Micromorphology and anatomy in systematics of Asteraceae. An old-fashioned approach?

Abstract:
The comparative study of plant morphology, intertwined with anatomy, has always been the basis for plant systematics, which strives to explain diversity, evolution and phylogeny of plants. In the molecular era, some authors diminish importance of morphology and especially anatomy in systematic and phylogenetic studies of plants. However, are molecular data exclusively a primary and self-sufficient approach in taxonomic research of plants? This review paper addresses this issue through specific examples. Studies of some Asteraceae taxa showed that morphological, micromorphological and anatomical data are extremely important in systematics. New opportunities for systematic morphology, micromorphology and anatomy in case of Asteraceae taxonomy, but certainly also in other plant groups, that were not present in the premolecular era, are opening regarding synergistic multidisciplinary taxonomic, evolutionary and phylogenetic studies that combine molecular with morphological, anatomical and other analyses (e.g. chemophenetics - describes a given taxon phenetically using specialized metabolites as phytochemical characters), keeping in the throne these “old fashioned” approaches.

Key words: morphology, taxonomy, characters, synergy

Introduction

The comparative study of plant structure has always been the backbone of plant systematics, which strives to elucidate plant diversity, phylogeny and evolution (Endress et al., 2000). Plant taxonomic studies traditionally use morphological and karyological (Stebbins, 1953), as well as micromorphological characters (Hayat et al., 2009; Bak & Ozcan, 2018). Micromorphological characters are of decisive importance in unrining taxonomic and phylogenetic relationships of various plant groups and have been successfully used in plant systematic studies for decades (Endress et al., 2000). For more than a

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century, comparative anatomy is used as a tool in the plant systematics. Anatomical characters are very important in perceiving systematic and phylogenetic relationships of particular plant groups. Indeed, anatomical features can provide useful characters which could help in identification of problematic plant taxa, as well as establishing their taxonomic relationships (Metcalfe & Chalk, 1957; Scatena et al., 2005; Makbul et al., 2011; Sosa et al., 2014; Karanović et al., 2015, Gavrilović et al., 2019a, b; Janačković et al., 2019).

On the other hand, the data provided by the phytochemists are extremely difficult to interpret in a cladistic context. Even so, integration of phytochemical and macro-molecular characters can be of prominent benefit, and can help, for example, in the delineation of clades so far only supported by DNA sequence data (Enke et al., 2012). In order to overcome the confusion in the interpretation of phytochemical characters (specialized metabolites that actually describe the given taxon phenetically) as phylogenetic characters and “under the umbrella” of the term chemotaxonomy (which is mistakenly identified with chemosystematics), the new term chemophenetics has been proposed by Zidorn, (2019). Nevertheless, chemophenetic studies contribute to the phenetic description of taxa, similarly to anatomical, morphological and karyological approaches, which have already been recognized as of major importance for establishing “natural” systems and which continue to be of the highest importance for the description of organisms classified with the help of modern molecular methods (Zidorn, 2019).

Asteraceae (Compositae), habitually known as the daisy or sunflower family, represent one of three mega-diverse families which jointly count more than 25% of all extant angiosperm species (Mandel et al., 2019). Asteraceae, counting 25000–35000 species, comprise 10% of all flowering plant species. Members of the sunflower family occur on every continent including Antarctica (Smith & Richardson, 2011) and inhabit nearly every type of habitat on Earth with the largest concentration of species in deserts, prairies, steppes, montane regions, and areas with Mediterranean-like climates (Mandel et al., 2019). According to newer fossil data and recent molecular clock dating, Asteraceae likely originated during the Late Cretaceous: ~83 MYA (Mandel et al., 2019). According to Mandel et al. (2019) the family consists of 13 subfamilies and 47 tribes. Asteraceae also includes species of wide economic interest, e.g. vegetables, sources of oil, medicinal plants, insecticides and many horticultural and garden ornamentals. However, some species of Asteraceae constitute a big problem for agriculture as noxious weeds. As a one of the largest, natural (with a combination of several specialized morphological characteristics e.g., capitula, highly reduced and modified flowers, syngenesious anthers, inferior ovaries and very unique in the plant kingdom fruit - cypsela) and economically most important families of flowering plants Asteraceae has been researched for centuries. Characters related to form, whether gross morphology, micromorphology, anatomy, embryology, palinology and so forth, regarded as morphological data, have larger impact on the cladistics and classification of the Asteraceae than other characters e.g. chemical and molecular data (Schönberger, 2002; Stuessy, 2009). Molecular data are significant but also insufficient, that is why, in some cases, better phylogenetic reconstructions of the Asteraceae are obtained taking into account also a morphological data (Pornponggrungrueng et al., 2007; Gruenstaeudl et al., 2009; Wang et al., 2013). More recent, modern-day studies of Asteraceae taxa have shown that morphological, micromorphological and anatomical data are still extremely important in systematics of Asteraceae family (Makbul et al., 2011; Wang et al., 2013; Sosa et al., 2014; Karanović et al., 2015; Bombo et al., 2016; Ginko et al., 2016; Batista and De Souza, 2017; Gavrilović et al., 2017; Gavrilović et al., 2018a, b; Gavrilović et al., 2019a, b; Janačković et al., 2019).

Brief history and modern-day micromorphology and anatomy in systematics of Asteraceae

Micromorphology

A different sets of characters has been used to demarcate the Asteraceae. Certainly, one of the pioneer work regarding micromorphological approaches to Asteraceae classification was done by Cassini (1821). These summarize many of the microscopic traits on which Cassini based his tribes. Of these, pronate versus recurved mature style branches, stigmatic surfaces, truncate to enlarged style appendages, bases of the anther thecae with or without tails, shape of the anther collar and form of the corolla are most important. The stigmatic surfaces of many tribes (Mutisieae, Lactuceae, Vernoniaeae, Arctoitideae, Eremothamneae, Cardueae) are consistently continuous over the inner surface of the style branch. In other tribes (Eupatorieae, Anthemideae, Asterae, most Inuleae, most Heliantheae, most Seneconieae) the stigmatic surface is divided into two lines.

The nature of endothecial tissue of the stamens, which could be polarized or radial, showed to be good character in taxonomy within Seneconieae (Dormer, 1962). In subtribes Seneconieae and Ohonninae, the endothecium is radial in all genera except Dauresia, Graphistylis, and perhaps Synotis. In...
Tussilagininae s.str. a polarized endothecium is the rule, but the radial type has been recorded in several genera (Tephrosperis, Nemosenecio, Psaciopsis, Psacallum, Arnoglossum); in Sinosenecio both types and an intermediate pattern seem to occur (Jeffrey & Chen, 1984). Moreover, the anthers of Cichorieae members vary considerably in length, but this variation probably occurs repeatedly within many genera and is therefore only of taxonomic relevance at the species level (Kilian et al., 2009). In Arctotideae morphological and micromorphological characters confirmed close relationships between the Gorteria clade and Berkheya clade (Karis et al., 2009).

Microcharacters of involucral bracts are considered very helpful for delimitation in certain taxonomic groups of Asteraceae (e.g., for subtribes of Cardueae, with spiny pectinate-fimbriate appendages in Cardopatiniae; usually spiny, innermost exappendiculate or with rudimentary appendages in Carduiniae; inner often conspicuous and coloured in Carliniae; scarious, fimbriate, pectinate, spiny or unarmed appendage in Centaureinae; and in many rows in Echinopsinae (Robinson, 2009; Susanna & Garcia-Jacas, 2009).

Certain floral microcharacters (anther size, shape of the anther apical appendage, configuration of stigmatic areas on the inner surface of the style branch, and configuration of the endothecial thickenings and of the filament collar) of 36 taxa of Sinosenecio showed that these floral characters are highly consistent with evidence from molecular systematics and cytology and provide the most important diagnostic characters in the tribe Senecioneae, as in the family at large and strongly suggest a polyphyletic nature of this genus, as well as the need of a taxonomic change at generic level (Liu & Yang, 2011).

Still nowadays, micromorphological investigations of Asteraceae could provide some novel characters (Erbar & Leins, 2015; Gavrilović et al. 2017, 2019b). Investigating style morphology of 395 species of 258 genera (covering all, in that time, 44 tribes of the Asteraceae), Erbar & Leins (2015) found a new microstructural feature, namely, often conspicuous cuticular patterns on the stylar hairs (involved in secondary pollen presentation) and stylar appendages. They determined five different patterns of cuticular striation and when they put these patterns onto a generalized phylogenetic tree (based on molecular data), they concluded that there is considerable homoplasy in these features. Nevertheless, cuticular patterns are still useful in characterizing some clades within the family. Gavrilović et al. (2017) investigating involucral bract micromorphology found, for the first time, a large number of densely packed crystals on the involucral bract surface. Also, the presence of nonglandular, curly trichomes and biseriate glandular trichomes on the bract surface, as well as the sylvite crystals on the petal surface of X. cylindraceum, clearly differentiates this species from X. annuum (Gavrilović et al., 2017). Comparative micromorphological analyses were conducted on five members of the Xerantheminae, both perennial (Amphorcarpos excel and Shangwu masarica) and annual (Chardinia orientalis, Siebera pungens and Xeranthemum inapertum), showing that micromorphological traits link together perennial species, some link annual ones, some are species-specific, and some are common to all taxa (Gavrilović et al., 2019b).

We could conclude that morphology and micromorphology of florets (e.g., style base, anther appendages, trichomes on corollas), and inflorescence (involucral bracts characters, e.g., crystals and glandular and nonglandular trichomes on their wall) were used as major distinguishing features for subtribal and generic delimitation, even though these characters can sometimes be significant at the species level.

**Anatomy**

At the beginning of the twentieth century Col (1899-1901), in light of anatomy, reviewed in considerable detail distribution of laticiferous versus resiniferous tissue throughout the Asteraceae. Taxa with latex in canals or sacs occur in several tribes of the subfamily Cichorioideae and consistently in the Lactuceae, but very rarely in the Asteroideae, where resin sacs and resin canals are common. Carlquist (1966) investigated the basic plan of the wood anatomy of Asteraceae (focusing on four tribes, Anthemideae, Ambrosieae, Calenduleae, and Arctotideae), which provided useful tribal characters and minor intertribal variation. Even though Carlquist (1966) stated that Asteraceae members share a basically specialized wood plan and that wood anatomy is not likely to reward one with tribal or subtribal characters, certain characters are of systematic value within Anthemideae, Ambrosieae, Calenduleae, and Arctotideae (e.g., carbonized resins in intercellular spaces, secretory canals in rays, patterns of crystal occurrence are characters which may be of specific or generic value).

Metcalfe & Chalk (1957) noted some particular anatomical traits, which showed to have taxonomic importance within the family, e.g., presence of secretory and laticiferous canals, types of nonglandular and glandular trichomes, occurrence of medullar and cortical vascular bundles and presence of anomalous secondary thickening. Also, anatomical characteristics observable in Asteraceae are: (a) presence of various types of glandular and non-glandular tri-
Anatomical data can also contribute in resolving complex taxonomy of certain genera, e.g. *Artemisia*. In anatomical investigation of five *Artemisia* species, Janačković et al. (2019) showed that some characters link together *A. absinthium* and *A. arborescens* from the same section; some other connect species belonging to different sections (*A. campestris* and *A. arborescens*; *A. absinthium* and *A. judaica*; *A. judaica* and *A. herba-alba*), while some could be considered as species-specific.

We could summarize that certain anatomical characters, such us distribution of laticiferous versus resiniferous tissue is useful on subfamily or tribal level, while patterns of crystal occurrence might be significant at species or genus level. Occurrence of cortical vascular bundles seems to be important on species level. Root and rhizome anatomical traits have proven to be useful on tribal and species, but not on the genus level. Leaf anatomical epidermal character are diagnostic and can be used for delimiting species. Also, qualitative anatomical characters may have role in understanding and solving phylogenetic relationships, which are reflected in the systematics of given taxa.

**Micromorphology and anatomy of cypsela**

The cypsela is a special form of dry indehiscent fruit in which the seed coat (testa) and fruit wall (pericarp) are tightly attached to one another and is exclusive characteristic of the family Asteraceae (Roth, 1977). As an exclusive fruit of the family cypsela and its features have been attracted by Tournefort (1694), Vaillant (1719), Cassini (1819), Lessing (1832), Bentham (1873), Hoffman (1894), Cronquist (1955), Robinson (1977), Bremer (1994), Nordenstam (1994), Rao & Datt (1996), Robinson (1999), Nordenstam et al. (2006), Lack (2007) and Mukherje & Nordenstam (2004, 2010). Cypsela morphology (macro- and micromorphology) and anatomy have been widely used in illuminating taxonomic relationships in Asteraceae and still represents a source of valid taxonomic characters (Lavialle, 1912; Stebbins, 1953; Wagenitz, 1976; Dittrich 1977; Barthlott, 1984; Singh & Pandey, 1984; Dittrich, 1985; Bruhl & Quinn, 1990; Glynis, 1993; Geng et al., 1994; Blanca & Díaz de la Guardia, 1997; Petit, 1997; Häffner, 2000; Zhu et al., 2006; Garg & Sharma, 2007; Pandey & Kumari, 2007; Zarembo and Boyko, 2008; Abid & Qaiser, 2009; Abid & Ali, 2010; Inceer et al., 2012; Ozcan & Akincı, 2019). This is why micromorphology and anatomy of cypsela are separated herein. Bremer (1987) stated the importance of cypselae characters at lower taxonomic levels but not at the tribal level. In Asteraceae, the anatomy and micromorphology of...
cypsela characters are distinct between the genera and are also useful for delimiting species.

Conclusion and future prospects

The breakthrough of molecular tools in plant systematics and its contribution to phylogenetic frameworks was and it is still a tremendous stimulus for comparative morphology and anatomy.

One should have in mind that the structure and biology of a majority of Asteraceae members are far from sufficiently investigated, thus combining morpho-anatomical, phytochemical, and molecular studies are necessary to explore them.

Although this overview represents only a glimpse of a role of micromorphological and anatomical approaches to Asteraceae systematics, it gives an valuable insight and perspective of this topic. Thus, the accumulated knowledge and permanent investigation of Asteraceae taxa using micromorphological and anatomical methods will put light on branching topologies of phylogenetis trees which molecular data established.

New opportunities for systematic morphology, micromorphology and anatomy in case of Asteraceae taxonomy, but certainly also in other plant groups, which were not present in the premolecular era, are now opening regarding synergistic multidisciplinary taxonomic, evolutionary and phylogenetic studies which combine molecular with morphological, anatomical and other approaches (e.g. chemophenetics), keeping in the throne these "old fashioned" approaches.

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