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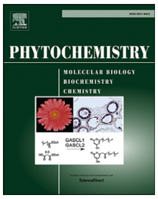

# Metabolomic analysis applied to chemosystematics and evolution of megadiverse Brazilian Vernonieae (Asteraceae)

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## ABSTRACT

*Vernonia sensu lato* is the largest and most complex genus of the tribe Vernonieae (Asteraceae). The tribe is chemically characterized by the presence of sesquiterpene lactones and flavonoids. Over the years, several taxonomic classifications have been proposed for *Vernonia s.l.* and for the tribe; however, there has been no consensus among the researches. According to traditional classification, *Vernonia s.l.* comprises more than 1000 species divided into sections, subsections and series (*sensu* Bentham). In a more recent classification, these species have been segregated into other genera and some subtribes were proposed, while the genus *Vernonia sensu stricto* was restricted to 22 species distributed mainly in North America (*sensu* Robinson). In this study, species from the subtribes Vernoniinae, Lepidaploinae and Rolandrinae were analyzed by UHPLC-UV-HRMS followed by multivariate statistical analysis. Data mining was performed using unsupervised (HCA and PCA) and supervised methods (OPLS-DA). The HCA showed the segregation of the species into four main groups. Comparing the HCA with taxonomical classifications of Vernonieae, we observed that the groups of the dendogram, based on metabolic profiling, were in accordance with the generic classification proposed by Robinson and with previous phylogenetic studies. The species of the genera *Stenocephalum*, *Stilpnopappus*, *Strophopappus* and *Rolandra* (Group 1) were revealed to be more related to the species of the genus *Vernonanthura* (Group 2), while the genera *Cyrtocymura*, *Chrysolaena* and *Echinocoryne* (Group 3) were chemically more similar to the genera *Lessingianthus* and *Lepidaploa* (Group 4). These findings indicated that the subtribes Vernoniinae and Lepidaploinae are non-chemically homogeneous groups and highlighted the application of untargeted metabolomic tools for taxonomy and as indicators of species evolution. Discriminant compounds for the groups obtained by OPLS-DA were determined. Groups 1 and 2 were characterized by the presence of 3′,4′-dimethoxyluteolin, glaucolide A and 8-tigloyloxyglaucolide A. The species of Groups 3 and 4 were characterized by the presence of putative acacetin 7-*O*-rutinoside and glaucolide B. Therefore, untargeted metabolomic approach combined with multivariate statistical analysis, as proposed herein, allowed the identification of potential chemotaxonomic markers, helping in the taxonomic classifications.

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## 1. Introduction

Current metabolomic approaches have been applied as a potential chemotaxonomic tool (Kim et al., 2016; Martucci et al., 2014; Sandasi et al., 2012) and as a tool for understanding species evolution (Dos Santos et al., 2017) and biogeographic trends in plant diversification (Padilla-González et al., 2017). Furthermore, obtaining metabolomic fingerprints associated with multivariate statistical analysis has had wide applications in natural products research, such as quality control of medicinal plants, solving phylogenetic problems and discovery of bioactive compounds (Chagas-Paula et al., 2015; Cox et al., 2014; Yuliana et al., 2011).

*Vernonia* Schreb. *sensu lato* (Asteraceae) is the largest and most complex genus of the tribe Vernonieae Cass. (Keeley et al., 2007). The "*Vernonia* problem" (Bremer, 1994) arose because several species from the tribe Vernonieae, 1000 of the 1500 species, were

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placed under the genus *Vernonia s.l.* due to the great morphological homogeneity in their reproductive characters and a wide vegetative plasticity (Keeley and Jones, 1977). The generic limits between *Vernonia s.l.* and its several satellite genera were based on obvious differences on pappus and inflorescences (Robinson, 1999a). Thus, the genus *Vernonia s.l.* was defined by “what is not” instead of by any set of specific characters (Keeley and Robinson, 2009).

Over the years, several taxonomical classifications have been proposed for Vernonieae and the genus *Vernonia s.l.* According to traditional classification (Bentham, 1873), Vernonieae has two subtribes and the genus *Vernonia s.l.* comprises more than 1000 species and is divided into sections, subsections and series (Baker, 1873; Bentham, 1873; Jones, 1977). Harborne and Williams (1977), Jones (1977) and Robinson et al. (1980) developed seminal studies that gathered cytogenetic, chemical, micromorphological and macromorphological information into the Vernonieae taxonomy. These researches were the basis for the new circumscription for Vernonieae and *Vernonia s.l.* proposed by Robinson (1999a, 1999b). In this series of studies, Robinson segregated several species of *Vernonia s.l.* into new genera, reestablished others and restricted *Vernonia sensu stricto* only to 22 species distributed mainly in North America. This re-circumscription of the genus was also accompanied by a big change at the subtribal level, from few subtribes to currently 21 subtribes (Keeley and Robinson, 2009; Robinson, 1999a, 1999b).

Vernonieae has two centers of diversity, one in Brazil and the other in tropical Africa (Jones, 1977). Brazilian Vernonieae is represented by 11 subtribes, 55 genera and 434 species, which ca. 40% are endemic species (BFG, 2015; Semir et al., 2011). Vernonieae is diverse in Cerrado, Atlantic Forest and Pampa phytogeographic domains, however there are some endemic species from Caatinga and Pantanal. Lepidaploinae and Vernoniinae are the most diverse subtribes in Brazil, while Rolandrinae comprises only a few species (BFG, 2015).

The current knowledge on Vernonieae has increased since the beginning of this revolution in classification. However, there are some disagreements about these drastic changes in the classification of Vernonieae and *Vernonia s.s.*. Several challenges have not been totally resolved (Bremer, 1994; Keeley et al., 2007; Keeley and Robinson, 2009; Robinson, 2007), which reflects the lack of consensus in the adoption of the current generic limits (Robinson, 1999a). In such complex cases, as in Vernonieae, multiple evidences have contributed to a better understanding of the evolutionary patterns. Thus, improving taxon sampling using chemical characterization together with morphological, cytogenetic, ecological and phylogenetic studies may offer an approach to clarify the evolution of specialized metabolites and the classification of Vernonieae.

Several classes of compounds have been used as chemotaxonomic markers in the Asteraceae family, mainly flavonoids and sesquiterpene lactones (Alvarenga et al., 2001; Emerenciano et al., 2001; Seaman, 1982). Besides, differential patterns of specialized metabolites accumulation were found in species of the genus *Vernonia s.l.*, which highlighted the application of metabolomic approaches as potential chemotaxonomic tool (Martucci et al., 2014).

In this study, we analyzed 79 species from nine genera, which belongs to the subtribes Vernoniinae Cass. ex. Dumort., Lepidaploinae S.C Keeley & H. Rob. and Rolandrinae Cass. ex Dumort., aiming to determine the main specialized metabolites by means of an untargeted metabolomic approach followed by multivariate statistical analysis (PCA, HCA and OPLS-DA). Then, we compared the obtained results with the classifications proposed by Bentham (1873) and Robinson (1999a, 1999b) to evaluate the use of chemical characters for taxonomic classifications and as indicators of species evolution.

## 2. Results and discussion

### 2.1. Multivariate statistical analysis

Metabolomic analysis was carried out using unsupervised and supervised statistical analysis. Hierarchical cluster analysis (HCA) showed that the species were segregated into four main groups (Fig. 1). Groups 1 and 2 appeared in the first branch of the dendogram. Group 1 was composed of species of the genera *Stenocephalum* Sch. Bip., *Stilpnopappus* Mart. ex DC., *Strophopappus* DC., *Rolandra* Rottb. and some *Lessingianthus* H. Rob. *(L. brevifolius* (Less.) H. Rob., *L. vepretorum* (Mart. ex DC.) H. Rob.*, L. zuccarinianus* (Mart. ex DC.) H. Rob., *L. secundus* (Sch. Bip. Ex Baker) H. Rob.) and *L. roseus* (Mart. ex DC.) H. Rob.) (Figs. 1 and 2). Group 2 was composed exclusively of species of the genus *Vernonanthura* H. Rob. (Figs. 1 and 3). Groups 3 and 4 appeared in the second branch of the dendogram, with the genera *Cyrtocymura* H. Rob., *Chrysolaena* H. Rob. and *Echinocoryne* H. Rob. belonging to the Group 3 (Figs. 1 and 4) and genera *Lessingianthus* H. Rob. and *Lepidaploa* (Cass.) Cass. belonging to Group 4 (Figs. 1 and 5).

According to the groups obtained from HCA, we established a link between the different genera that were analyzed. In general, the genus *Vernonanthura* was chemically more similar to the genera *Stenocephalum*, *Stilpnopappus*, *Strophopappus* and *Rolandra,* while the genera *Lessingianthus* and *Lepidaploa* showed a close relationship between each other and to the genera *Cyrtocymura*, *Chrysolaena* and *Echinocoryne*.

Beyond the relationship between the genera, we observed (Figs. 3 and 5) that most of the populations of a given species were clustered, such as those from *Vernonanthura brasiliana* (L.) H. Rob. (VB098, VB099, VB100 and VB101), *V. densiflora* (Gardner) A.J. Vega & M. Dematt. (VD104, VD105, VD106, and VD107), *V. montevidensis* (Spreng.) H. Rob. (VM123, VM124 and VM126), *V. petiolaris* (DC.) H. Rob. (VP132, VP133 and VP134), *Lessingianthus coriaceous* (Less.) H. Rob. (LC052 and LC053), *L. durus* (Mart. ex Dc.) H. Rob. (LD055, LD056, LD057 and LD058) and *Lepidaploa chamissonis* (Less.) H. Rob. (LC032 and LC033).

Some species of *Lessingianthus (L. brevifolius*, LB084; *L. vepretorum*, LV078; *L. zuccarinianus*, LZ079; *L. secundus*, LS077 and *L. roseus*, LR07 and LR076) were clustered into different groups from the main *Lessingianthus* (Group 4). These species showed a close relationship with the genera *Stenocephalum, Stilpnopappus* and *Strophopappus*, which belong to subtribe Lepidaploinae, like the genus *Lessingianthus*. However, these species did not exhibit any morphological difference compared to other *Lessingianthus* species.

Comparing the results of HCA with taxonomical classifications of Vernonieae proposed by Bentham (1873) and Robinson (1999a, 1999b), we observed that the groups of the dendogram were in agreement with the generic circumscriptions proposed by Robinson (1999a, 1999b), who segregated *Vernonia s.l.* to several other genera. Moreover, Robinson's proposals were also corroborated by palynologic (Angulo and Dematteis, 2010; Mendonça et al., 2010, 2009; Vega and Dematteis, 2011), cytogenetic (Angulo and Dematteis, 2012; De Oliveira et al., 2007; Salles-de-Melo et al., 2010; Vega and Dematteis, 2012), morphological (Angulo et al., 2015; Angulo and Dematteis, 2014; Redonda-Martínez et al., 2012) and phylogenetic (Keeley et al., 2007; Loeuille et al., 2015) evidences, as well as for phytochemical characterization (Bohlmann et al., 1982, 1981; Igual et al., 2013; Jakupovic et al., 1986; Lusa et al., 2016; Martucci et al., 2014). Thus, Robinson's classification may best indicate the taxonomical differences and similarities among the species and genera of Vernonieae and its evolutionary relationships.

Regarding the division according to the subtribes, we observed

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