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YC suggested the idea; AP said let's do it; YC and AP collected specimens; AP designed, performed, and interpreted the measurements and YC wrote the manuscript

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## INVITED ORIGINAL RESEARCH PAPER

# Fibonacci spirals in a brown alga [*Sargassum muticum* (Yendo) Fensholt] and in a land plant [*Arabidopsis thaliana* (L.) Heynh.]: a case of morphogenetic convergence

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**Abstract**

In this article, the morphology of a brown alga is revisited and compared to the phyllotaxis of land plants. The alga, *Sargassum muticum* (Yendo) Fensholt has a highly organized thallus with a stipe, the stem-like main axis, and hierarchically organized lateral branches of successive orders. Around each of these axes, the lateral organs: blades, side-branches, and receptacles grow in a spiral disposition. As in land plants, this organization is related to an apical mode of growth. Measurements performed along the mature differentiated axes as well as in their meristematic regions confirm the similarity of the large-scale organization of this brown alga with that of the land plants. In particular, the divergence angle between successive elements has similar values and it results from the existence around the meristem of parastichies having the same Fibonacci ordering. This is remarkable in view of the fact that brown algae (Phaeophyceae) and land plants (Embryophyta) are two clades that diverged approximately 1800 million years ago when they were both unicellular organisms. We argue that the observed similarity results from a morphogenetic convergence. This is in strong support of the genericity and robustness of self-organization models in which similar structures, here Fibonacci related spirals, can be obtained in various situations in which the genetic and physiological implementation of development can be of a different nature.

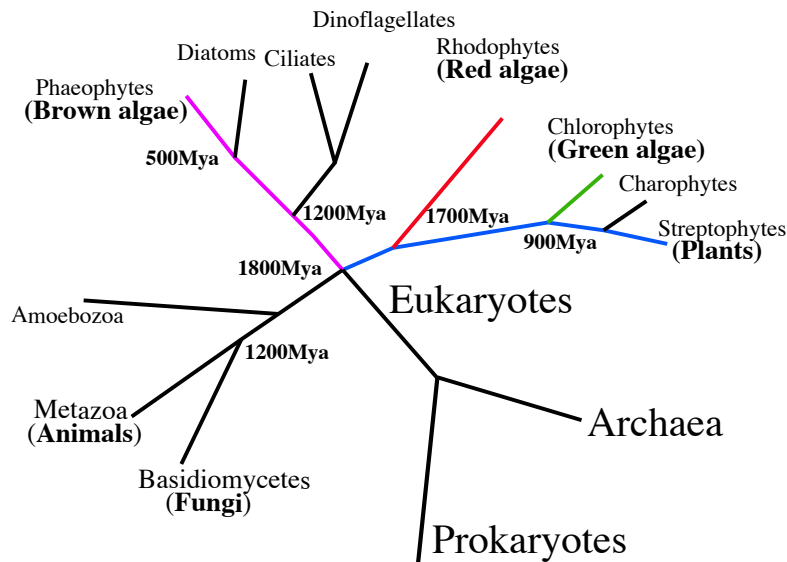
**Keywords**

phyllotaxis; Fibonacci; *Sargassum*; brown algae; self-organization; convergence

**Introduction**

Etymologically, phyllotaxis concerns the arrangement (*taxis*) of the leaves (*phyllon*) around the stems of plants. With this definition there can be, strictly speaking, no phyllotaxis in brown algae (Phaeophyceae). They do not belong to the same lineage as land plants and they do not have structures that are equivalent anatomically or physiologically to stems or leaves. Furthermore, it is now established by phylogenetic analysis that the most recent common ancestors to green plants and brown algae were unicellular eukaryotic organisms and that the divergence between these two clades (Fig. 1) occurred approximately 1800 million years ago (Mya) [1–3]. The transitions to multi-cellularity occurred independently in the two lineages therefore their large-scale structures cannot result from a common inheritance.

However, some brown algae have a morphology that resembles that of the aerial part of land plants. In particular, they usually have an axisymmetric stem-like structure (a stipe) with lateral branches. These primary branches have themselves lateral



**Fig. 1** Simple sketch of a phylogenetic diagram of the Eukaryotes showing the four main clades that have developed multicellularity: the brown algae, the red algae, the Chloroplastida (green algae, mosses and vascular plants), the metazoans (animals), and the fungi. This sketch and in particular the estimates for the times of divergence of the branches was done using the data available in references [1–3]. The common ancestor to brown algae and plants was a monocellular organism. The same is true for the common ancestor of brown and red algae. The estimated times for both divergences vary in the range from 1500 to 2000 Mya depending on the authors, but are now sufficiently similar.

appendages, blades and secondary branches and the repetition of this process gives the alga its global organization. At each level of the hierarchy, the lateral appendages are spirally organized around the axis on which they grow. This had been observed long ago and was described and discussed by Church in 1920 [4]. At that time, the huge phylogenetic distance separating plants and brown algae had not been measured. Mosses and algae were seen by Church as precursors of land plants, in which phyllotactic-like organizations had appeared first [4]. While this hypothesis may still be valid for mosses [5], it has become unrealistic for brown algae. Because of the evolutionary distance between brown algae and land plants, their organizations cannot be homologous in the biological sense of the term. It is our aim here to examine how analogous they can still be, and what could be the origin of this analogy.

It is useful to recall first the main characteristic of the classical spiral phyllotactic patterns observed in land plants to which we will refer. The first step in their investigation was the geometrical approach of Schimper [6], Braun [7], and A. and L. Bravais [8]. They showed that if the leaves along the stem are linked by their order of appearance they are disposed on a single spiral (the generative spiral). The basic quantity here is the divergence  $\phi$ , the angle between the orthoradial positions of two consecutive elements. Measurements of divergences on mature plants showed that they were surprisingly close to the golden section:  $\Phi = 2\pi(1 - \tau) \approx 137.5^\circ$ , where  $\tau = (-1 + \sqrt{5})/2 = 0.61803\dots$  is the golden mean. The other striking result is that in structures where a secondary elongation has not taken place (e.g., inflorescences) the elements (e.g., florets) are tightly packed and the structure is better characterized by parastichies, the conspicuous spirals linking each element to its nearest neighbors in space. The pattern is formed of a number  $i$  of spirals running in one direction, and  $j$  in the other. The striking feature is that  $i$  and  $j$  are usually two consecutive numbers of the Fibonacci series:  $\{F_k\} = \{1, 1, 2, 3, 5, 8, 13, 21, 34, \dots\}$  in which each term is the sum of the two previous ones (the first two terms being 1 and 1). When microscopic observations of the shoot apical meristem became possible, they revealed [9] how the future leaves appear as primordia in the annular region surrounding the central meristem. In this region in which the secondary elongation has not yet taken place, the primordia are tightly packed, the relative positions of two successive ones can be

characterized by the divergence angle  $\varphi$  between them and the plastochrone ratio  $R = r_n + 1/r_n$  (see below Fig. 4) introduced by Richards [10]. As for the global structure, it is better characterized by parastichies. This led Hofmeister [9] then Snow and Snow [11] to put forward hypotheses in which the spiral organization resulted from the iterative formation of successive repulsively interacting primordia around the central apical meristem. Correlatively, van Iterson [12] suggested that there was a geometric origin to this particular type of organization and that it was related to the possible dispositions of elements of finite size around a disk or along a cylinder. Using these hypotheses it was demonstrated (Douady and Couder [13]) that the Fibonacci spirals could be obtained by pure self-organization during the iterative formation of successive finite size primordia in the periphery of a stable apical meristem. The relevance of this self-organization process was demonstrated by obtaining phyllotactic patterns in a purely physical experiment in which no biological process was involved [13]. This was the precursor for systematic numerical simulations [14–16]. They showed that all the phyllotactic modes of plants: spiral, whorled, bijugates, etc., could be obtained with the same principles. Comparison of the results of this self-organization model with botanical data revealed that this hypothesis led to precise predictions as concerns the phyllotactic evolution during the whole ontogeny of a plant [17].

After this reminder we can turn to brown algae [18–21]. There are unfortunately relatively few quantitative measurements of the algae architecture. Traditionally, the main effort in phycology went into taxonomy and more recently into genetics. From both these viewpoints architecture is not trustworthy. For instance, in plants, taxonomists do not use phyllotaxis as a classification criterion. This mistrust is justified: on a lilac tree, while most branches grow in a decussate mode, some can be whorled or spiral, not due to a mutation, but simply after a severe pruning (Y. C. unpublished data). The genetic control on such assets as phyllotaxis is indirect since it creates the conditions in which a physical self-organization takes place. This also means that in reverse, as in the present work, observing a similar organization in two genera is in no way a guarantee of a genetic or evolutionary proximity.

The review article by Popper et al. [22] suggests that the green algae ancestors of land plants acquired their multicellularity 750 Mya, while the present brown algae (Phaeophyceae) became multicellular only 200 Mya, so that as multicellular structures, they would be younger than plants. Common brown algae present a much larger set of possible architectures than plants [18]. Many of them have a uniseriate filamentary structure, while others develop complex parenchymatous thalli. In the latter case, many different morphologies are observed, resulting from different meristematic types of growth. We limit ourselves to the spiral structures observed in Sargassaceae. This family, the largest in the class of Phaeophyceae (now approximately 350 species) was initially established by C. Agardh (1820) [23] as one of the five families of the Fucoideae.

An early description of the spiral disposition of laterals by Church [4] concluded to a relation to Fibonacci organization, but was limited to a single specimen of *Cystocleira ericoides* (Linnaeus) Agardh. A much wider set of data on organization of lateral branches in Sargassaceae can be found in the taxonomic study by Yoshida [24,25] for the Japanese species. They are mostly spiral but Yoshida describes them as having 1/2, 1/3, or 2/5 phyllotaxes, depending on the species. This terminology initially introduced by Schimper [6] refers to the angular positions of the lateral organs, such as leaves, around the stem. The denominator is the number of successive leaves separating two leaves having the same angular position, the numerator the number of turns of the generative spiral around the stem in between them. If the coincidence in angular positions was indeed perfect, the divergence angle would be in these three modes 180°, 120°, or 144°, respectively, and the organization would be linked to the rational numbers.

An origin of such organizations in Sargassaceae could lie in a global symmetry of the branch. The sections of the branches, as described by Yoshida [24], are often not cylindrical. Alternate dispositions 1/2 are observed in *Sargassum nigrifolium* or *S. giganteifolium* where the branches are flattened, 1/3 dispositions are found in *S. microcanthum*, in which the section is triangular. These observations seemed to suggest that a rational divergence angle could result from a global symmetry of the axis. In such a case the spiral organization would be different from the Fibonacci phyllotaxis which

is linked to irrational numbers. However, the terminology using fractions ( $1/2$ ,  $1/3$ , or  $2/5$ ) is ambiguous, being often used as a rational approximate of a really non-rational organization in which there is only near coincidence. To lift the ambiguity and assess the possible analogy with land plants, we re-examine in this article the spiral organization using quantitative measurements.

For that purpose we revisit the structure of *Sargassum muticum* (Yendo) Fensholt. This particular species has been widely investigated recently [26–29], mainly because of its invasive character. Originally from Japan, it reached western America in the 1940s and Europe in the 1970s. In both cases, it had been brought with Japanese oysters imported for aquaculture. A description of its global morphology can be found in Yoshida [24] and Critchley [29]. It can be summarized as follows.

This brown alga has a hierarchically organized thallus (Fig. 2). It is anchored on a substrate (e.g., a rock) by a holdfast, above which grows a stipe, a short cylindrical main axis that is perennial. This axis grows slowly through the activity of its apical meristem and generates primary laterals (fronds). These lateral branches are only locally submitted to the dominance of the apex of the stipe. Some distance from it they assume a fast growth during summer. By a large inter-nodal elongation, they become the largest structures of the seaweed and can be several meters long. Each of these primary lateral branches has a meristematic apex that produces lateral structures: leaf-like blades and axillary branches. The secondary lateral branches that they generate have a more limited growth, being submitted to apical dominance, but they also produce lateral tertiary branches. This hierarchical construction can continue to higher orders for very large specimens that were not investigated here. The branches of any order carry flattened blades attached to them by short stalks that make them look like leaves. Associated to these blades, the lateral branches of the next order grow in axillary position. On fertile fronds the reproductive receptacles are also axillary. A simple inspection of the branch organization shows that, at all hierarchical levels, the lateral elements are spirally arranged (Fig. 2). It also shows that the branches have a cylindrical section so that the global axisymmetry of the system is respected. We will measure as accurately as possible the orthoradial positions of the laterals on mature primary or secondary branches as well as the disposition of the primordia in the apical region.

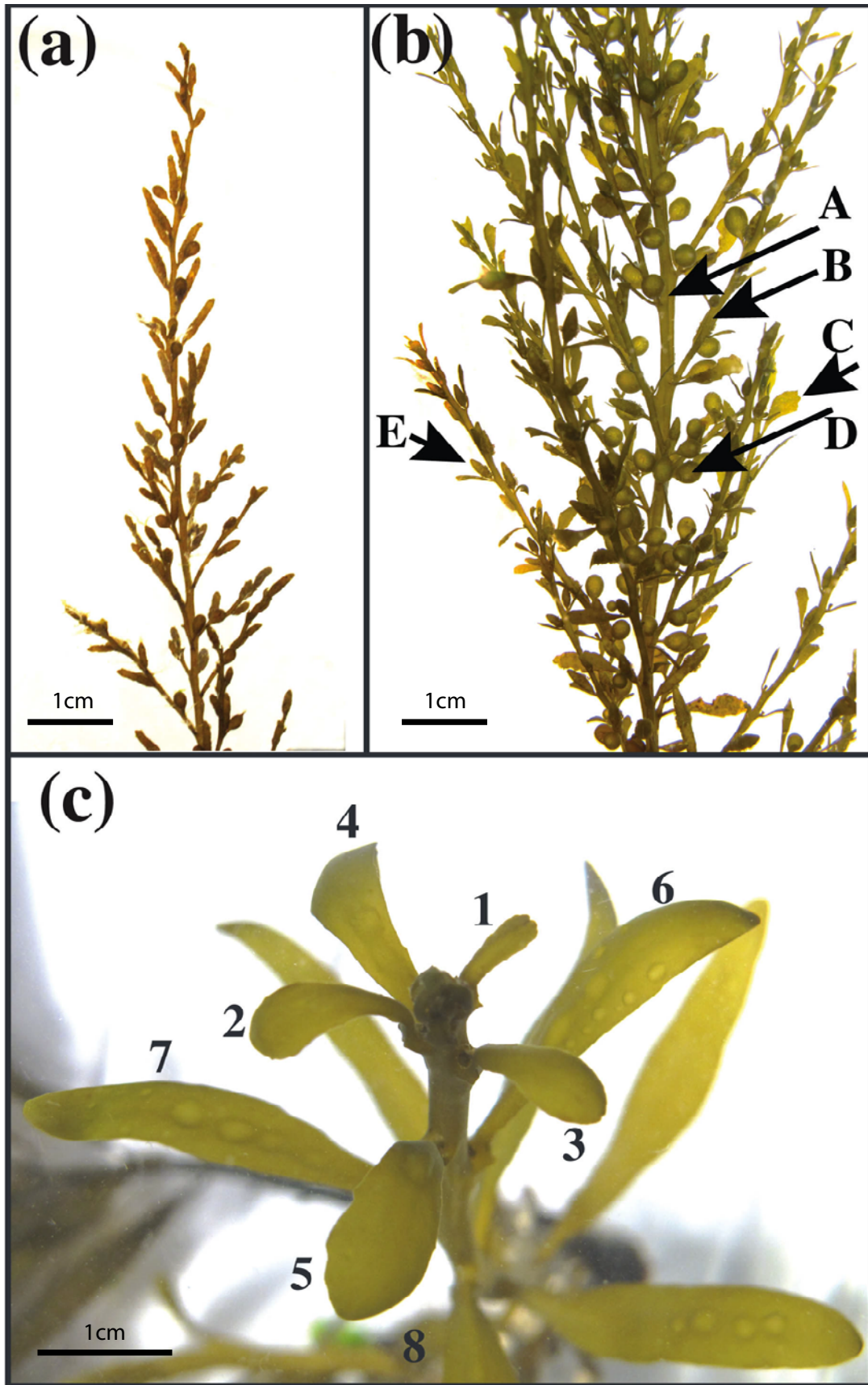
## Material and methods

The specimens we examined were collected in March in Roscoff (North Brittany, France), in April in Berder (south Brittany, France), and in the beginning of October in Dieppe (Normandy, France). They were collected at low tide in intertidal rock pools. They were kept in a cool seawater aquarium and examined shortly after returning to the laboratory. The specimens collected at different times of the year differed by the length of their fronds. Those collected in fall had lost most, but not all, of their longest reproductive fronds.

For the sake of comparison we also used in this work the specimens of a wild type ecotype *Ws Arabidopsis thaliana*. There were grown in greenhouses as described in Peaucelle [30].

Several types of measurement were done in order to correlate the spatial distribution of the lateral elements around the primary lateral branches with the structure of the apical region. The main difficulty in measuring the relative angular positions of the successive blades is linked to the lack of rigidity of algae (as compared to plants). For this reason we used a small apparatus described in Peaucelle [30] in which the branch is attached at both ends and held vertically. The axis of the branch coincides with the center of a 360° protractor. By sliding the protractor vertically we can measure the successive positions of the laterals.

The extremity of a branch is a tightly packed structure similar to the terminal bud of plants. The outer blades that cover it were cut so that the central region was observed with a low magnification microscope. After identification of the order of the successive primordia we measured their relative positions, using an ImageJ software [30,31].



**Fig. 2** Photographs of some parts of the thallus of *Sargassum muticum* (Yendo) Fensholt. **a** Terminal part of a fertile frond with its lateral appendages. **b** Close-up of mature region of a primary branch showing the various appendages. A – a primary lateral branch; B – an axillary secondary branch; C – a leaf-like blade; D – an air bladder (pneumatocyst) that contributes to keeping the branch buoyant; E – an axillary receptacle. **c** The extremity of a young primary branch showing the spiral arrangement of the blades (numbered by age).

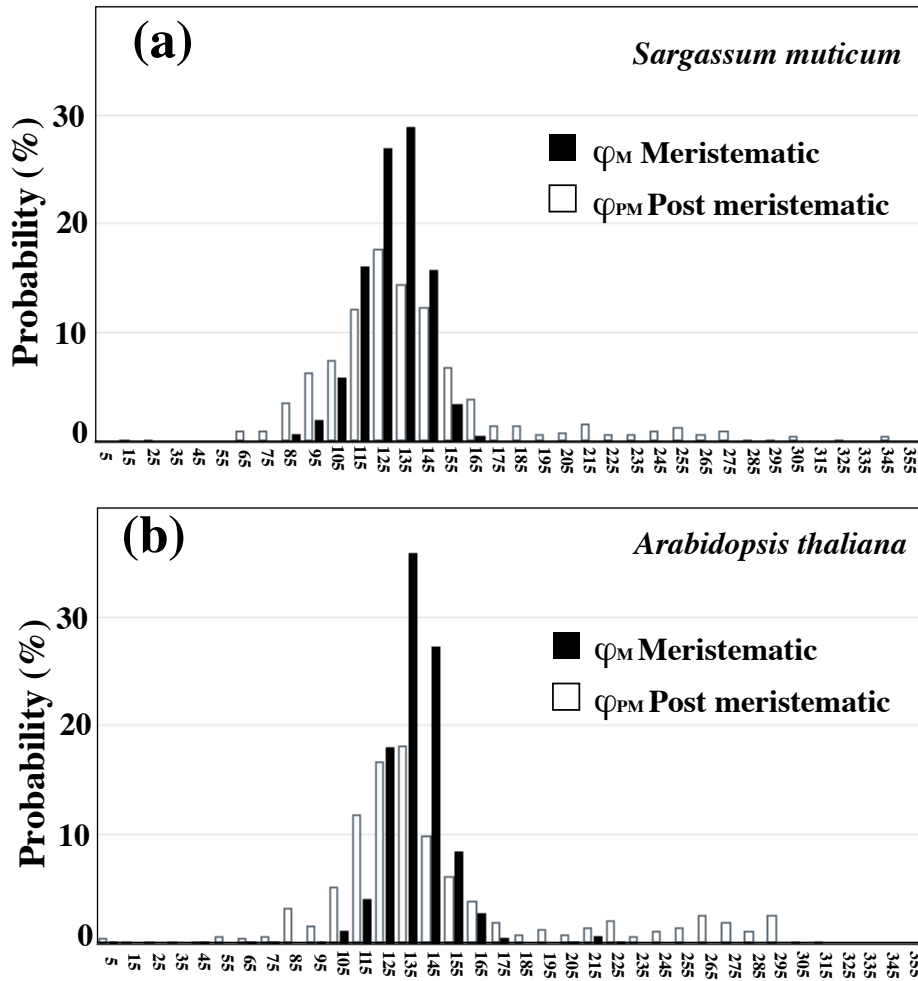
For cytology investigations, the *Sargassum* apices were fixed in sea-water FAA [formaldehyde 3.7%, acid acetic 5%, ethanol 50%, artificial sea water (Instant Ocean: 43, rue Gambetta, 57400 Srasbourg, France) 45%]. The *Arabidopsis* apices were fixed in pure water FAA (formaldehyde 3.7%, acid acetic 5%, ethanol 50%, water 45%). Both were dehydrated and imbedded in paraffin wax as described at <http://www.its.caltech.edu/~plantlab/protocols/insitu.htm>. Samples were then cut in 5- $\mu\text{m}$  slices using a Leica RM2245 Semi-Automated Rotary Microtom. Cuts were then positioned on Glass Probe One Plus (Fisher Biotechnology, USA) glass slides. The samples were then dewaxed using xylene, followed by rehydration using an ethanol series: 100% (v/v), 95%, 70%, 50%, 30%, and 10%, incubated in pure water for 20 minutes. Cell walls were stained using calcofluor white, nuclei using propidium iodide. Labeled sections were mounted in ProLong Gold Antifade Mountant (Life Technologies, USA) with a coverslip and sealed. Images were taken with a Zeiss LSM 700 confocal microscope equipped with a 203 0.8 numerical aperture (NA) dry objective.

## Results

As is usual for plants, a quantitative characterization of the spiral organizations was obtained by two types of measurements.

The first one concerns the disposition of the lateral appendages on a mature branch. Three photographs of the structure of a primary lateral are shown in Fig. 2. As in spirally organized land plants, the elements are disposed successively along the axis. The length of the “internodes” separating them is mostly approximately constant. In the samples that we measured it was of the order of 0.35 cm. It has been shown [27] that this elongation depends strongly on the season. If we link the appendages in their order of appearance, the resulting curve forms a single helix. This is analogous to the land plants generative spiral. Using a hundred specimens we measured the direction of winding of this helix and found that 54 grew clockwise and 46 anticlockwise. As for the land plants, the direction of winding is thus with equal probability left or right. We then measured the post-meristematic divergence angle, the orthoradial angular distance  $\varphi_{\text{PM}}$  between the bases of two successive blades when projected in a plane perpendicular to the main branch axis. Its measurement is more difficult than in land plants due to the lack of rigidity of the alga. For this reason, we had to stretch each branch vertically in the above-described experimental set-up before measuring all the divergence angles. We then repeated the procedure on several branches. Separate measurements were done on the disposition of the laterals along primary and secondary branches. The results being identical we could gather them in order to improve the statistics. We have thus collected 479 values of the divergence angle. Fig. 3a (white boxes) shows a probability distribution function of the measured values. The global average value for the angle is  $\langle \varphi_{\text{PM}}^{\text{Sarg}} \rangle = 143^\circ$ . As a comparison, we measured 467 divergences on *Arabidopsis thaliana* inflorescences in the same conditions and obtained  $\langle \varphi_{\text{PM}}^{\text{Arab}} \rangle = 155^\circ$  a result similar to that reported in Peaucelle et al. [30]. The distributions of the divergence angles are similar in *Arabidopsis* and *Sargassum*. In both cases, most of the values have a Gaussian distribution around a central value of approximately  $137.5^\circ$  but the average is shifted by the existence of long tails of very anomalous values. They are due to the local permutations of the order of primordia formation that will be discussed below.

All the branches of the alga are terminated by an apex of the type, shown in Fig. 4a. As in land plants, the most recently formed lateral blades cover and protect the meristem in a structure very similar to a plant terminal bud. When the older blades are micro-dissected the inner structure of the apex can be observed (Fig. 4b). As in land plants, there is a central zone equivalent to the apical dome. However, it is in the form of a pit instead of a dome. At low magnification, tightly packed multicellular primordia-like protrusions are observed, distributed around the central zone. The order of their formation can be deduced from both their distance to the center and their size so that they can be numbered by increasing age (see Fig. 4c). Their relative position can be measured by the plastochrone ratio and by the divergence angle  $\varphi_{\text{M}}$  between successive primordia. The mean value of the plastochrone ratio  $R = r_{n+1}/r_n$  in

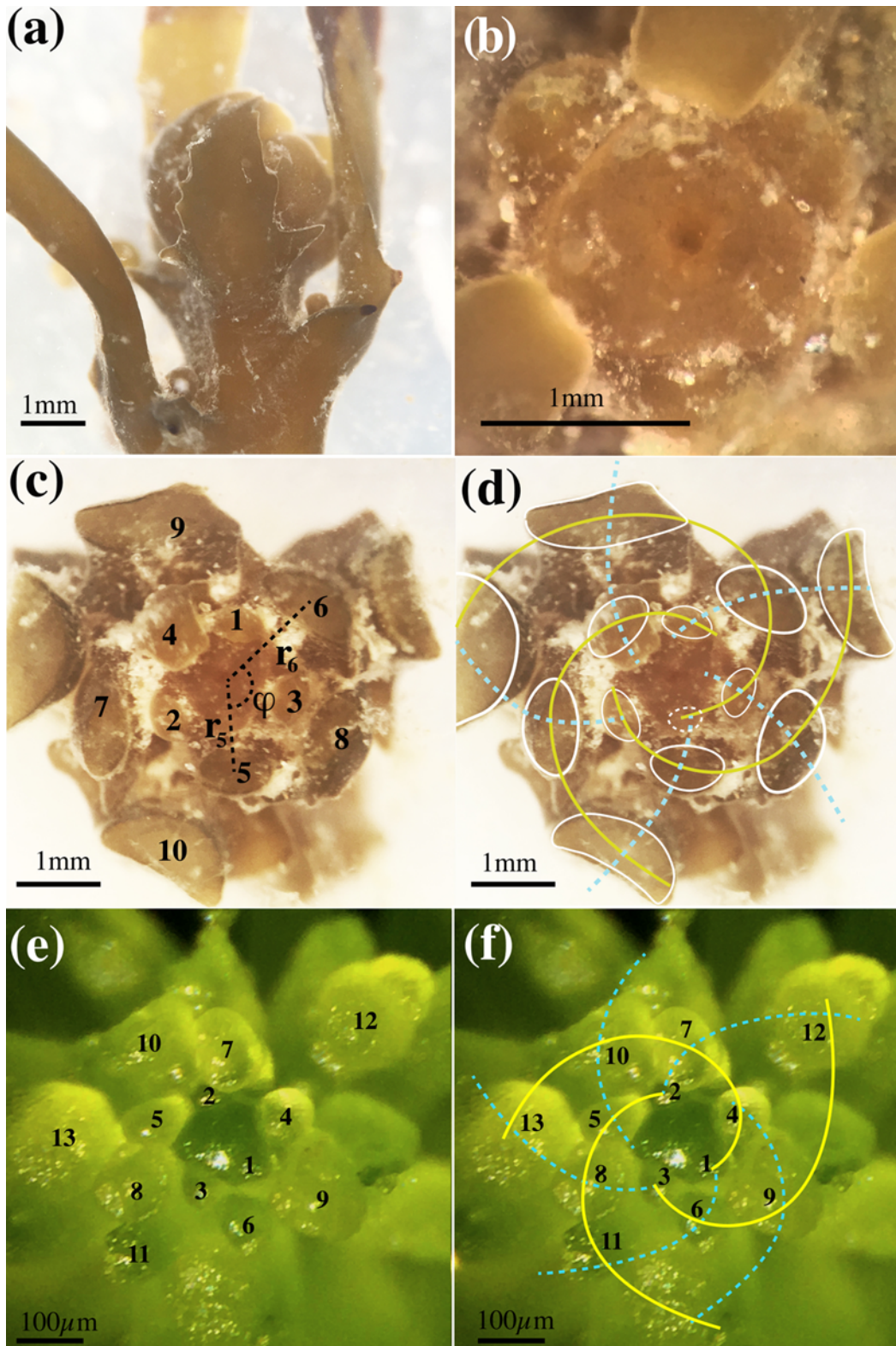


**Fig. 3** Probability distribution functions of the values of the divergence angle  $\varphi$ . **a** Measurements in *Sargassum muticum*. In white: statistical distribution obtained from 479 post-meristematic measurements (along the axis of branches); in black: statistical distribution from 474 apical measurements. **b** The same measurements in *Arabidopsis thaliana*. In white: results from 467 post-meristematic measurements; in black: from 551 apical measurements.

the case shown in Fig. 4c was  $\langle R^{Sarg} \rangle = 1.13$ . As for  $\varphi$ , we performed systematic measurements and Fig. 3a (black boxes) shows the histogram obtained from 474 of them. The mean value we obtained was  $\langle \varphi_M^{Sarg} \rangle = 138.8^\circ$  with a standard deviation of  $13^\circ$ . As a comparison, we measured 551 values of the divergence on *Arabidopsis thaliana* inflorescences (Fig. 3b, black boxes) and obtained  $\langle \varphi_M^{Arab} \rangle = 136.7^\circ$  with a standard deviation of  $14^\circ$ , a result similar to that reported in Peaucelle et al. [30]. The similarity of the distributions observed in *Sargassum* and *Arabidopsis* is striking. In both cases, all the measured divergence angles were distributed around the ideal angle  $137.5^\circ$ . Furthermore, both presented a similar variability.

We can also describe the primordia arrangements in the classical way used for plant apices. Because the primordia are tightly packed there, their distribution can be described by linking each element to its nearest neighbors. Two sets of intersecting parastichies with opposite directions of winding are thus defined. In Fig. 4d, we see the result of this geometrical construction on a *Sargassum* apex: it is characterized by three clockwise spirals and five anticlockwise spirals. In a usual terminology this is a regular ( $i = 3, j = 5$ ) Fibonacci pattern. This Fibonacci organization was observed in all the apices we measured. For comparison, we chose randomly the inflorescence apex of a wild type *Arabidopsis thaliana*, in which the mean plastochrone ratio turned out to be  $\langle R^{Arab} \rangle = 1.16$ , a value close to that in *Sargassum*  $\langle R^{Sarg} \rangle = 1.13$ . As can be seen by comparing Fig. 4c,d and Fig. 4e,f, and as expected from the self-organization models [14–16], the similarity of the values of  $\langle R \rangle$  leads to the same parastichy organizations ( $i = 3, j = 5$ ).





**Fig. 4** **a** Photograph of the apex of a primary lateral as seen from the side. As in plants the older lateral blades cover and protect the meristem in a global structure very similar to a bud. **b** The apex was micro-dissected and the older blades had been removed to reveal the pit-shaped apical “meristem”. **c,d** Organization of the “primordia” around the meristem. In (c) they are numbered with increasing age and their relative position is measured by their distance  $r_n$  to the center and by the divergence angle  $\phi$  that separates them. **d** The same apex on which the parastichies have been drawn. Note that if the phyllotaxis was a  $2/5$  organization with a  $144^\circ$  divergence, a primordium of order  $n$  would be radially aligned with the primordium of order  $n+5$ . This is clearly not what is observed. **e,f** The apical structure of *Arabidopsis thaliana* (wild type) inflorescence shows a very similar organization even though the meristem is a dome instead of a pit.

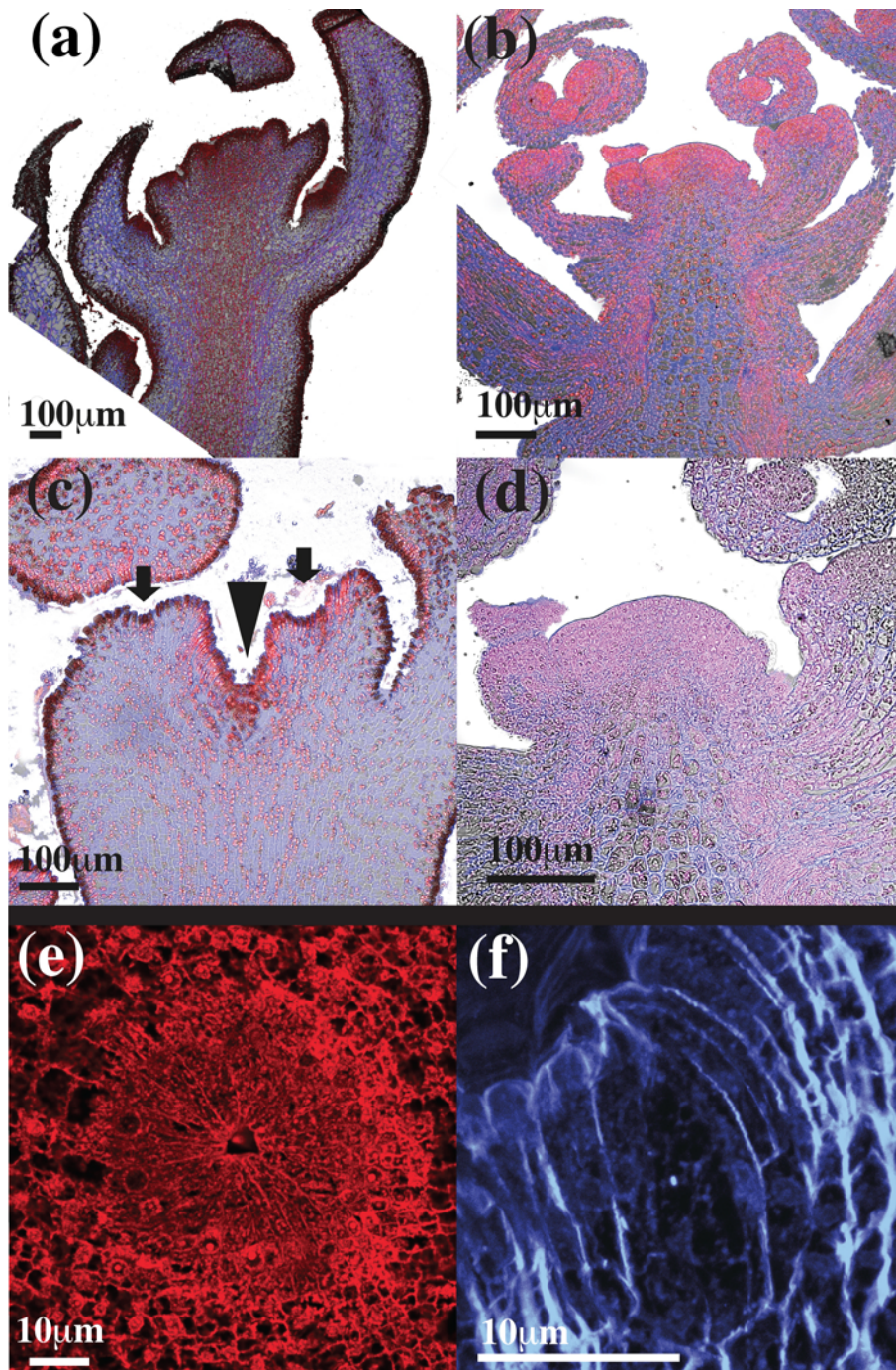
It is noteworthy that the parastichy order appears more regular than the values of the divergence angles and there is a difference in the probability distribution functions and average values obtained in the measurements of  $\varphi_{PM}$  and  $\varphi_M$ .

A first possible origin of the dispersion of the divergence values  $\varphi_{PM}$  is related to environment. In land plants, the stem is usually rigid, while the branches of *Sargassum* are very flexible. Being submitted to waves and tidal currents their axis can be twisted. The observation of drying branches shows that sometimes such twists have remained permanently. In this case, the measurements of  $\varphi_{PM}$  are disturbed. This could be at the origin of the slightly larger dispersion of the angles in *Sargassum* post-meristematic phyllotaxis than in the *Arabidopsis* plants grown in a windless environment.

However, the scatter in the values of  $\langle\varphi_{PM}\rangle$  and  $\langle\varphi_M\rangle$  has a more fundamental origin [15,17,32,33]. The phyllotactic patterns are generated sequentially by the formation of successive primordia. Each of them forms in an optimum position determined by its nearest neighbors. For this reason, the new primordium will contribute to the build up of an ordered and robust parastichy structure. However, the order in which successive sites become available around the meristem results from the existing pattern. It is thus determined by the history of the previous growth that built it up. When the phyllotaxis becomes complex, several locally favorable sites, all coherent with the parastichy order, become available around the meristem at approximately the same time. As was demonstrated by simulations [15] and by measurements performed on the stem of sunflowers [17], permutations in the order of formation of the primordia can then become frequent. The effect of a natural noise on phyllotaxis has been investigated recently by Mirabet et al. [32] and Refahi et al. [33] and was shown to generate such permutations, in excellent agreement with the botanical data. These permutations have little effect on the parastichy order but the plastochrone values as well as the sequence of angles are both strongly disturbed. This is why the histograms of  $\varphi_{PM}$  and  $\varphi_M$  differ slightly from each other (Fig. 3). The secondary elongation of the branches amplifies and reveals the fluctuations of the plastochrone. The rare permutations of the order of the laterals formation are thus easily observed along the axis. They are responsible for the very anomalous values ( $\varphi$  around  $\varphi \sim 85^\circ$  and  $\varphi \sim 220^\circ$ ) measured in the post-meristematic measurements. In contrast, these permutations remained usually undetected in the apical measurements because the irregularity of the plastochrone results in practically undetectable shifts in the primordia radial positions. The divergences due to these permutations can be filtered-out by eliminating in the post-meristematic measurements all the values of  $\varphi$  smaller than  $90^\circ$  and larger than  $180^\circ$ . The averages found on the truncated distributions are then  $\langle\varphi'_{PM}{}^{Sarg}\rangle \sim 134^\circ$  for *Sargassum* and  $\langle\varphi'_{PM}{}^{Arab}\rangle \sim 137^\circ$  for *Arabidopsis*. These values are in good agreement with those measured in the apex. They are also in agreement with the mean value  $\langle\varphi\rangle \sim 135.3^\circ$  measured by Church [4] on a single branch of *Cystoseira ericoides*.

So far we have seen that the phyllotactic pattern and its maintenance are strikingly similar between *Sargassum* and *Arabidopsis*. We will now examine the similarities and differences of the geometry in which these patterns are formed. At low magnification, as shown in Fig. 4b, the aspect of the apical structure with primordia forming at the periphery of a central zone appears very similar in the *Sargassum* and in the representative of a land plant. However, the observation of the meristematic region at high magnification reveals large differences. Fig. 5a–d shows a systematic comparison of two apices; longitudinal sections at two magnifications of a *Sargassum* apex (Fig. 5a,c) and similar sections of an apex of *Arabidopsis* during its transition to flowering (Fig. 5b,d). In *Sargassum*, the center of the meristem, instead of a dome, is a pit-like structure surrounded by smaller pits positioned in a phyllotactic pattern. The central pit is deeper than the surrounding ones. Each of these pits has a large cell at the bottom. The lateral pits are asymmetrical with an outer bulging part. These bulges grow fast to become the primordia-looking bumps seen in Fig. 4c, then the blades. The resulting structure will thus be similar to that observed in land plants differentiating into both a leaf-like structure (blade) and an axillary apex. However, in *Sargassum* the order of formation is reversed, the secondary pit (and thus the future lateral apex) forming before the blade primordium.

The shape of the cells at the bottom of the pits can be deduced by sections of the type shown in Fig. 5e,f. Its transverse section is triangular while its longitudinal section is oblong. This observation is in agreement with the central cells previously



**Fig. 5** a–d Comparison of longitudinal cuts through the apices of *Sargassum muticum* (a,c) and *Arabidopsis thaliana* (b,d) at different magnifications. These are composite images in which the gray scales correspond to transmission microscopy, the blue to calcofluor white (staining the cell walls), and the red to propidium iodide (staining the nuclei and the cell walls). (a) and (b) are low magnification cuts showing the terminal portions of the axes. (c) and (d) are zoomed-in images of the apical regions. In *Sargassum* (c), a large pit (arrow) is present in the center of the apical zone. It is surrounded by the thallus extensions containing smaller pits (arrows). In contrast, in *Arabidopsis* (d), the meristem is dome-shaped and surrounded by protruding primordia. e,f The central apical cell at the bottom of the pit in *Sargassum muticum*. In (e), a cut stained with propidium iodide shows its triangular transverse section. In (f) a longitudinal cut at a larger magnification and stained with calcofluor white shows its oblong section in this direction.

described in the Sargassaceae [4,18,19,25,34,35]. It can be noted that the terminal cell of *Sargassum* is in some aspects similar to the apical cells observed in mosses as first emphasized by Church [4]. There are differences however. In mosses the apical cell is tetrahedral and it is not at the bottom of a pit but forms the tip of a conical structure. Furthermore, as noticed by Church [4] the existence of this central cell of triangular section in Sargassaceae does not imply (as it does in mosses) a three-fold symmetry of the global structure. The phyllotactic organization appears here to be fixed at a larger scale by the growth of the global meristematic tissue surrounding this cell [34].

These first results call for a wider investigation of the organization of algae having an apically dominated growth. As noted in the introduction, other species of Sargassaceae were described as having 1/2 or 1/3 phyllotaxes. Some of them could be related to the deformations of the axis. However, in the cases of cylindrical symmetry, we believe that they are all Fibonacci related organizations, 1/2 being the distichous mode with  $\varphi = 180^\circ$  and 1/3 corresponding to apical parastichies ( $i = 1, j = 2$ ) that can have a divergence angle  $128^\circ \leq \varphi \leq 180^\circ$  [14]. This should be checked by observations of other apical structures, a task that could not be performed in the present work by lack of access to fresh specimens of different species. It can also be noted that after searching the available data on the morphologies of Sargassaceae we did not find any report on observations of whorled organizations (2,2) or (3,3) that are common in plants.

## Discussion and conclusion

The present investigation, though limited to one species, confirms a previous observation in another species of Sargassaceae [4] of Fibonacci spirals. Their similarity to those of land plants, necessarily result from two independent evolutionary processes, the last common ancestor of brown algae and land plants being a mono-cellular structure. It is thus a case of convergence. It is interesting to note that this convergence extends to other features of the architectures of these two distant groups of phototrophic eukaryotes. There is a clear similarity in the presence of axillary growth in both systems. Furthermore, a plant-like apical dominance has been demonstrated to exist in Sargassaceae [28].

Convergences can have two distinct origins. They can appear because they bring the same evolutionary advantage, or they can result from similar phenomena of self-organization. Here, it appears that the two types of convergences are present and cooperate to give a real similarity in form (homoplasy).

A well-known example of evolutionary advantage in the animal world is the acquisition of the ability to fly. There is a similarity in the acquired function but the means by which this function is obtained can be completely unrelated. For instance, there is no developmental or morphological relation between the wings of insects and those of birds.

There are such evolutionarily based similarities in land plants and brown algae leading to what looks like similar developmental “strategies”. As summarized by Bogaert et al. [20], brown algae and land plants, in spite of their phylogenetic distance, share many common developmental features. Both have an open-growth strategy, in which meristematic cells produce an unlimited sequence of new organs. In such lineages as the Sargassaceae, the meristems are able to divide in three dimensions generating parenchymatous tissues. This is due to mitotic divisions of cells that remain attached to each other, producing a multicellular tissue. These cells remain embedded in a rigid extracellular matrix having cellulose fibrils and maintain a cytoplasmic continuity through the formation of plasmodesmata. In both clades, the multi-cellularity has opened the possibility of building-up very large individuals that have two evolutionary advantages common to land plants and Sargassaceae. Lateral leaves or blades attached to branches are good organizations to collect light. Long branches are a way to release the reproductive structures in the regions of the surrounding fluid where they will be easily spread: by the wind, far from the ground for land plants and by water currents and waves, far from the sea floor, for algae. The incredibly rapid invasion of European coasts by *Sargassum muticum* is due to this efficiency.

As explored by D'Arcy Thompson [36], there is another possible origin of convergences. The organisms can use the same generic self-organization mechanisms. Living entities are part of the physical world and they use physical phenomena for their organization, but this exploitation of the same physical effects can be implemented by a different physiology.

The investigation of patterns resulting from growth phenomena in physics has revealed the existence of archetypes due to symmetries that generate morphological analogies in systems apparently unrelated to each other. This universality of morphological processes can be illustrated first by physical examples. In physics, two growth processes leading to the spontaneous formation of complex forms are viscous fingering and solidification (reviewed in Couder [37]). In the former, a fluid of weak viscosity pushes a fluid of high viscosity out of, e.g., a porous medium. In the latter, a crystal grows in, e.g., an undercooled salt solution. In both situations, there is propagation of an interface separating two media. A flat interface is not a stable solution so that it destabilizes and protrusions form and grow. The remarkable feature is that in spite of the different nature of these systems, in both of them these protrusions can have the same two possible morphologies [37]. If the front is homogeneous and isotropic, the protrusions will be smooth and destabilize dichotomously (by “a tip splitting instability“ for the physicists). If the system is anisotropic, or if it has localized singularities, the tips of the protrusions become stable and they form the axis of large structures endowed with lateral branches (“by a side-branching instability“ for the physicists).

The parenchymatous growth of either algae or land plants is characterized by the coexistence of two dominant morphologies. Amongst brown algae, the growth of a thallus characterized by repeated dichotomous divisions is frequent (as in, e.g., *Fucus vesiculosus*), but in Sargassaceae growth generates stable axes. The same two organizations exist in land plants. Dichotomous divisions are observed in liverworts (Marchantiophyta) while vascular plants have shoots forming stable axes of growth. There is thus in both clades coexistence of two morphologies with a remarkable analogy with those obtained in the above-mentioned physics experiments (as briefly noted in Couder [38]).

The idea of a convergence due to the existence of growth archetypes is reinforced by the possible observation of similar morphologies in a third clade, the red algae (Rhodophyceae). As shown in Fig. 1, they have diverged from Phaeophyceae 1800 Mya and from land plants approximately 1700 Mya. Some of the species [18] have developed a form of pseudo-parenchymatous growth. Although they are unrelated to brown algae, similar morphologies are observed. For example, in *Chondrus crispus* the growth is characterized, as in *Fucus*, by repeated dichotomous divisions. In *Laurencia*, there is a stable apex and growth of axes with lateral appendages. Furthermore, there is evidence (though poorly documented) of a spiral organization when the axes are cylindrical as for instance in *Laurencia papillosa* or in *Laurencia pinnatifida*. A drawing of a transverse section of an apex of the latter species by Kylin [39], quoted by Fritsch (see Fig. 204 in [18]) exhibits parastichies ( $i = 5, j = 8$ ) hinting that this may be one more case of Fibonacci organization. Unfortunately, information on the corresponding apical structures in red algae is very scarce.

The fact that Fibonacci spirals result from a self-organization was demonstrated by the possibility of obtaining them even in physics experiments having no relation to biology [13]. Naturally, this way of understanding the dynamics triggers many questions that concern the genetic and physiological processes by which the necessary geometry and interactions are implemented in plants, in brown algae and possibly in red algae. Owing to the remarkable robustness of the self-organization process, these implementations could be quite different from each other. They are well beyond the scope of the present article; we can only add a few comments.

We can first consider the possible origin of the very anisotropic growth of land plants and Sargassaceae. In land plants it has been clarified. There is a self-induced phenomenon in which the growth itself generates mechanical stresses that orient the cortical microtubules of the cells [40]. This is followed by an oriented deposition of cellulose microfibrils in the cell walls that corsets the stem below the apical meristem. There is (at present) no evidence in Sargassaceae for a generation of anisotropy by a similar mechanism since their vegetative cells do not have the same cortical

microtubule organization [41]. However, they have peripheral actin that acts on the cellulose deposition [41]. Another possibility is that, as noted in physics experiments [37,38], an anisotropic type of growth could also be triggered by a unique singularity at the tip. In Sargassaceae, the apical cell, always present at the center of the meristem, could form such a singularity and its presence could, by itself, be responsible for the stabilization of the axial growth

Several recent works have led to a better understanding of the formation and disposition of the laterals in plants. The formation of primordia is linked with a forced flow of auxin pumped from cell to cell by PIN proteins [42]. The concentration of auxin in convergence regions modifies the mechanical properties of the cellular tissue. As shown by Peaucelle et al. [43,44], the tissue is softened by the demethylesterification of the cell walls pectins in sub-epidermal cell layers, resulting in the formation of bulging multicellular primordia.

In algae, the investigation of the role of phytohormones (auxins, gibberellins, cytokinins) is complex [45,46], because of the diversity of the clades. A recent review can be found in Tarakhovskaya et al. [47]. As concluded in this article, there is no doubt that these hormones play a role in growth and morphogenesis, and tantalizing data indicates that their polar transport is also important in differential growth [48]. It will be interesting to see if the signaling pathways involving auxin and its polar transport could contribute to the convergence of morphologies at the multicellular level.

It is remarkable that similar global morphologies are observed in land plants and in brown algae. In both clades, the evolution of multicellularity has led to comparable modes of growth. However, competition has not (or has not yet) led to the same distribution of the various morphologies. On land, some of the non-vascular liverworts (Marchantiophyta) have a morphology (see, e.g., *Metzgeria furcata*) comparable to the dichotomous organizations common amongst brown and red algae. However, they are only present in limited ecological niches and the vascular plants with an axial structure have become dominant almost everywhere. This is not the case in the sea where competition between algae has not led to such a strong domination of one type of morphology over the others. However, the invasive character of *Sargassum* could be an indication that in this environment too axial structures are favored. Will exobiology reveal that on outer planets, as on Earth, the axial growth and Fibonacci organization are dominant amongst motionless multicellular entities? For the time being, there is little risk of seeing this hypothesis disproved!

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### References

1. Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D. A molecular timeline for the origin of photosynthetic eukaryotes. *Mol Biol Evol.* 2004;21:809–818. <https://doi.org/10.1093/molbev/msh075>
2. Keeling PJ, Burger G, Durnford DG, Lang BF, Lee RW, Pearlman RE, et al. The tree of eukaryotes. *Trends Ecol Evol.* 2005;20:671–676. <https://doi.org/10.1016/j.tree.2005.09.005>
3. Hedges SB, Marin J, Suleski M, Paymer M, Kumar S., Tree of life reveals clock-like speciation and diversification. *Mol Biol Evol.* 2015;32:835–845. <https://doi.org/10.1093/molbev/msv037>
4. Church AH. On the interpretation of phenomena of phyllotaxis. London: Humphrey Milford and Oxford University Press; 1920. (Botanical Memoirs).
5. Shaw AJ, Szövényi P, Shaw B. Bryophyte diversity and evolution: windows into the early evolution of land plants. *Am J Bot.* 2011;98:352–369. <https://doi.org/10.3732/ajb.1000316>

6. Schimper KF. Beschreibung des *Symphytum Zeyheri* und seiner zwei deutschen Verwandten der *S. bulbosum* Schimp und *S. tuberosum* Jacq. Heidelberg: Winter; 1835.
7. Braun A. Vergleichende Untersuchung über die Ordnung der Schuppen an den Tannenzapfen. *Nova Acta Academiae Caesareae Leopoldino-Carolinae*. 1831;15:195–402. <https://doi.org/10.5962/bhl.title.69200>
8. Bravais L, Bravais A. Essai sur la disposition des feuilles curvisériées. *Annales des Sciences Naturelles. Botanique*. 1837;7:42–110.
9. Hofmeister W. Allgemeine Morphologie der Gewächse, Handbuch der Physiologischen Botanik. Leipzig: Engelmann; 1868.
10. Richards FJ. Phyllotaxis: its quantitative expression and relation to growth in the apex. *Philos Trans R Soc Lond B*. 1951;235:510–564. <https://doi.org/10.1098/rstb.1951.0007>
11. Snow M, Snow R. Minimum area and leaf determination. *Proc R Soc Lond B Biol Sci*. 1952;139:545–566. <https://doi.org/10.1098/rspb.1952.0034>
12. van Iterson G. Mathematische und microscopisch-anatomische Studien über Blattstellungen, nebst Betrachungen über den Schalenbau der Miliolinen. Jena: G. Fisher; 1907.
13. Douady S, Couder Y. Phyllotaxis as a physical self-organized growth process. *Phys Rev Lett*. 1992;68:2098–2101. <https://doi.org/10.1103/PhysRevLett.68.2098>
14. Douady S, Couder Y. Phyllotaxis as a dynamical self organizing process Part I: the spiral modes resulting from time-periodic iterations. *J Theor Biol*. 1996;178:255–273. <https://doi.org/10.1006/jtbi.1996.0024>
15. Douady S, Couder Y. Phyllotaxis as a dynamical self organizing process Part II: the spontaneous formation of a periodicity and the coexistence of spiral and whorled patterns. *J Theor Biol*. 1996;178:275–294. <https://doi.org/10.1006/jtbi.1996.0025>
16. Douady S, Couder Y. Phyllotaxis as a dynamical self organizing process Part III: the simulation of the transient regimes of ontogeny. *J Theor Biol*. 1996;178:295–312. <https://doi.org/10.1006/jtbi.1996.0026>
17. Couder Y. Initial transitions, order and disorder in phyllotactic patterns: the ontogeny of *Helianthus annuus*, a case study. *Acta Soc Bot Pol*. 1998;67:129–150. <https://doi.org/10.5586/asbp.1998.016>
18. Fritsch FE. The structure and reproduction of the algae. Cambridge: Cambridge University Press; 1945.
19. Charrier B, Le Bail A, de Reviers B. Plant proteus: brown algal morphological plasticity and underlying developmental mechanisms. *Trends Plant Sci*. 2012;17(8):468–477. <https://doi.org/10.1016/j.tplants.2012.03.003>
20. Bogaert KA, Arun A, Coelho SM, de Clerck O. Brown algae as a model for plant organogenesis. *Methods Mol Biol*. 2013;959:97–125. [https://doi.org/10.1007/978-1-62703-221-6\\_6](https://doi.org/10.1007/978-1-62703-221-6_6)
21. Jensen JB. Morphological studies in Cystoseiraceae and Sargassaceae (Phaeophyceae), with special reference to apical organization. Berkeley, CA: University of California Press; 1974. (University of California Publications in Botany; vol 68).
22. Popper ZA, Michel G, Herve C, Domozych DS, Willats WGT, Tuohy MG, et al. Evolution and diversity of plant cell walls: from algae to flowering plants. *Annu Rev Plant Biol*. 2011;62:567–590. <https://doi.org/10.1146/annurev-arplant-042110-103809>
23. Agardh CA. Species algarum rite cognitae, cum synonymis differentiis specificis et descriptionibus succintis. Vol. 1, Part 1. Lundae: E. Mavritii; 1820.
24. Yoshida T. Japanese species of *Sargassum* subgenus *Bactrophyucus* (Phaeophyta, Fucales). *Journal of the Faculty of Science, Hokkaido University. Series 5, Botany*. 1983;13(2):99–246.
25. Yoshida T, Majima T, Marui M. Apical organization of some genera of Fucales (Phaeophyta) from Japan. *Journal of the Faculty of Science, Hokkaido University. Series 5, Botany*. 1983;13(1):49–56.
26. Norton TA. The growth and development of *Sargassum muticum*. *J Exp Mar Bio Ecol*. 1977;26:41–53. [https://doi.org/10.1016/0022-0981\(77\)90079-X](https://doi.org/10.1016/0022-0981(77)90079-X)
27. Kane DF, Chamberlain AHL. Laboratory growth studies on *Sargassum muticum* (Yendo) Fensholt. I. Seasonal growth of whole plants and lateral sections. *Botanica Marina*. 1978;22:1–9. <https://doi.org/10.1515/botm.1979.22.1.1>
28. Chamberlain HL, Gorham J, Kane DF, Lewey SA. Laboratory growth studies on

- Sargassum muticum* (Yendo) Fensholt. II. Apical dominance. *Botanica Marina*. 1978;22:11–19.
29. Critchley AT. *Sargassum muticum*: a morphological description of European material. *J Mar Biol Assoc U K*. 2009;63:813–824. <https://doi.org/10.1017/S002531540007123X>
  30. Peaucelle A, Morin H, Traas J, Laufs P. Plants expressing a *miR164*-resistant *CUC2* gene reveal the importance of postmeristematic maintenance of phyllotaxy in *Arabidopsis*. *Development*. 2007;134(6):1045–1050. <https://doi.org/10.1242/dev.02774>
  31. Yang W, Schuster C, Beahan CT, Charoensawan V, Peaucelle A, Basic A, et al. Regulation of meristem morphogenesis by cell wall synthases in *Arabidopsis*. *Curr Biol*. 2016;26:1404–1415. <https://doi.org/10.1016/j.cub.2016.04.026>
  32. Mirabet V, Besnard F, Vernoux T, Boudaoud A. Noise and robustness in phyllotaxis. *PLoS Comput Biol*. 2012;8(2):e1002389. <https://doi.org/10.1371/journal.pcbi.1002389>
  33. Refahi YG, Farcot E, Jean-Marie A, Pulkkinen M, Vernoux T, Godin C. A stochastic multicellular model identifies biological watermarks from disorders in self-organized patterns of phyllotaxis. *eLife*. 2016;5:e14093. <https://doi.org/10.7554/eLife.14093>
  34. Klemm MF, Hallam ND. Branching patterns and growth in *Cystophora* (Fucales, Phaeophyta). *Phycologia*. 1987;26:252–261. <https://doi.org/10.2216/i0031-8884-26-2-252.1>
  35. Katsaros CI. Apical cells of brown algae with particular reference to Sphacelariales, Dictyotales and Fucales. *Phycological Res*. 1995;43:43–59. <https://doi.org/10.1111/j.1440-1835.1995.tb00004.x>
  36. D'Arcy Thompson W. On growth and form. Vols 1 and 2. Cambridge: Cambridge University Press; 1917. <https://doi.org/10.5962/bhl.title.11332>
  37. Couder Y. Viscous fingering as an archetype for growth patterns. In: Batchelor GK, Moffat HK, Worster MG, editors. *Perspectives in fluid dynamics*. Cambridge: Cambridge University Press; 2000. p. 53–104.
  38. Couder Y. Patterns with open branches or closed networks: growth in scalar or tensorial fields. In: Fleury V, Gouyet JF, Leonetti M, editors. *Branching in nature*. Berlin: EDP Sciences and Springer; 2001. p. 1–22. [https://doi.org/10.1007/978-3-662-06162-6\\_1](https://doi.org/10.1007/978-3-662-06162-6_1)
  39. Kylin H. Studien über die Entwicklungsgeschichte der Florideen. Stockholm: Almqvist & Wiksells; 1923. [Kungliga Svenska Vetenskapsakademiens Handlingar; vol 63(11)].
  40. Katsaros C, Karyophyllis D, Galatis B. Cytoskeleton and morphogenesis in brown algae. *Ann Bot*. 2006;97:679–693. <https://doi.org/10.1093/aob/mcl023>
  41. Hamant O, Heisler MG, Jönsson H, Krupinski P, Uyttewaal M, Bokov P, et al. Developmental patterning by mechanical signals in *Arabidopsis*. *Science*. 2008;322:1650–1655. <https://doi.org/10.1126/science.1165594>
  42. Heisler MG, Hamant O, Krupinski P, Uyttewaal M, Ohno C, Jönsson H, et al. Alignment between PIN1 polarity and microtubule orientation in the shoot apical meristem reveals a tight coupling between morphogenesis and auxin transport. *PLoS Biol*. 2010;8:e1000516. <https://doi.org/10.1371/journal.pbio.1000516>
  43. Peaucelle A, Louvet R, Johansen JN, Salsac F, Morin H, Fournet F, et al. Transcription factor BELLRINGER modulates phyllotaxis by regulating the expression of a pectin methylesterase in *Arabidopsis*. *Development*. 2011;138:4733–4741. <https://doi.org/10.1242/dev.072496>
  44. Peaucelle A, Braybrook SA, Le Guillou L, Bron E, Kuhlemeier C, Höfte H. Pectin-induced changes in cell wall mechanics underlie organ initiation in *Arabidopsis*. *Curr Biol*. 2011;21:1720–1726. <https://doi.org/10.1016/j.cub.2011.08.057>
  45. Augier, H. Les hormones des algues. Etat actuel des connaissances. VII. *Applications conclusions et bibliographie*. *Botanica Marina*. 1978;21:175–197. <https://doi.org/10.1515/botm.1978.21.3.175>
  46. Bradley PM. Plant hormones do have a role in controlling growth and development of algae. *J Phycol*. 1991;27:317–321. <https://doi.org/10.1111/j.0022-3646.1991.00317.x>
  47. Tarakhovskaya ER, Maslov YI, Shishova MF. Phytohormones in algae. *Russ J Plant Physiol*. 2007;54:163–170. <https://doi.org/10.1134/S1021443707020021>
  48. Sun H, Basu S, Brady SR, Luciano RL, Muday GK. Interactions between auxin transport and the actin cytoskeleton in developmental polarity of *Fucus distichus* embryos in response to light and gravity. *Plant Physiol*. 2004;135:266–278. <https://doi.org/10.1104/pp.103.034900>