



Plant structure ontology: How should we label plant structures with doubtful or mixed identities? *

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Abstract

This paper discusses problems with labelling plant structures in the context of attempts to create a unified Plant Structure Ontology. Special attention is given to structures with mixed, or doubtful identities that are difficult or even impossible to label with a single term. In various vascular plants (and some groups of animals) the structural categories for the description of forms are less distinct than is often supposed. Thus, there are morphological misfits that do not fit exactly into one or the other category and to which it is difficult, or even impossible, to apply a categorical name. After presenting three case studies of intermediate organs and organs whose identity is in doubt, we review five approaches to categorizing plant organs, and evaluate the potential of each to serve as a general reference system for gene annotations. The five approaches are (1) standardized vocabularies, (2) labels based on developmental genetics, (3) continuum morphology, (4) process morphology, (5) character cladograms. While all of these approaches have important domains of applicability, we conclude that process morphology is the one most suited to gene annotation.

Key words: Character cladogram, Continuum, Evo-devo, Flower, Flowering plants, Gene ontology, Intermediate organs, Leaf, Morphological nomenclature, Organ identity, Plant ontology, Plant structure ontology, Root

Introduction

For practical investigation of morphology and phylogeny reconstruction, most botanists still work with metaphors or concepts that view morphological characters as “frozen in time,” as static “slices” of a continuous process.

Ingrouille & Eddie (2006, p. 133)

Modern plant science involves a multidisciplinary approach to understanding plant structure, function and development. The results of these investigations are summarized in botanical textbooks such as Cutler *et al.* (2008), which provide comprehensive overviews of all aspects of plant structure, phylogeny and genetics. Much of this exciting work involves molecular/genetic research, which is beginning to provide answers to longstanding questions. Yet these advances should not blind us to the epistemological and linguistic shortcomings that can creep into the work from old ways of thinking. We should not apply outmoded ways of thinking to these new areas without carefully weighing the consequences. Categorical thinking is one mode of thought of which we should be cautious.

That categorical thinking can be a problem has been recognized by Ingrouille & Eddie (2006, p. 123) in their introductory textbook *Plants—Diversity and Evolution*: “We see the world of plants as discrete objects rather than as processes. Individuals too become further divided into discrete parts or characters, which are, in essence, symbolic rather than biological. These categories form the basis of all homology concepts. This has powerful implications for the way we classify plants and study their evolution and phylogeny, for we are liable to confuse the metaphor with the real thing.” In other words, the use of categorical concepts may shape our perception to the point that we begin to believe that the natural parts of plants are the parts we name. This is the old problem of essentialism in a new guise.

Thoughtful and critical essays on how we perceive and conceive of the plant world have been written by a number of authors (Kirchoff 2001; Vergara-Silva 2003; Kirchoff *et al.* 2007; Faria 2008; among others). Faria (2008) points out that “there are many concepts in biology, which the widespread use seems to legitimate and vulgarize, but are still very problematic, lacking a precise definition.” With respect to biological systematics Sober (1993, p. 153) mentions “We must recognize that our concepts are not logically perfect. They, like organisms themselves, get along reasonably well in their normal habitats but may be seriously ill-suited to coping with unusual circumstances.” Even in earlier decades biologists have been aware of these problems. For example, Arber (1950), Bertalanffy (1955), and Sattler (1986) emphasised the relativity of scientific categories.

The present paper stresses the fact that—at least in plant biology—most structural categories used for describing and understanding organisms are less clearly and crisply defined than is often thought. In the three case studies presented below we describe leaves, roots, and flowers that transcend to some degree the typical plan of construction of plant organs found in model organisms such as *Antirrhinum*, *Arabidopsis* and *Zea*. We need to be aware of the poorly defined nature and relativity of even such common terms as these. Understanding the limitations of categorical terms has important consequences for future research in evolutionary and developmental genetics.

Morphological nomenclature: The Plant Structure Ontology

The Plant Structure Ontology (PSO: www.plantontology.org) is a controlled vocabulary of botanical terms describing morphological and anatomical structures representing organ, tissue and cell types, and their relationships (Ilic *et al.* 2007). It was developed in response to the rapid proliferation of molecular sequences and databases, which has created data access problems for biologists. Index terms used in a specific database are

often unique to that database, and may not have a one-to-one correspondence with terms in other databases that index sequences of the same type. For instance, the maize genome database uses the word "lodicule" to refer to what is called the "perianth" in the Arabidopsis database. The PSO was created to address this problem. It began through the integration of terms from three species-specific ontologies for rice (Gramene: Ware *et al.* 2002a; Ware *et al.* 2002b), maize (MaizeGDB: Leszek *et al.* 2003) and Arabidopsis (TAIR: Huala *et al.* 2001; Rhee *et al.* 2003), and was later extended to terms needed for the families Solanaceae and Fabaceae. The intent is to create a unified language that can be used to describe spatial and temporal aspects of gene expression.

Each term in the PSO is associated with a definition, an accession number (ontology ID), and has a specific relationship to at least one parental term. Terms may have more, but not less, than one parent. Circular references are not permitted.

There are three possible relationships between terms in the PSO. These relationships are described with the labels "is_a," "part_of," and "develops_from." The "is_a" relationship is one of generalization, where the child is a type, or instance, of the parent. For example, a "seed" is_a "sporophyte." The relationship "part_of" indicates a composition relationship between the daughter and parent. A "shoot" is part_of a "sporophyte." Finally, the relationship "develops_from" indicates that the structure designated by the daughter term develops from, or is derived from the structure indicated by the parent.

Each of the following three case studies illustrates difficulties in the use of the PSO. Each will be introduced by a set of definitions for structural categories as given by the PSO (www.plantontology.org/amigo/go.cgi), and will be followed by examples that show the limitations of the terms. We refer to the version of the PSO which was available on the web in August 2008.

Case studies

"Leaves"

The Plant Structure Ontology presents under PO:0009025 the term leaf (syn. foliage leaf), defined as follows: "Commonly thought of as one of the three basic parts of the seed plant body, a structure usually of determinate growth." One of the daughter terms is (PO:0020043) compound leaf: "a leaf having two or more distinct leaflets that are evident as such from early in development;" which has the daughter term leaflet (PO:0020049), defined as "one of the ultimate segments of a compound leaf."

Provided with this terminology, we now explore the compound foliage leaves of Ash (*Fraxinus excelsior*: Oleaceae). Typical Ash leaves are arranged in opposite pairs along the stem. Each leaf is imparipinnate, having 4–6 pairs of lateral leaflets, and a terminal leaflet. When the main shoot tip of this plant becomes infected with the gall mite, *Aceria fraxinivora* (Eriophyidae), the leaves produced after the infection have an altered morphology (Anthony & Sattler 1990). Unlike typical ash leaves, the altered leaves (Fig. 1A) can become doubly pinnate and lack a terminal leaflet. In this example, the terminal leaflet is replaced by a distal bud similar to those found on shoots. The bud grows out to produce a continuously growing compound "leaf." Is this modified "leaf" still a leaf? According to the PO definition it falls in the leaf category because of the word "usually." This, of course, implies that leaves can be indeterminate, though they rarely are.

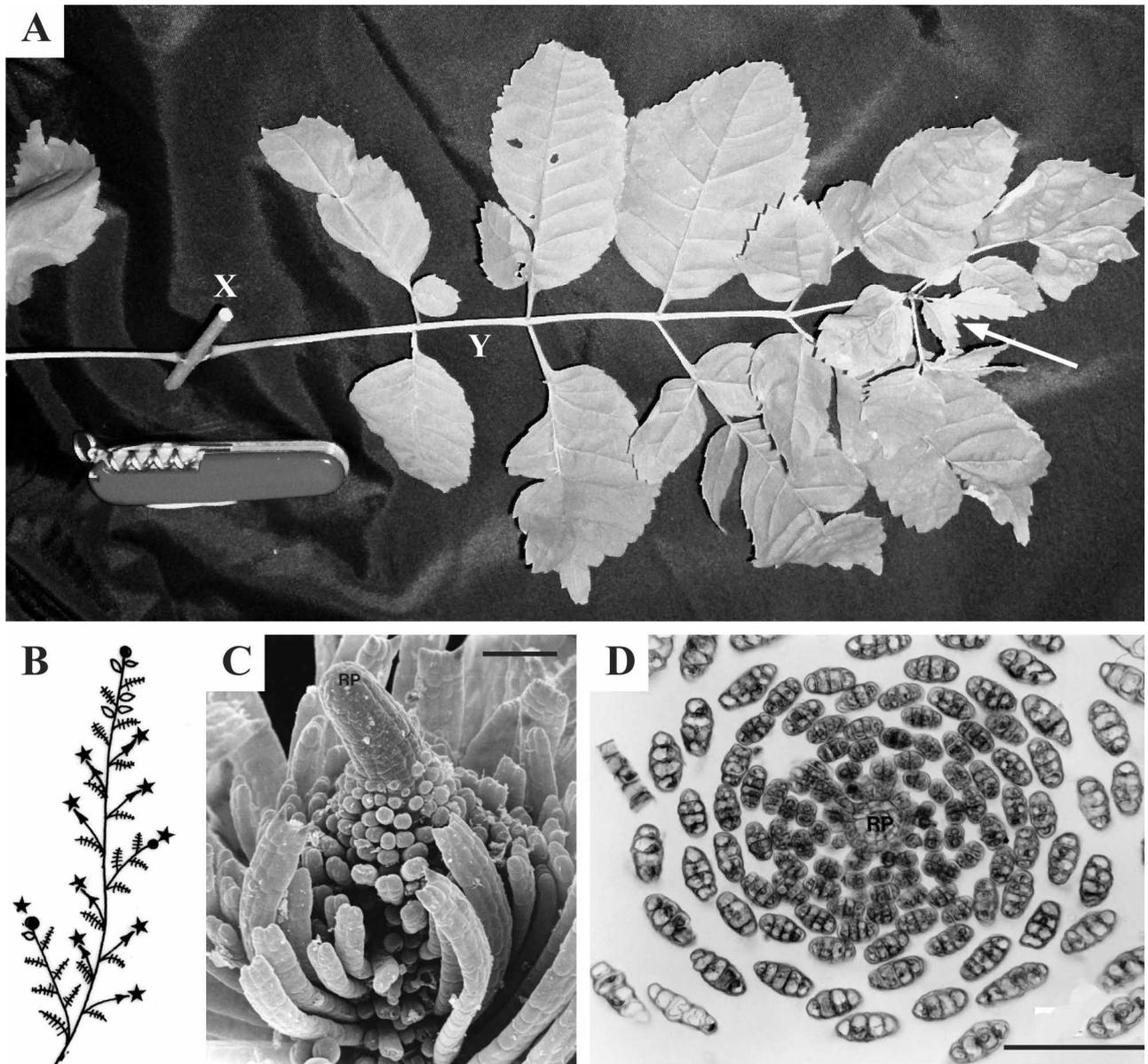


FIGURE 1. “Leaves” with mixed identities. A. Compound foliage leaf of ash (*Fraxinus excelsior*: Oleaceae) infected with the gall mite, *Aceria fraxinivora* (Nalepa, 1909). Unlike typical ash leaves, this leaf is partially doubly pinnate and lacks a terminal leaflet. The leaf tip is occupied by a bud (arrow), which strongly resembles buds found on shoots. X = stem, Y = leaf rachis. B – D. *Indotristicha ramosissima* (Podostemaceae). B. Drawing of a portion of a long shoot with lateral ramuli: ca. 3 cm long determinate, feather-like appendages in the positions where leaves normally occur. Similar to typical leaves, most ramuli subtend axillary shoots. Scales are found as lateral stem appendages, especially towards the shoot tips. Stars = tips of axillary shoots, each subtended by ramulus. C. Apical region of a ramulus at a young developmental stage (250 µm long). The apical meristem (RP) is elongated and slightly curved. Most of the globular primordia on flank of the apex will grow into linear, bifid scales. Scale bar = 30 µm. D. Cross-section of a ramulus tip with the apical meristem in the centre (RP) surrounded by linear scales consisting of a single cell layer. Scale bar = 100 µm. C, D modified from Rutishauser & Huber (1991).

Such indeterminate leaves (or shoots in the position of leaves) are rarely found in *Fraxinus*. However, they are the rule in bladderworts (*Utricularia*: Lentibulariaceae), as well in *Guarea* and *Chisocheton* (Meliaceae) (Fisher & Rutishauser 1990; Fisher 2002), which have indeterminate, compound leaves. In *Guarea* and

Chisocheton the leaf tips (or LAMs = leaf apical meristems) may behave similarly to indeterminate shoot apical meristems (SAMs) continuing the formation of lateral leaflets for several years.

From these examples, and from the definition itself, we know that leaves can be indeterminate. They can even have terminal buds. To annotate a gene to the terminal bud on the leaf of *Guarea* we must find a term for the apical bud of a leaf. According to the rules of the ontology this term must be a daughter of the term “leaf.” Unfortunately, there is no such term in the Ontology. Leaves do not currently have terminal buds in the PSO. This seems easy to solve. We can add the term “bud” as a daughter of “leaf.” Unfortunately, the term “bud” is already a daughter of “shoot,” and circular references are not permitted. This means that, in order to add a term for the indeterminate apex of *Guarea*, we must create a new term (call it “leaf-bud”). The use of this new term implies that “leaf-buds” are not homologous (because not homotopous) to normal buds, which creates problems if we find, as seems likely, that similar patterns of gene expression occur in buds and leaf-buds. The use of different, categorical terms for these indeterminate structures raises issues of structural and gene homology, but these issues are not real. They exist only because current naming rules require hierarchical categorical names, do not allow circular referencing of categories, and ignore the morphological and genetic similarity of the structures.

A second “leaf” example will highlight another problem. Certain organs are difficult to assign to a category. A typical leaf is usually photosynthetic, dorsiventrally flattened, determinate, and is usually provided with an axillary bud that may grow out as a lateral branch. All three of these leaf characters are observable in the ramuli of *Indotristicha* and *Tristicha* (Podostemaceae), flowering plants adapted to river rapids in the tropics (Fig. 1B – 1D). Unfortunately, ramuli also have characteristics more typically found in shoots. Plant structures with mixed identities or blurred boundaries are observed more often in aquatic than terrestrial plants, probably because distinct roots, stems and leaves fit in a terrestrial adaptive landscape better than in an aquatic one (Ingrouille & Eddie 2006).

A portion of a long shoot (ca. 60 cm) of *Indotristicha ramosissima* shows the lateral position of ca. 3 cm long ramuli (Fig. 1B). Similar to typical leaves, ramuli occur in a lateral position, have determinate growth, and subtend axillary shoots (Fig. 1B, stars). Unlike the leaflets of typical compound leaves, the subunits of the ramuli are linear, narrow scales, which are inserted all around the axis. These scales consist of a single cell layer, and completely lack vascular tissue. In *Tristicha trifaria* the scales are arranged in three rows, instead of completely encircling the axis. In both species the ramulus apex is radially symmetrical. Both the three-dimensional arrangement of the scaly subunits and the radial symmetry of the ramuli apices are typical stem features. The only dorsiventrality in the ramuli is the slight curvature of the elongated apical meristem (Fig. 1C).

Given these features, it is not surprising that there are contradictory views on what to call the ramuli. (i) Perrier de la Bâthie (1929) concluded that the ramuli are leaves. (ii) Warming (1899) and Engler (1930) described the ramuli as photosynthetic shoots. (iii) Jäger-Zürn (1970, 1992) adopted a slightly modified view, concluding that the ramuli are the free distal portions of several basally fused shoots. (iv) Rutishauser & Huber (1991) and Rutishauser (1995) emphasized the fact that the ramuli share developmental traits of both typical leaves and typical stems. They viewed the ramuli as developmental mosaics, or leaf-stem intermediates. (v) A final interpretation of the ramuli was suggested by Cusset & Cusset (1988). They saw the ramuli as *de novo* formations (organs *sui generis*) which are not homologous to leaves or stems.

Within the current PSO genes cannot be annotated to the ramuli in a way that does justice to their structure. Since there has not yet been any genetic work on these plants, this is not an immediate problem. However, eventually it will be. Changes to the structure of the PSO are needed to accommodate organs such as these.

“Roots”

The Plant Structure Ontology, under PO:0009005, provides the following definition of the term root: “An axial system which is usually underground and more or less positively geotropic, does not bear leaves and only rarely shoots, is endogenous in origin, indeterminate in growth and often with secondary thickening. Commonly thought of as one of the three basic parts of the seed plant body.” The root cap (PO:0020123) is defined as follows: “A group of cells that covers the apical meristem of the root.” Root caps are a part_of roots, meaning that a specific root may or may not have a root cap. Provided with these definitions we will explore typical and atypical roots of vascular plants (Fig. 2A–H).

Typical roots are observable in most higher plants (Fig. 2A). They typically have a prominent root cap and a more proximal zone with root-hairs. Cap-less roots are quite rare in angiosperms. In the carnivorous butterworts (*Pinguicula*: Lentibulariaceae), some species have caps (e.g., *P. vulgaris*, Fig. 2B) and some lack them (e.g., *P. moranensis*, Fig. 2C). Both conditions are easily accommodated by the PSO. Typical root apical structure in angiosperms has recently been reviewed by Heimsch & Seago (2008), although without any mention of cap-less roots.

Unlike typical roots, most Podostemaceae (river-weeds) have dorsiventrally flattened attachment organs. These organs are attached to submerged rocks by adhesive hairs that are restricted to the lower (ventral) side of the structure (Fig. 2G). In some cases, these attachment structures are ribbon-like (Fig. 2D, G) with a central cylinder reduced to a stripe-shaped area. Xylem and phloem elements are inconspicuous or even lacking. This reduced vascular system is typical for most axial elements in the Podostemaceae. In some river-weeds the attachment organs have root cap-like structures (e.g. *Podostemum distichum*, Fig. 2E, F), whereas others lack these structures (e.g., *Ledermanniella bowlingii*, Fig. 2H). When present, the caps are strongly asymmetrical, and are prominent only on the upper (dorsal) side of the organ tip (Fig. 2E, F). As in typical roots, shoots arise from endogenous buds inside the cortex of the attachment organs (Fig. 2F). Unlike typical roots, the flattened attachment organs of some river-weeds show exogenous branching, resulting in lateral lobes, and occasionally in completely crustose attachment organs (Fig. 2H).

As with the unusual “leaves” discussed above, there exist contradictory views on the morphological significance of the attachment structures in the Podostemaceae. (i) A number of botanists (e.g. Troll 1941; Rutishauser 1997; Jäger-Zürn 2003; Kita & Kato 2004) accept the attachment ribbons and crusts as highly modified roots, though the exogenous branching present in some taxa argues strongly against this interpretation. When the structures are accepted as roots, terminological hybrids such as “thalloid roots” or “root thallus” are often used in order to emphasize their extraordinary structure. (ii) The attachment structures of river-weeds may be viewed as root-shoot (or root-stem) intermediates (Rutishauser *et al.* 2008). This interpretation accommodates most of the structural features of the attachment organs, though does not provide a clear categorization of the structures. (iii) An even more neutral solution is to dispense with the term “root.” Ota *et al.* (2001) and Sehgal *et al.* (2002) suggest that the rooting ribbons and crusts of river-weeds are structural novelties transcending the classical root-shoot model of typical angiosperms.

How can these attachment organs be accommodated in the Plant Structure Ontology? Neither the definition of root nor root cap corresponds well to the structures found in these organs. An annotation that relates gene expression involved in exogenous branching to the crustose roots of *Ledermanniella bowlingii* would likely cause difficulties to scientists seeking for root specific genes. The problem is the association between certain growth characteristics and non-overlapping categorical terms. The terms defined in the PSO are, in most cases, assumed to refer to structural categories, which are only secondarily associated with gene expression patterns. An organ is a root because it belongs to a specific structural category, not because it possesses an association of structural and developmental characteristics that are recognized as being somewhat plastic and susceptible to expression in various positions and at various times during the life of a plant. True, an organ fits in the classificatory scheme based on its structural and developmental characteristics, but this is not the problem. The problem is the rigid association of characteristics that are used to define the organ. Although the judicious use of terms like “more or less,” “usually” and “typically” softens these associations, the current

PSO is not well suited for the description of organs where the associations are broken. A study of "morphological misfits" such as those found in the Podostemaceae highlights these problems. Developmental processes can become disassociated, and can be expressed in various positions and at various times in the life of a plant (see additional examples in Rutishauser *et al.* 2008). The failure to take this into account is a shortcoming of current attempts to establish a plant structure ontology.

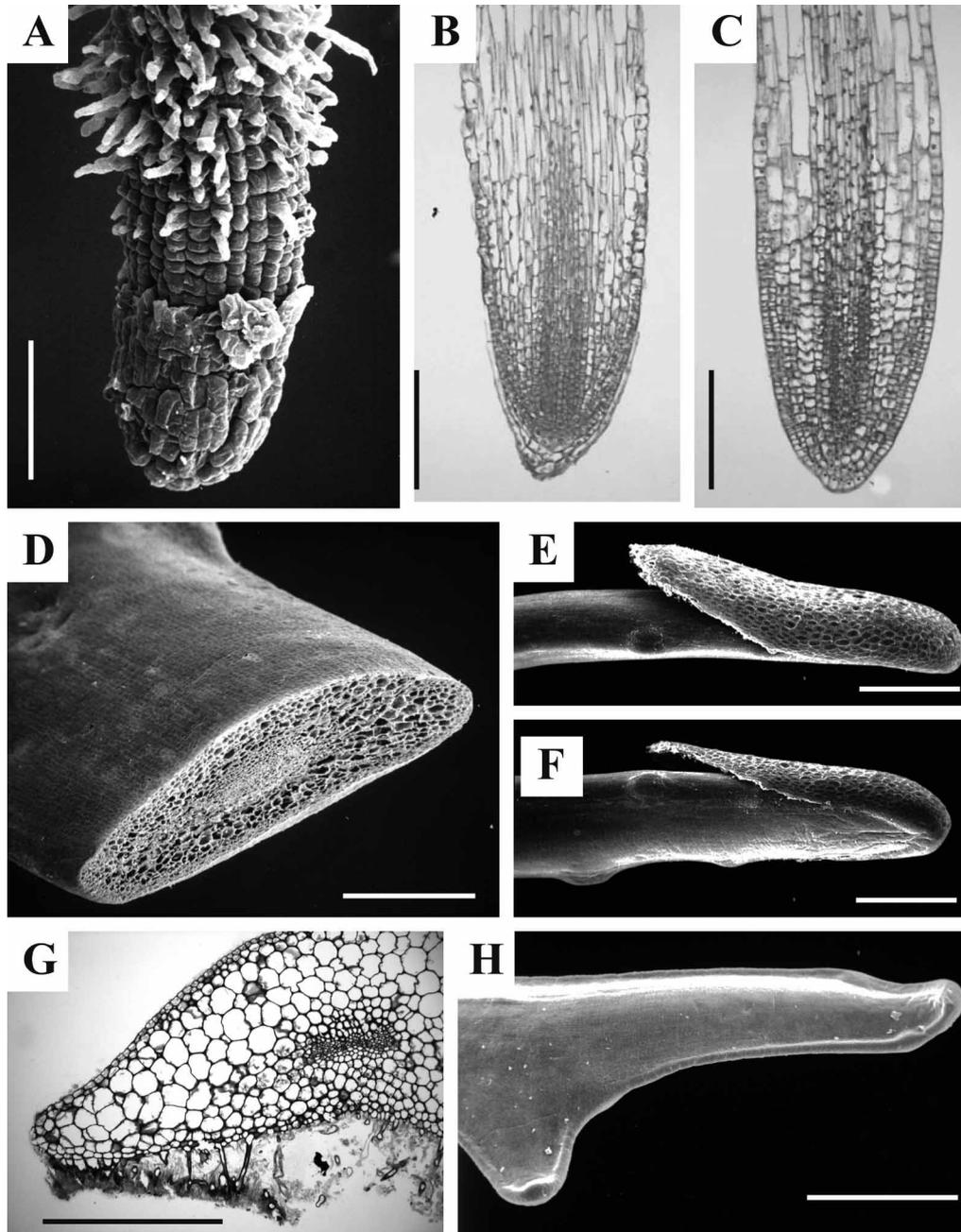


FIGURE 2. Typical and atypical roots. A. Cylindrical root of *Asplenium sandersonii* (Aspleniaceae) showing prominent root cap and more proximal zone with root-hairs. Scale bar = 250 μm . B–C. Longitudinal sections of *Pinguicula* root-tips (Lentibulariaceae). B. *Pinguicula vulgaris* with root-cap. Scale bar = 200 μm . C. *Pinguicula moranensis* lacking root-cap. Scale bar = 200 μm . D–F. Ribbon-like root of *Podostemum distichum* (Podostemaceae). D. Cross-section of ribbon-like root. Scale bar = 400 μm . E, F. Two views of root-tip with asymmetrical root cap, shown from slightly above and slightly below. Scale bars = 500 μm . G–H. Ribbon-like root of *Ledermanniella* spp. (Podostemaceae). G. *Ledermanniella bosii* (microtome, transversal section) showing adhesive hairs (“root-hairs”) along ventral side. Scale bar = 250 μm . H. *Ledermanniella bowlingii*. Ribbon-like root shown from ventral side, with exogenous lobe and root-tip lacking cap. Scale bar = 2 mm.

”Flowers”

According to the PSO a flower (PO:0009005) is “The characteristic reproductive structure of angiosperms. A heterosporangiate strobilus, typically consisting of androecium, gynoecium, usually surrounded by a perianth and borne on an axis or receptacle.” The androecium (PO:0009061) is “collectively the stamens of one flower,” while the female counterpart of the androecium, the gynoecium (PO:0009062), is “collectively the carpels of a flower.” A stamen (PO:0009029) is defined as “a microsporophyll bearing one or more microsporangia,” and a stamen primordium (PO:0004705) is “the very first appearance of a stamen.” Since the term stamen is defined in terms of microsporangia, a microsporangium (PO:0009070), which is synonymous with anther lobe and pollen sac, is “a sporangium producing microspores, usually many in number.” The PSO also defines the term inflorescence (PO:0009049), which is “that part of the axial system of plants above the uppermost foliage leaf/pair of foliage leaves that bears flowers.” The term strobilus, which is used for defining the term flower (PO:0009005) is not included in the PSO.

In this section we explore the male flowers of the castor-oil plant *Ricinus communis* (Euphorbiaceae) with regard to this terminology. The male flowers of this species are found in the proximal portion of the inflorescence, below the female flowers, which are borne distally. Both female and male flowers have a simple perianth consisting of sepals fused into a collar that covers the young buds. The androecium consists of stalked, branched compound structures that bear many anthers (Fig. 3). The development of both sexes has been studied by Prenner *et al.* (2008), but here we restrict ourselves to a discussion of male flower development.

A male flower consists of approximately 11 branched stamens with the central ones having longer stalks than the peripheral (Fig. 3G). Young floral apices are initially triangular, and soon form the approximately 11 primary primordia that will produce the same number of branching stamens (Fig. 3A–B). As the floral apex enlarges, these primary primordia divide to form secondary and tertiary primordia, which remain connected below (Fig. 3C–D). Each primary primordium thus produces a branching stamens (Fig. 3E). A mature branched stamen looks like a small tree, with the distal branchlets provided with multiple thecae separated by elongated appendages of the connective (Fig. 3F). When the androecium reaches anthesis, the calyx opens and the numerous anthers dehisce explosively (Fig. 3G).

There are at least three distinct views of the morphological nature of the branched stamens. (1) The first interpretation is that each *Ricinus* stamen has a compound or fascicled architecture, and is thus equated with a compound, or pinnate foliage leaf (Lam 1948; Meeuse 1966; van Heel 1966). According to Prenner *et al.* (2008), the term fascicled stamen emphasizes the fact that each primary stamen primordium occupies the position of a simple, unbranched stamen in other Euphorbiaceae, such as *Mercurialis*. (2) The superficially similar term stamen fascicle is sometimes used based on the view that each anther is equivalent to a single stamen, while the complete fascicle is a condensed aggregate of many stamens, as found in polyandrous angiosperms (Prenner *et al.* 2008). (3) The branched stamens of *Ricinus* are sometimes equated with a reduced flower or inflorescence, as in the cyathium of the related genus *Euphorbia* (Delpino 1889). Prenner *et al.* (2008, p. 741) speculate “This tendency for loss of determinacy in the stamen could be related to the obscure flower-inflorescence boundary in some Euphorbiaceae, which is particularly noticeable in the cyathium of *Euphorbia*.”

How would one annotate a gene to the branched stamens of *Ricinus*? Depending on the gene expression, any of the terms defined above could conceivably be used. The problem arises in knowing which part of the male flower to label with a specific PSO term. Even the term “flower” is ambiguous in this case. Does this term apply to the whole “male flower” (Fig. 3G), or to a single branched stamen (Prenner & Rudall 2007)? Which term we use depends on our interpretation of the branched stamens, and potentially on the homology of the genes that are found and their distribution in the male flower.

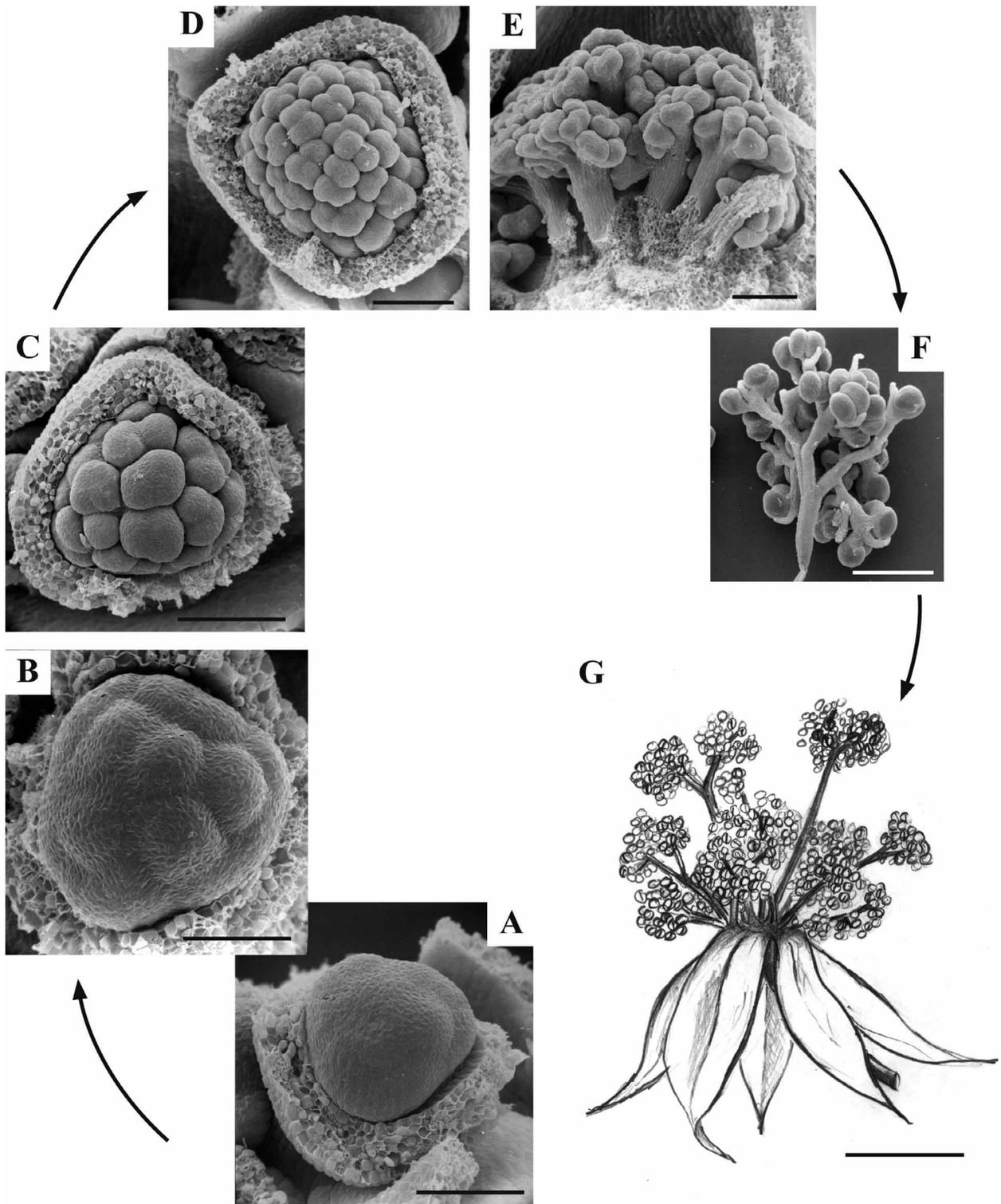


FIGURE 3. *Ricinus communis* (Euphorbiaceae). Development of complex androecial units inside the calyx of male flowers (calyx removed in Figs. A–E). A. Young male flower bud. Triangular floral apex starting to form primary primordia. Scale bar = 150 μm . B. Next floral developmental stage, showing ca. 11 primary primordia. Scale bar = 80 μm . C, D. Two consecutive stages of androecium development. The primary primordia divide to form secondary and tertiary primordia. Scale bars = 150 μm . E. Young branching stamens seen from the side. Scale bar = 400 μm . F. Portion of mature branched stamen showing primary, secondary and tertiary branches. Note anthers with thecae separated by elongated connective appendages at ends of the distal branches. Scale bar = 1 mm. G. Mature male flower with calyx divided into 5 reflexed lobes. Several branched stamens are shown: the central ones with long stalks, the peripheral ones with short stalks. Scale bar = 3 mm.

As an example, let us consider the potential distribution of the B and C class MADS box genes in a male *Ricinus* flower (Glover 2007). If these genes are expressed throughout the centre of the male flower, then we might conclude that the expression should be annotated to the flower, meaning in this case the whole set of branched stamens (Fig. 3G). If B and C class genes are expressed in the branched stamens, but not in the regions separating them, then the genes might still be annotated to the flower, but this case a single branched stamen would be meant. One could equally well annotate genes with this distribution to the stamen, or to both the stamen and the flower.

The problem in this case is not that the branched stamens are intermediate organs, but that their morphological nature remains in doubt. This makes it difficult to annotate genes to them. This is a problem for the PSO because the nature of the ontology makes it necessary to know which term to apply to each structure. Structures like the branched stamens of *Ricinus* make this process very difficult.

More examples of flowering plant structures that do not fit exactly into a single category are given by Rutishauser (1995, 1999), Rutishauser & Isler (2001) Lacroix *et al.* (2003), Rutishauser & Moline (2005), and Rutishauser *et al.* (2008).

Morphological nomenclature: five complementary approaches

In this section we present five complementary approaches to naming morphological structures. The first two are the most commonly used approaches, employing crisply defined terms and often accepting only total homology in an either-or manner. A plant structure is either this or that, but not both. These approaches are widely used in the study of model plants with typical organs (Rutishauser & Isler 2001). The final three approaches, continuum morphology, process morphology, and character cladograms are attempts to overcome some of the shortcomings of the categorical approaches, either by allowing intermediates, mixed identities and complementary views (continuum morphology), by the replacement of all structural categories by sets of developmental process terms (process morphology), or by creating hierarchical sets of homologous parts (character cladograms). These three approaches permit the classification of plant structures that do not fit into mutually exclusive categories (Jeune *et al.* 2006).

Approach 1 = Standardized vocabularies

In the three case studies, the Plant Structure Ontology was taken as an example of a standardized vocabulary. A major reason for establishing this ontology was the “variable terminology that is used to describe plant anatomy and morphology in publications and genomic databases for different species. The same terms are sometimes applied to different plant structures in different taxonomic groups (Ilic *et al.* 2007).” The PSO is intended to be a unified vocabulary of anatomy and morphology for flowering plants so that the structural features can be “correctly understood and uniformly described (Ilic *et al.* 2007).”

The case studies illustrate some of the shortcomings of an approach based on standardized terminology. Descriptive terminology based on sharply defined terms is not adequate for describing structures with blurred or mixed identities. Although the examples we have used might be considered atypical and thus easily ignored, Arber (1950) has demonstrated that even normal leaves and shoots intergrade with each other.

Approach 2 = Developmental genetics

Regardless of how much faith one has in anatomical definitions, they should not be taken as more than a means of communication prior to subsequent genetic analysis.

Scheres *et al.* (1996, p.963)

If structural categories do not provide adequate descriptions of plant structure, perhaps it is possible to define structures based on developmental genetics. At least some developmental geneticists are aware of a certain

degree of fuzziness in plant development. They have used fuzzy concepts such as the “leaf-shoot continuum model” (Sinha 1999), “mixed shoot-leaf identity” (Baum & Donoghue 2002), and “leaf-shoot indistinction” (Albert & Jobson 2001) to describe plant structures. If there is a one-to-one correspondence between structural units (e.g. leaves, roots, flowers) and the “molecular players behind the characters” (Koentges 2008), it should be possible to identify the structural units by the expression of well-characterized marker genes. In order to do this we would only need to look for master control genes or organ identity genes in order to clearly define the structural categories. For example, the KNOX/ARP module would help with the determination of the leaf as determinate unit, and the shoot as an indeterminate module (Langdale & Harrison 2008).

This approach seems to have promise in the cases where control genes for organ identity have been shown to exist; for instance *Pax6*, the master control gene for eye development in arthropods and vertebrates (Wolpert *et al.* 2002). However, adopting this approach for all organs leads to some unusual conclusions, for structural homologues do not always have the same underlying molecular genetic machinery (Jaramillo & Kramer 2007). Examples from both botany and zoology show that homologous structures can result from different genetic controls (Butler & Saidel 2000; Wilkins 2002; Minelli 2003). For instance, leaflet formation in pinnately compound leaves of many eudicots is correlated with *KNOX1* expression in leaf primordia (Kim *et al.* 2003; Kessler & Sinha 2004). In contrast, in pea (*Pisum sativum*) the formation of compound leaves depends on the expression of the *PEAFLO* gene, the pea homologue of *LEAFY* from *Arabidopsis* (Hofer *et al.* 2001). Moreover, pinnation in pea is independent of *PHANTASTICA* (*PHAN*), in contrast to *PHAN* dependent pinnation in tomato. If we accept a one-to-one correspondence between compound leaves and underlying genes, we must conclude that pinnate leaves of pea (Fabaceae) are not homologous to pinnate leaves in other eudicots such as tomato (Bharathan *et al.* 2002).

Although the possibility of using genetic means to determine structural categories is intriguing, grave difficulties remain. The most serious of these is that it is first necessary to define structural categories before the underlying genetic mechanisms can be investigated in them. We must know what an organ is before we can investigate gene expression in that organ, let alone use gene expression to define that category. Gene expression can thus be most easily used to subdivide categories, but not to create them *de novo*.

The second problem relates to the lack of one-to-one correspondence between structural categories and gene expression. While this lack of correspondence may be due to imprecise morphological homology assessments, it may also arise from the reuse of existing genetic resources in novel contexts. Transcription and signalling factors are often used multiple times in context-specific combinations within an organism (Weiss 2005, p.41). The case studies point to plant structures that are difficult to explain by a simple one-to-one correspondence between structure and gene function. We suspect that genetic study of these organisms will show that at least some of the phenotypic fuzziness results from overlapping developmental programs, i.e. from partially indistinct developmental genetic networks.

Approach 3 = Continuum Model and Fuzzy Morphology

Where organs can be seen to represent variations on a theme, it is only a short step to blur the boundaries between them and imagine intermediate forms. This “fuzzy” approach to plant morphology fits perfectly with the idea, propounded by Darwin, that organisms were formed by gradual transitions between types.

Glover (2007, p.15)

Structural categories are often less distinct than is commonly supposed. These “morphological misfits” transcend traditional structural categories, and cannot be placed fully into one or the other category. In these cases it becomes very difficult, or even impossible, to accept just one name for an organ or appendage. In addition to the plant examples cited above, examples of structures with mixed identities are known from animals (Minelli 2003). Based on examples from comparative developmental biology Minelli (2003) supports a combinatorial view of homology. In this section we briefly explore a continuum approach in which terms are used

as “fuzzy sets,” allowing some degree overlap with related terms. This approach is similar to the concept of partial homology that was championed by Sattler (1990, 1992, 1994).

The continuum approach accepts that there are intermediates between traditional structural categories and tries to find a way to represent these intermediates in a classificatory system. Much of the work done by the advocates of this approach has been directed at establishing the fact that intermediates exist. Less attention has been directed at developing methods for accommodating these intermediates in a classificatory system. Arber (1950), for instance, elegantly described leaf-stem intermediates in her partial-shoot theory of the leaf, but did not propose a new terminology by which these intermediates could be accommodated into a classification system.

One method of accommodating intermediate structures is to allow hyphenated terms to describe the intermediate forms. For instance, the indeterminate leaves of Ash described above could be classified in a “leaf-stem” category. This appellation is meant to indicate that these structures have some features of leaves, and some of stems. Although allowing intermediate categories is an advance over categorical approach that does not permit these categories, the use of hyphenated terms is still a categorical approach to naming structures and suffers from the same shortcomings as other categorical approaches. For instance, it does not specify which features of the organ are leaf-like, and which are stem-like. It is possible, therefore, that the leaf-stem category could be used for a heterogeneous assemblage of structures with no real relationship to each other. While this is a definite shortcoming, perhaps it is not as detrimental as it first appears. For example, in current usage, the term “stipule” almost certainly refers to a heterogeneous category that contains many, non-homologous organs (Ilic *et al.* 2007). Only a categorical system that is tightly linked to phylogenetically-defined monophyletic groups has any chance of overcoming this problem. But even in this case structural reversions within a clade are likely to make the categories invalid.

A second approach can be developed from “fuzzy morphology,” i.e. the possibility of using terms as fuzzy sets with blurred boundaries. The PSO already incorporates some of this approach by including qualifying terms like “usually” and “more or less” in its definitions. These qualifiers soften the categories and make them more applicable to a wide range of plant structures. Two examples of this approach are given here. (1) Indeterminate leaves can be categorized as “leaves” with the understanding that the “leaf” category is not rigidly tied to a definition of a leaf as a determinate structure. The PSO allows this. (2) The term “inflorescence” (in the sense of an anthoclade) can be used for flower-producing shoots even when all the bracts (and bracteoles) are identical to foliage leaves. At present, this is not allowed in the PSO.

Approach 4 = Process morphology, i.e. dynamic morphology

The organism has no static qualities or properties. It is a complex of flow, not a thing.
Ingrouille & Eddie (2006, p. 132).

Process morphology (syn. dynamic morphology) as developed by Sattler (1990, 1992, 1994) uses sets of developmental processes instead of structural categories to describe plant structures, including heterogeneous continua of plant forms. Following up on these ideas, Sattler & Jeune (1992), Jeune & Sattler (1992), Lacroix *et al.* (2003, 2005), and Jeune *et al.* (2006) have used quantitative approaches to represent structures as combinations of developmental processes. Their multivariate analyses show that in flowering plants structures occur that are intermediate between typical roots, stems, leaves, and trichomes (plant hairs). We will use an example taken from Jeune *et al.* (2006) to illustrate this method.

Utricularia foliosa (Lentibulariaceae) is a floating aquatic found in ponds and ditches. It consists of a main shoot, called a watershoot, branch watershoots, leaves, and inflorescences with bracts (Fig. 4). Watershoots are indeterminate structures that grow continuously, while they rot proximally. What we will call a

“leaf” in *U. foliosa* is a determinate lateral outgrowth of a watershoot (Taylor 1989; Sattler & Rutishauser 1990). The developmental morphology of watershoots (Fig. 4A, B), leaves (Fig. 4C) and bracts (Fig. 4D) of *U. foliosa* is described in the legend to Figure 4 and in Table 1, and will not be repeated here.

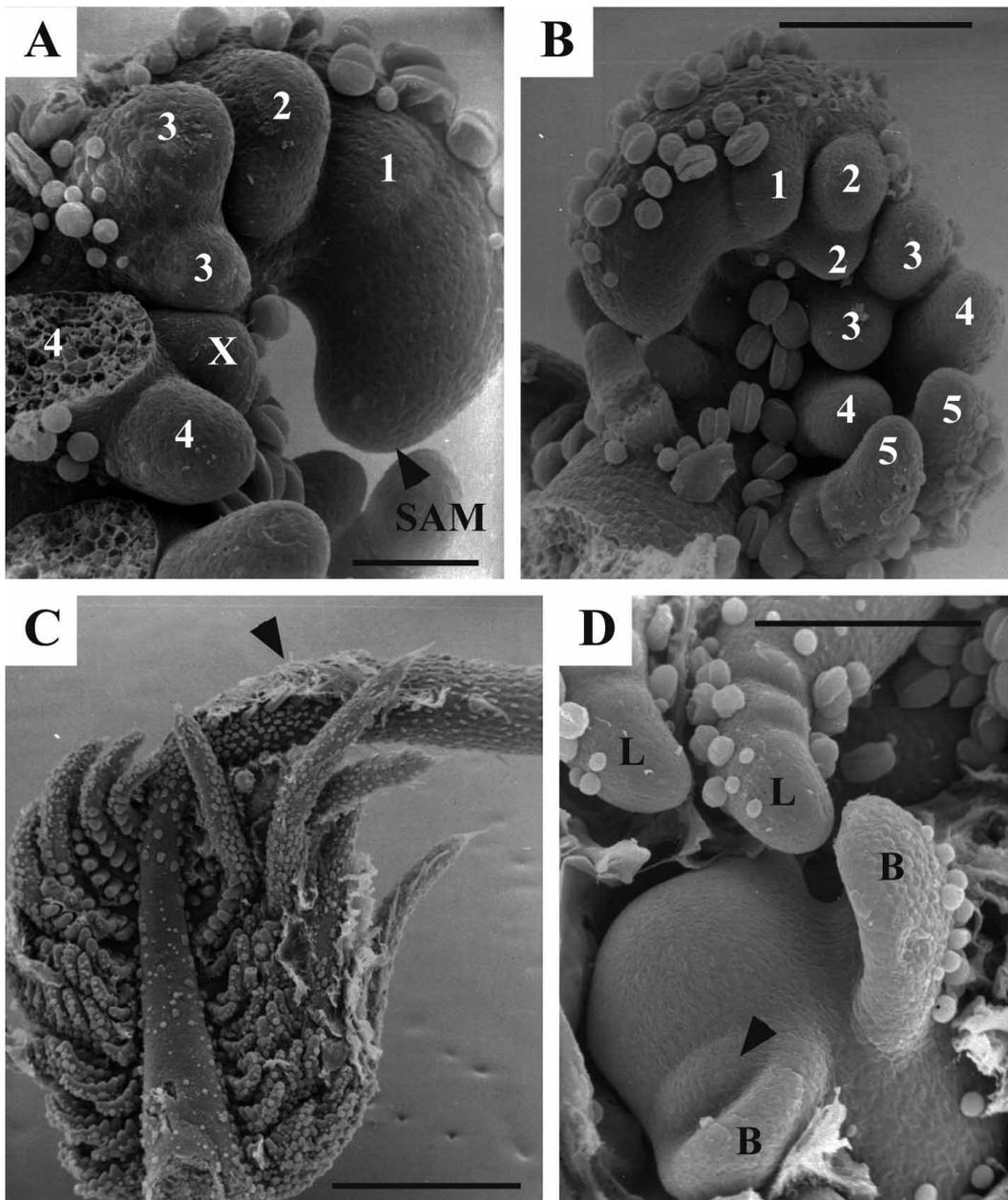


FIGURE 4. Developmental aspects of submerged plant parts (lacking roots) of *Utricularia foliosa* (Lentibulariaceae). A, B. Growing watershoot tips with conspicuously curved indeterminate shoot apical meristems (SAM). The meristematic tips of the lobes, which continue to divide, are less curved than the SAM of the watershoots. Sessile two-celled hairs are visible on the convex and concave sides of the young watershoots. 1–5 = “leaves” = outgrowths along watershoot flank, each one dividing to form two determinate lobes. X = inflorescence primordium. Scale bars = 50 μm (A) and 100 μm (B). C. Determinate ends of leaf tip (arrowhead) and primary leaflet tips. The curved tips elongate considerably by precocious differentiation before ceasing growth. Scale bar = 500 μm . D. Racemose inflorescence apex forming bract (B) primordia, with floral apices (arrowhead) in their axils. Note presence of slightly curved tips of leaf primordia (L). Scale bar = 100 μm . Fig. 4A modified from Sattler & Rutishauser (1990).

Jeune *et al.* (2006) used multivariate statistical methods to project plant structures, including the watershoots, leaves and bracts of *U. foliosa*, into a morphospace that represents all possible forms of related organs.

The theoretical morphospace includes all possible process combinations for seed-plants, whereas the empirical morphospace contains only those process combinations which are realized in nature (Jeune *et al.* 2006). Each axis of the morphospace corresponds to a variable which describes some developmental processes of an organism, or its parts. Representations of the space can be projected onto two (Fig. 5) or three dimensions for ease of interpretation. Individual organs can be identified and plotted in the morphospace as specific process combinations. When this is done, each point in the morphospace represents an individual plant structure such as the watershoots, leaves or bracts of *U. foliosa*. Plotting the organs of *U. foliosa* clearly shows that watershoots (Fig. 5, black circle) and leaves (Fig. 5, cross) are more similar in their process combinations than either is to inflorescence bracts (Fig. 5, black diamond).

TABLE 1. Developmental processes used in the Principal Component Analysis (PCA) of *Utricularia foliosa* by Jeune *et al.* (2006), and represented in the planar representation of the morphospace (Fig. 5). For further explanations of processes see Jeune *et al.* (2006).

	Watershoot (Fig. 4A, B)	Leaf (Fig. 4C)	Bract (Fig. 4D)
Positioning: main axis (-0.5), axillary (+0.5), axillant (+1.0), other (+1.5)	1.5	1.5	1.0
Growth period: indeterminate (-0.5), determinate (+0.5)	-0.5	0.5	0.5
Developmental symmetry: variable (-0.5), stable (e.g. consistently dorsiventral) (+0.5)	0.5	0.5	0.5
Final symmetry: radial (-0.5), dorsiventral (+0.5)	0.5	0.5	0.5
Branching: no branching (-1.0), branching with distichous phyllotaxis (+1.0)	1.0	1.0	-1.0
Growth distribution: acropetal (0), mixed = acropetal and basipetal (+0.25), basipetal (+0.5), diffuse (+1.0)	0.0	0.0	0.25

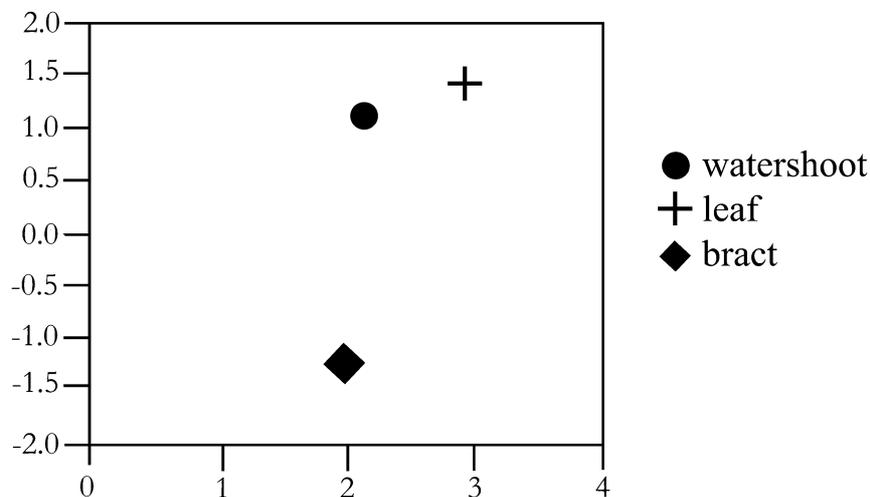


FIGURE 5. Planar representation of the morphospace of shoots, foliage leaves and bracts in flowering plants (after Jeune *et al.* 2006). The process combinations of watershoot leaves and bracts of the aquatic plant *Utricularia foliosa* (Lentibulariaceae) are visualized by Principal Components Analysis of the developmental processes shown in Table 1 (see Jeune *et al.* 2006 for a more detailed explanation). Each point in the morphospace represents an individual plant structure. The process combinations of watershoots (black circle) and leaves (cross) are more similar to each other than either is to the process combination of bracts (black diamond).

One of the great strengths of this approach is that categorical terms are only used to identify organ posi-

tion within the morphospace, not to create the space itself. All that is necessary to include an "organ" in the analysis is to be able to recognize and isolate the organ from the context of the total morphology of plant. Once this is done, the ontological nature of that organ becomes unimportant. All that is important is the ability to recognize the processes that are expressed in that organ. When they have been recognized, these processes can be plotted in the morphospace and a name applied to the cluster of points that includes this process combination. The name serves only as a placeholder for the combination of developmental processes which locate the organ in the morphospace. It does not define the organ.

Gene expression patterns can be annotated to the morphospace by associating the expression pattern with the combination of processes that are found in the part in which the gene is expressed. For example, if a gene were expressed in the bracts of *U. foliosa*, it would be associated with the coordinates 2 and -1.25 in the two-dimensional morphospace represented in Fig. 5. Here the term "bract" is only meant to refer to a set of processes that are associated in the morphospace, not a categorically defined plant organ. This is an extremely flexible mechanism for annotating genes. It allows almost infinite variety in the types of organs that are recognized. Because terms are only used as placeholders, there are no categories and no possibility that intermediate organs, or organs of dubious identity, will be misscategorized.

Approach 5 = Character cladograms

Who, I ask, in their right mind would condemn a picture which, it is clear, expresses things much more clearly than they can be described with any words of the most eloquent men? . . . It is certain that there are many plants which cannot be described by any words so as to be recognized, but which, being placed before the eyes in a picture, can be recognized immediately at first sight.

Fuchs (1542, p. xiii; translation: Kusakawa 1997)

Kirchoff and his collaborators have recently proposed a visual method of representing structural categories (characters) that is not dependent on terminology (Kirchoff *et al.* 2004; Kirchoff *et al.* 2007). In this method, photographs of homologous parts are compared and arranged into hierarchical diagrams (character cladograms) that represent the structural similarities among the parts (Fig. 6). None of the nodes are labelled in these visual morphospaces, and no terminology is used either to create or communicate the categorical information. Although the example shown here is hierarchical, non-hierarchical character cladograms can also be created (Kirchoff *et al.* 2004).

The use of hierarchical character cladograms to represent structural categories overcomes most of the difficulties identified above. Since no terms are used in the creation of the cladograms, their use obviates most of the problems associated with terminology. For instance, sets of images can be used instead of terms to represent structural categories. This removes any doubt as to the domain of applicability of the term by making group membership explicit. New structures can easily be added to a structural category by placing them on the cladogram in positions where they show the most similarity. For instance, if ovary structure in another species of *Costus* became available, it could be inserted on the character cladogram in the position where it showed the most similarity, presumably next to *Costus dubius* (Fig. 6J). Intermediate organs can be easily accommodated on character cladograms, as can organs of uncertain identity. For instance, group (I,(H,G)) (Fig. 6) shows characteristics that are intermediate between groups (((K, J),(L,M)),(A,B)) and ((C,D),(E,F)). Its position in the character cladogram represents this intermediate structure better than could any set of terms. Organs of dubious identity can be accommodated in character cladograms because it is not necessary to know what something "is" in order to know what it is similar to. Similarity, not ontological identity is used to create the morphospace.

Annotating a gene to a character cladogram can be accomplished by associating a reference to the gene with the character cladogram for the part of the organism in which the gene is expressed. If photographs of in

situ hybridization are available, they can be placed on the character cladogram. If multiple genes are annotated to the same terminal, the terminal can be enlarged from a single taxon/photograph to a group of photographs representing that taxon. Each photograph is an instance of the expression of a specific gene in that taxon. Its position in the morphospace shows its relationship to the expression of other genes in homologous “organs.” The use of the term “organ” here is meant to indicate a specific character cladogram, not a structural category.

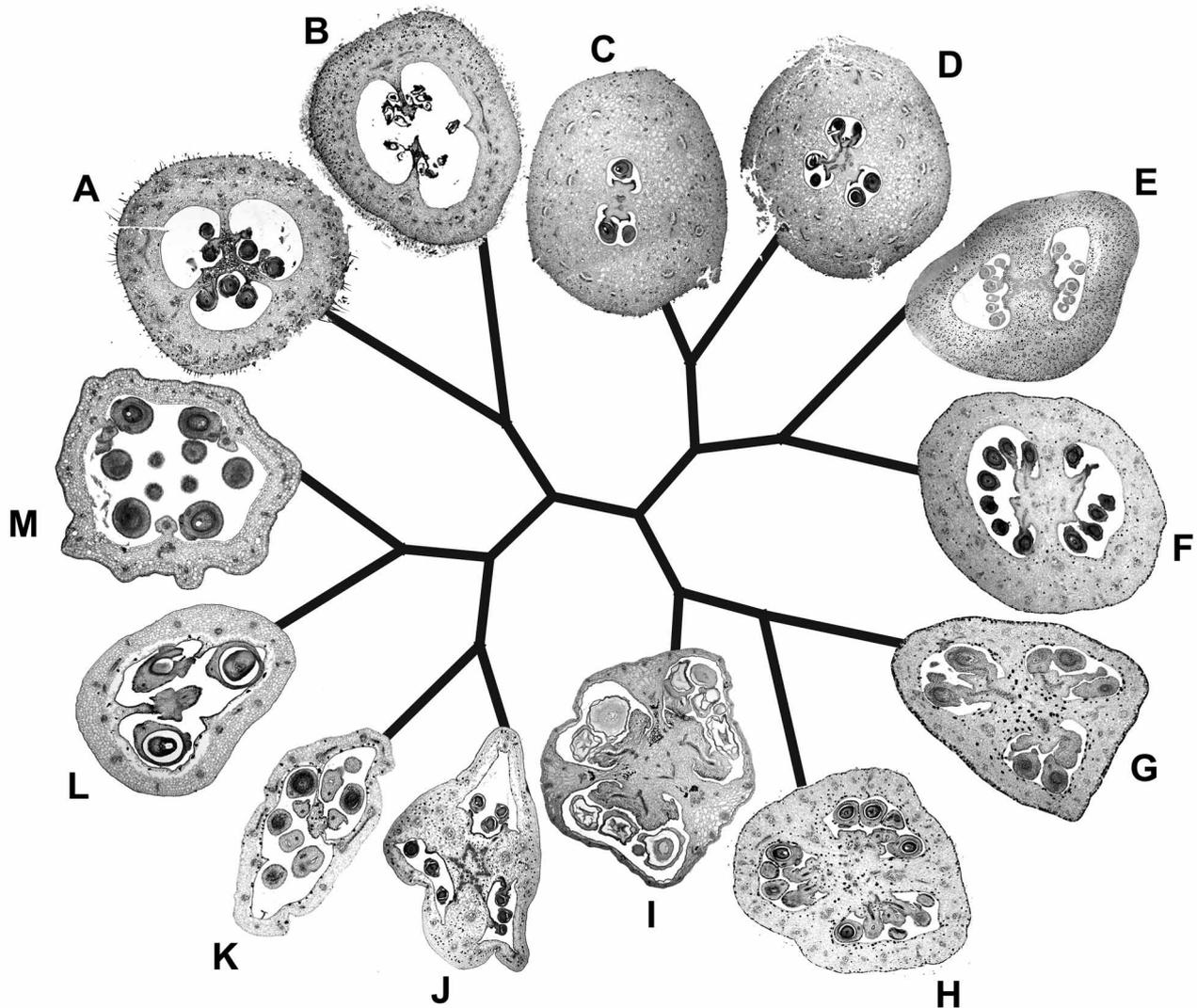


FIGURE 6. Visual morphospace (character cladogram) for ovary structure at the mid-locular level of the Zingiberaceae and Costaceae (modified from Kirchoff *et al.* 2007). A visual morphospace was created by assembling standardized photographs of homologous parts and arranging them according to similarity. The groups are hierarchical, and are not associated with terminology. Both intermediate structures and structures of unknown identity can easily be accommodated in the morphospace. A. *Alpinia calcarata* (Zingiberaceae). B. *Etilingera elatior* (Zingiberaceae). C. *Riedelia* sp. (bilocular, Zingiberaceae). D. *Riedelia* sp. (trilocular, Zingiberaceae) E. *Dimerocostus strobilaceus* (Costaceae). F. *Hedychium coronarium* (Zingiberaceae). G. *H. gardnerianum* (Zingiberaceae). H. *H. flavescens* (Zingiberaceae). I. *Zingiber zerumbet* (Zingiberaceae). J. *Costus dubius* (Costaceae). K. *Kaempferia atrovirens* (Zingiberaceae). L. *Scaphochlamys kunstleri* (Zingiberaceae). M. *Globba marantiana* (Zingiberaceae).

Conclusions and outlook

Most botanical textbooks and bio-ontologies ignore the fact that the terms used to signify structures and developmental patterns in living plants distort evolutionary reality to some degree. We should not confuse our favourite metaphors (including concepts such as root, shoot, leaf and flower) with reality, which is more complex. Plant description based on categorical terminology is not sufficient for understanding plant structures with blurred boundaries, mixed identities, and/or ambiguous interpretations. Complementary interpretations such as those presented above highlight the discrepancy between accepting a single interpretation as the right one, and the realistic view that more than one type of structural description is needed for understanding all aspects of plant structure and development. Developmental processes that are normally associated with each other can become disassociated, and can be expressed in different positions and at various times in the life of a plant (Rutishauser *et al.* 2008). However, recognizing this does not solve the immediate problem of what type of descriptive system to use for annotating gene expression. In closing, we briefly discuss each of the above approaches and provide a final evaluation of their usefulness as methods for gene annotation.

Standardized vocabularies are already in use, but can be improved through the addition of terms that allow intermediate structures and structures of doubtful identity to be accommodated. Examples of these terms are “ectopic” or “heterotopous” buds (i.e. buds that occur in sites not predicted by the classical root-shoot model); “epiphyllous shoots” (i.e., shoots arising from leaf tissue); and “homocratic flowers” (i.e., flowers that arise in unpredictable positions, e.g. from endogenous buds in the stem cortex, as in African Podostemaceae). The addition of the term anthoclade would also be useful, as it allows to the recognition of a leafy region with flowers in the axils of foliage leaves as an inflorescence. This type of inflorescence occurs in many plant families, such as Solanaceae, Lamiaceae, and Costaceae.

The developmental genetic approach to annotating plant structures does not seem to us to have as much potential as other methods, unless it is used as in the sense of process terminology. In this case it intergrades with process morphology, to be discussed below.

Although continuum morphology offers some hope of being able to create intermediate terms, hyphenated terms by their very nature are categorical and suffer from the same problems as other categorical terms. We see little hope for the use of hyphenated terms as a method of annotating gene expression. The use of fuzzy morphology is more promising. The fact that some terms in the PSO (e.g., leaf = PO:0009025) are already to some degree fuzzy attests to this potential.

Process morphology (or dynamic morphology) *sensu* Sattler (1990, 1992, 1994) and Jeune *et al.* (2006) allow us to dispense with all structural categories and characterize phenotypes by sets of developmental processes. Although using process combinations to describe plant structures may make communication among scientists difficult, the use of a single morphospace to which gene expression can be annotated is appealing, especially so since its use would remove most, if not all of the problems described here. Unlike rigid categorical vocabularies, process morphology should allow better hypotheses about the “molecular players behind the characters” (Koentges 2008).

Character cladograms as visual morphospaces, while potentially useful, suffer from the problem that independent cladograms are needed for each set of homologous organs. The time and effort needed to create and access these cladograms makes the method less useful than process morphology. Like process morphology it has the strength of being completely independent of categorical terms.

Although it seems to us that process morphology has clear advantages as a reference system for gene annotation, this does not mean that the other methods are without value. Accepting a single valid description of plant structure and rejecting all others is an inflexible approach that will ultimately prove fruitless. It is much wiser to adopt descriptive systems that are suited to the tasks we wish to perform. Since the goals of the tasks are varied, we should expect that we will need to employ a variety of descriptive systems.

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