Marinoan glaciations compared to other freshwater lineages of that era (Žárský et al., 2022).

One possible scenario of zygnematophytes evolution would be a gradual process resulting in progressive losses of thallus multicellularity, branching, oogamy and flagella - ultimately resulting in a single cell status. Instability and reversibility of early stages of multicellularity evolution are predicted also based on mathematical models of genome - environmental stress interactions (Isaksson et al., 2025). However, basal Zygnematophyceae - represented by the extant genus *Spirogloea* - are simple single-cell algae, there are no extant representants of intermediate stages of thallus reduction and there is also no obvious phylogenomic evidence for the stepwise evolutionary reduction from ancestral multicellularity of anydrophytes to unicellularity of zygnematophytes. On the contrary - the genetic equipment of these organisms harbour till today clear vestiges of multicellular origin (Cheng et al., 2019; Žárský et al., 2022).

We thus hypothesise that this reduction process is currently best explained by a macro-evolutionary mechanism (“evolutionary quantum leap”) - initiation of independent stress resistant single-cell lineage either by mitotic division of haploid precursor or mitotic division of diploid precursor - zygote. Mitotic divisions of a haploid precursor and further development is poised to result in the formation of haploid thallus undergoing an oogamous haplontic life cycle. We consider this possibility less probable in contrast to evolutionary advantageous diploid starting genomic arrangement in case of mitotically dividing zygote as a starting stage (Fig.1A). It is generally accepted that anydrophyte zygotes evolved ability to divide mitotically by skipping meiosis producing diploid embryo/initial stage of sporophyte evolution (Bower 1908; Rensing 2020). We propose that stress-resistant diploid zygotes of a multicellular common zygnematophytes and embryophytes ancestor, able to skip meiosis and initiate mitotic divisions, are also source for the immediate establishment of a new single-cell lineage - Zygnematophyceae (Fig 1A), which in contrast to the embryophyte ancestor has intermittently lost sexual reproduction, rendering number of developmental genes redundant and thus allowing for a rapid gene loss. This mechanism is well congruent with the loss of oogamy, flagella and de novo evolution of a new conjugation-based sexual process as a land drought/aridity stress adaptation (Stebbins and Hill 1980). Moreover, it is explaining an ancient whole-genome duplication event indicated in the common ancestor of Zygnematophyceae (Cheng et al., 2019, Fig. 2C). **If so, sporophyte of Embryophyta and Zygnematophyceae would share related evolutionary origins in dividing zygotes of Anydrophyta ancestor.** While dividing diploid zygote origin of plant sporophyte is generally accepted, our concept of origin of zygnematophytes via related process of mitotic zygote division is surprising and opens new possible evo-devo perspectives on the plant terrestrialization and land plant evolution. It is also congruent with the diploidy of vegetative cells documented in *Closterium ehrenbergii* (and possibly other zygnematophytes Hamada 1987; Haig 2010).

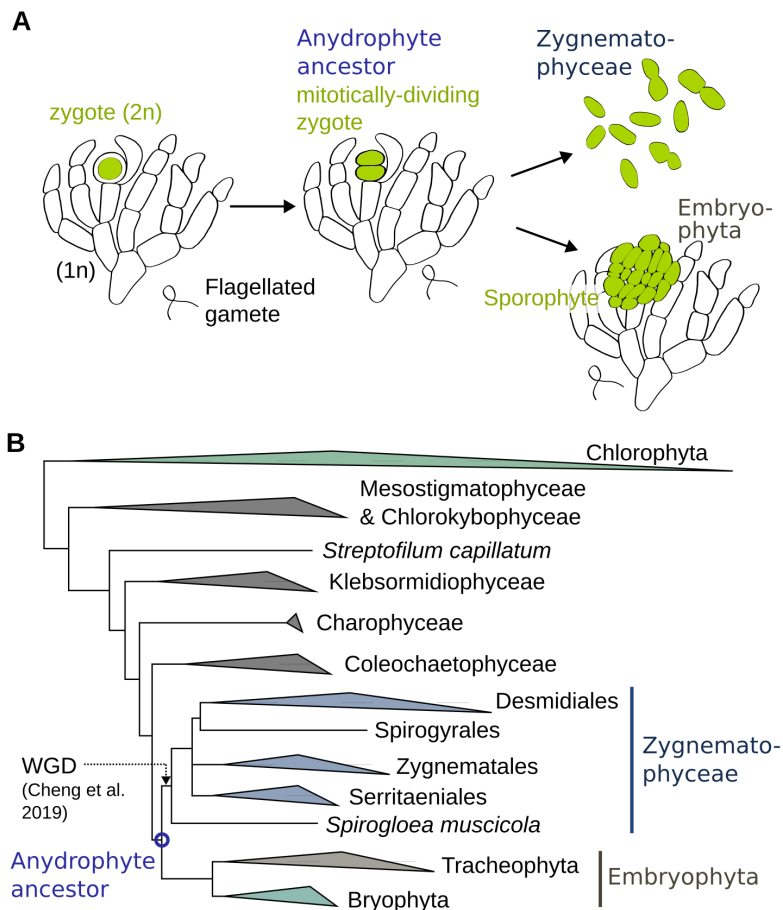


Figure 1. (A) Shared origins of the zygmatophytes and embryophyte sporophyte from mitotically-dividing zygotes of the anydrophyte ancestor. **(B)** A phylogenetic tree of streptophytes based on a 96-gene phylogenomic matrix (modified from Žárský and Eliáš 2025). A possible whole-genome duplication inferred by Cheng et al. (2019) is indicated.

Unusually short evolutionary branch leading from Anydrophyta to Zygnemato-phyceae

A recent phylogenomic analysis of streptophyte lineage (Žárský and Eliáš 2025; Fig. 1B) highlights again an interesting feature of the phylogenetic tree of Streptophyta after the split of Zygnemato-phyceae and the ancestor of land plants - i.e. very short branch leading to an immediate evolutionary radiation of Zygnemato-phyceae as compared to pre-embryophytes with crown group creation much later in the late Proterozoic/early Paleozoic (Su et al., 2021; Hu et al., 2025; Bastürk et al., 2026). This indicates, that: 1) If the common ancestor of zygmatophytes was indeed highly reduced single-cell lineage, as supported by several independent studies, this reduction must have happened fast not only in geological time, but also relative to the anagenesis of the zygnemato-phyceae lineage, which is well explained by above-described macro-evolutionary mechanism of abrupt Zygnemato-phyceae algal single cell origin. 2) Descendants of the stem Embryophyta lineage have experienced higher rates of extinction compared to the Zygnemato-phyceae lineages, which could be attributed to the higher vulnerability of multicellular organisms to mass extinctions during shifting climates (Luo et al. 2024).

Multicellular sporophyte-like genomic equipment of unicellular conjugating algae - some cell division regulators of diploid land plant embryo are active also in zygnemato-phytes

As we have concluded earlier (Žárský et al., 2022) and as implied in several previous phylogenomic analyses (e.g. Cheng et al. 2019), Zygnemato-phyceae genetic set up bears distinct vestiges of original anydrophyte multicellularity exemplified by number of transcription factors and receptor kinases. This multicellularity-related genetic hoard of

Anydrophyta was at the same time most probably the starting point for the further cellular elaboration after the abrupt Zygnematophyceae establishment - return to unicellularity. At the same time many genes and whole gene families were lost (e.g. Cheng et al., 2019; Petroll et al., 2025) - including those necessary for the biogenesis and the function of flagella. For our hypothesis it is important that zygote activating pair of transcription factors KNOX-BELL underwent evolutionary innovation in Anydrophytes with the establishment of clear KNOX1 family isoform and respective BELL partner co-evolution - and their recruitment for zygote cell division regulation in sporophyte of Embryophyta (Frangedakis et al., 2017; Dierschke et al., 2021 and Petroll et al., 2025). These KNOX-BELL partners are still expressed also in Zygnematophyceae along with the Anydrophyta originating WOX and MYB-3R cell cycle/cell division regulators functioning in production of sporophyte diploid multicellularity. Moreover, other transcription or epigenetic regulators contributing to sporophyte cell proliferation regulation are also expressed in zygnematophytes (GRAS, LDL/FLD, LOB2; Petroll et al., 2025). This indicates activity of a part of zygote mitotic division machinery also in zygnematophytes. This overall genomic set-up of Zygnematophyceae shared with embryophytes is well explained by the above proposed process of their sudden establishment by the initiation of anydrophyte zygote mitotic divisions and is in accord with the surprising close relationship between zygnematophytes and land plants.

***De novo* conjugation, sex evolution, and ploidy changes in the Zygnematophyceae life cycle**

The necessity to evolve new sexual mechanisms was a clear constraint imposed by the establishment of a mitotically dividing diploid cell lineage and the loss of oogamy and flagella in zygnematophytes (Sekimoto et al., 2023). In this scenario Zygnematophyceae ancestor initially relied exclusively on vegetative propagation by cell division and the conjugation process - allowing the re-establishment of the sexual process - evolved later. It might be relevant in this respect that sexual reproduction in the extant basal Zygnematophyceae *Spirogloea* clade is currently unknown (Cheng et al., 2019). It was proposed that sexual reproduction is an important driver of mobile genetic element proliferation; e.g. seemingly asexual streptophyte alga *Klebsormidium* features an extraordinarily low incidence of transposons in the genome. In this respect it might be also relevant that proportion of transposable elements in zygnematophyte genomes is distinctly lowest in basal *Spirogloea* as compared to *Mesotaenium* and *Penium* (Jiao et al., 2020). Importantly, the origin of Zygnematophyceae from a dividing diploid zygotes is biologically well explaining the unusual ploidy changes observed in few well studied cases of Zygnematophyceae life cycle (Haig 2010). Currently, the best description of the lifecycle dynamics of the DNA content available is that for placoderm desmid *Closterium ehrenbergii* (Hamada 1987). During the induction of gametogenesis in *Closterium*, 2C pre-gametes reduce non-meiotically DNA content to 1C level (1C - DNA content of gamete nuclei). 2C zygotes replicate DNA to 4C level before meiosis, which produces two “gones” - each with one surviving and one degenerating 1C nuclei. The surviving nucleus replicates first to 2C DNA and then to 4C content without cell division. After the division of the surviving 4C cells, the 2C status of vegetatively multiplying *Closterium* is restored, so that vegetative cells of *Closterium* are diploid (Hamada 1987). These complicated DNA content shifts during the life cycle seem to be widely occurring (e.g. in *Spirogyra*; Haig 2010) and might have evolved in relation to the original diploid status of the first Zygnematophyceae and the *de novo* sexual process evolution.

Timing and ecological context of Zygnematophyceae-Embryophyta split - convergent evolution of Zygnematophyceae and the zygomycetous fungi in Cryogenian?

Timing of crucial events in the Earth geobiology history is essential to understand environmental constraints to the evolution of life on our planet, but this challenging quest is objectively less and less supported by data as we move deeper into the past. Not surprisingly also the timing of Anydrophyta evolution and Embryophyta-Zygnematophyceae split - crucial processes in plant terrestrialization - is currently a highly controversial topic, as molecular clock analyses date events of plant evolution mostly much deeper than palaeobotanical inference (e.g. Su et al., 2021; Hu et al., 2023; Bastürk et al., 2026 vs. Boyce and Nelsen 2025). Based on the critical comparison and evaluation of published plant terrestrialization time-scenarios we proposed as a most probable time of Anydrophyta evolution and Embryophyta-Zygnematophyceae split Cryogenian Snowball Earth habitats - under variable drought stress imposed by a ten of millions of years of freezing conditions (Žárský et al., 2022). While multicellular, oogamous and flagellated pre-embryophytes survived in a relatively warmer, humid and mineral rich refugia, Zygnematophyceae adapted to oligotrophic glacial bare ice and supraglacial sediments by the switch to unicellularity and loss of flagella (Žárský et al., 2022). Conjugation sex might have evolved in the same period also in zygomycetous fungi (Naranjo-Ortiz and Gabaldón, 2019, “white scenario”) so that the loss of flagella and evolution of sex by conjugation in these two unrelated groups of organisms might represent an example of convergent Cryogenian epoch adaptation in organisms living on a stressful glaciated Rodinia continent (Žárský et al., 2022). The Cryogenian timing of initial plant terrestrialization events is now supported by new phylogenomic analyses (e.g. Bowles et al. 2024; Hu et al., 2025) and integrated into the hypothesis on polar-alpine ancestry of the extant surface biosphere ecosystem initiated by the relocation of ecosystems evolved on Snowball Earth (Hoffman 2025).

Conclusions

Surprising similarities between land plants and Zygnematophyceae cell biology machineries and physiology were summarized several times recently (e.g. Permann and Holzinger 2024) and are all well understandable within the evolutionary framework proposed here - the origin of Zygnematophyceae conjugating algae from mitotically dividing diploid anydrophyta zygotes. Whatever is the timing of the first multicellular streptophyte algae evolution, it is well supported, that already a common ancestor of Coleochaetophyceae and anydrophyta was a multicellular organism in the early stages of multicellularity evolution well corresponding to the theoretical framework of unstable multi- vs. uni- cellularity models of evolution in response to drought (Isakson et al., 2025). As from the extant organisms the class Coleochaetophyceae is the closest relative of Anydrophyta, it is an imperative to intensify functional biological analyses of these algae from all aspects of molecular cell biology to ecology to create a more informative basis for the understanding of Anydrophyta evolution and later Embryophyta-Zygnematophyceae split. Therefore, the life cycle and especially the zygotes of Coleochaetophyceae deserve utmost attention of plant biologists in the future as the sister clade of Anydrophyta. Based on our hypothesis - esp. functionality of KNOX-BELL, WOX and MYB-3R homologs of zygnematophytes should be tested in appropriate embryophyte mutants (both bryophyte and tracheophytes models). Bower’s idea (1908) of mitotically dividing zygote of a Coleochaete-like organism as the origin of sporophyte might well be extended also for the origin of Zygnematophyceae (Fig. 1A). Possible shared evolutionary origins of sporophyte and

conjugating zygnematophyte algae opens new perspective on basic processes of plant terrestrialization.

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References

- Baştürk, M. N., Kunz, C. F., Darienko, T., Pröschold, T., de Vries, J., & Chen, H. (2026). Phylogenomic frameworks from deep archaeplastid evolution to embryophyte diversification. *Current Biology*, 36(11), R522-R544.
- Bower FO. (1908). The origin of land flora: A theory based upon the facts of alternation. London : MacMillan & Co.
- Bowles, A. M. C., Williamson, C. J., Williams, T. A., & Donoghue, P. C. J. (2024). Cryogenian Origins of Multicellularity in Archaeplastida. *Genome biology and evolution*, 16(2), evae026.
- Boyce, C. K., & Nelsen, M. P. (2025). Terrestrialization: toward a shared framework for ecosystem evolution. *Paleobiology*, 51(1), 174-194.
- Celakovsky L. 1874. Ueber die verschiedenen Formen und die Bedeutung des Generationwechsels der Pflanzen. *Sitzungsberichte der koeniglichen Boehmischen Gesellschaft der Wissenschaften in Prague* 2: 21-61.
- Dierschke, T., Flores-Sandoval, E., Rast-Somssich, M. I., Althoff, F., Zachgo, S., & Bowman, J. L. (2021). Gamete expression of TALE class HD genes activates the diploid sporophyte program in *Marchantia polymorpha*. *Elife*, 10, e57088.
- Frangedakis, E., Saint-Marcoux, D., Moody, L. A., Rabbinowitsch, E., & Langdale, J. A. (2017). Nonreciprocal complementation of KNOX gene function in land plants. *New Phytologist*, 216(2), 591-604.
- Haig, D. (2010). What do we know about Charophyte (Streptophyta) life cycles?. *Journal of Phycology*, 46(5), 860-867.
- Hamada, J. (1987). Diploidy in DNA content in vegetative cells of *Closterium ehrenbergii* (Chlorophyta). *Journal of Phycology*, 23: 541-546.
- Hoffman, P. F. (2025). Ecosystem relocation on Snowball Earth: Polar– alpine ancestry of the extant surface biosphere?. *Proceedings of the National Academy of Sciences*, 122(20), e2414059122.

Hu, S. Y., Shi, G., Yang, C. A., Van de Peer, Y., Li, Z., & Xue, J. Y. (2025). Comprehensive sampling of mitochondrial genomes substantiates the Neoproterozoic origin of land plants. *Plant Communications*, 6(11).

Cheng, S., Xian, W., Fu, Y., Marin, B., Keller, J., Wu, T., ... & Melkonian, M. (2019). Genomes of subaerial Zygnematophyceae provide insights into land plant evolution. *Cell*, 179(5), 1057-1067.

Isaksson, H., Lind, P., & Libby, E. (2025). Adaptive evolutionary trajectories in complexity: Transitions between unicellularity and facultative differentiated multicellularity. *Proceedings of the National Academy of Sciences*, 122(4), e2411692122.

Jiao, C., Sørensen, I., Sun, X., Sun, H., Behar, H., Alseekh, S., ... & Rose, J. K. (2020). The *Penium margaritaceum* genome: hallmarks of the origins of land plants. *Cell*, 181(5), 1097-1111.

Luo, G., Liu, D., & Yang, H. (2024). Microbes in mass extinction: an accomplice or a savior? *National Science Review*, 11(1), nwad291.

Naranjo-Ortiz, M. A., & Gabaldón, T. (2019). Fungal evolution: major ecological adaptations and evolutionary transitions. *Biological Reviews*, 94(4), 1443-1476.

Permann, C., & Holzinger, A. (2024). Zygospore formation in Zygnematophyceae predates several land plant traits. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 379(1914).

Petroll, R., Varshney, D., Hiltemann, S., Finke, H., Schreiber, M., de Vries, J., & Rensing, S. A. (2025). Enhanced sensitivity of TAPscan v4 enables comprehensive analysis of streptophyte transcription factor evolution. *The Plant Journal*, 121(1), e17184.

Pringsheim N. (1876). Über den Generationswechsel der Thallophyten und seinen Anschluss an den Generationswechsel der Moose. *Monatsberichte der königlich Preussischen Akademie der Wissenschaften zu Berlin* 1876: 869-911.

Rensing, S. A. (2020). How plants conquered land. *Cell*, 181(5), 964-966.

Sekimoto, H., Komiya, A., Tsuyuki, N., Kawai, J., Kanda, N., Ootsuki, R., ... & Nishiyama, T. (2023). A divergent RWP-RK transcription factor determines mating type in heterothallic *Closterium*. *New Phytologist*, 237(5), 1636-1651.

Stebbins, G. L., & Hill, G. J. C. (1980). Did multicellular plants invade the land?. *The American Naturalist*, 115(3), 342-353.

Su, D., Yang, L., Shi, X., Ma, X., Zhou, X., Hedges, S. B., & Zhong, B. (2021). Large-scale phylogenomic analyses reveal the monophyly of bryophytes and neoproterozoic origin of land plants. *Molecular Biology and Evolution*, 38(8), 3332-3344.

Timme, R. E., Bachvaroff, T. R., & Delwiche, C. F. (2012). Broad phylogenomic sampling and the sister lineage of land plants. *PLoS one*, 7(1), e29696.

Turmel, M., Ehara, M., Otis, C., & Lemieux, C. (2002). Phylogenetic Relationships among Streptophytes as Inferred from Chloroplast Small and Large Subunit rRNA Gene Sequences. *Journal of Phycology*, 38(2), 364-375.

Wodniok, S., Brinkmann, H., Glöckner, G., Heidel, A. J., Philippe, H., Melkonian, M., & Becker, B. (2011). Origin of land plants: do conjugating green algae hold the key? *BMC Evolutionary Biology*, 11(1), 104.

Žárský, J., Žárský, V., Hanáček, M., & Žárský, V. (2022). Cryogenian glacial habitats as a plant terrestrialisation cradle-the origin of the anydrophytes and Zygnematophyceae split. *Frontiers in Plant Science*, 12, 735020.

Žárský, V., & Eliáš, M. (2025). Phylogenomics defines *Streptofilum* as a novel deep branch of streptophyte algae. *Current Biology*, 35(5), R171-R172.