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Bayesian inference of phylogeny, morphology and range evolution reveals a complex evolutionary history in St. John's wort (*Hypericum*)

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ABSTRACT

The genus *Hypericum* L. ("St. John's wort", Hypericaceae) comprises nearly 500 species of shrubs, trees and herbs distributed mainly in temperate regions of the Northern Hemisphere, but also in high-altitude tropical and subtropical areas. Until now, molecular phylogenetic hypotheses on infra-generic relationships have been based solely on the nuclear marker ITS. Here, we used a full Bayesian approach to simultaneously reconstruct phylogenetic relationships, divergence times, and patterns of morphological and range evolution in *Hypericum*, using nuclear (ITS) and plastid DNA sequences (*psbA-trnH*, *trnS-trnG*, *trnL-trnF*) of 186 species representing 33 of the 36 described morphological sections. Consistent with other studies, we found that corrections of the branch length prior helped recover more realistic branch lengths in by-gene partitioned Bayesian analyses, but the effect was also seen within single genes if the overall mutation rate differed considerably among sites or regions. Our study confirms that *Hypericum* is not monophyletic with the genus *Triadenum* embedded within, and rejects the traditional infrageneric classification, with many sections being para- or polyphyletic. The small Western Palearctic sections *Elodes* and *Adenotrias* are the sister-group of a geographic dichotomy between a mainly New World clade and a large Old World clade. Bayesian reconstruction of morphological character states and range evolution show a complex pattern of morphological plasticity and inter-continental movement within the genus. The ancestors of *Hypericum* were probably tropical shrubs that migrated from Africa to the Palearctic in the Early Tertiary, concurrent with the expansion of tropical climates in northern latitudes. Global climate cooling from the Mid Tertiary onwards might have promoted adaptation to temperate conditions in some lineages, such as the development of the herbaceous habit or unspecialized corollas.

1. Introduction

Bayesian inference techniques have become very popular in phylogenetics because of the relative ease with which these techniques allow biologists to infer evolutionary patterns using complex and realistic models (Ronquist, 2004). Markov Chain Monte Carlo Bayesian approaches have now been developed to answer evolutionary questions, ranging from the time and place of origin of lineages to inferring the evolution of morphological traits, while accounting for phylogenetic and model uncertainty (Drummond and Rambaut, 2007; Huelsenbeck and Bollback, 2001; Lemey et al., 2009; Ronquist and Sanmartín, 2011; Sanmartin et al., 2008). Here, we use this full Bayesian approach (Ronquist, 2004) to simultaneously reconstruct phylogenetic relationships, lineage divergence times and ancestral areas in the old worldwide distributed plant genus *Hypericum* (Nürk and Blattner, 2010; Robson, 1981), while integrating out uncertainty concerning tree topology and other model parameters.

Hypericum L. represents one of the 100 largest angiosperm genera of the world (Carine and Christenhusz, 2010), with over 496 species (including other Hypericeae genera (Nürk et al., 2012), or 500 in the most recent Robson's (2012) revision) of trees, shrubs and herbs. The genus is distributed in almost every continent and ecosystem, being absent only in the poles, arid deserts, and low-altitude tropical areas (Fig. 1) (Robson, 1977). *Hypericum* is a relatively old genus as suggested by its fossil record dating back to the Early–Mid Tertiary, ca. 37–34 Ma (Meseguer and Sanmartín, 2012). Some *Hypericum* species, such as *Hypericum perforatum* L. (common St. John's wort), are economically important in pharmacology because of their active compounds hypericine and pseudo-hypericine, which are used as painkillers, antidepressants or anticancer treatments (Barnes et al., 2001). In this aspect, a phylogenetic hypothesis for the genus *Hypericum* could be interesting for bioprospecting.

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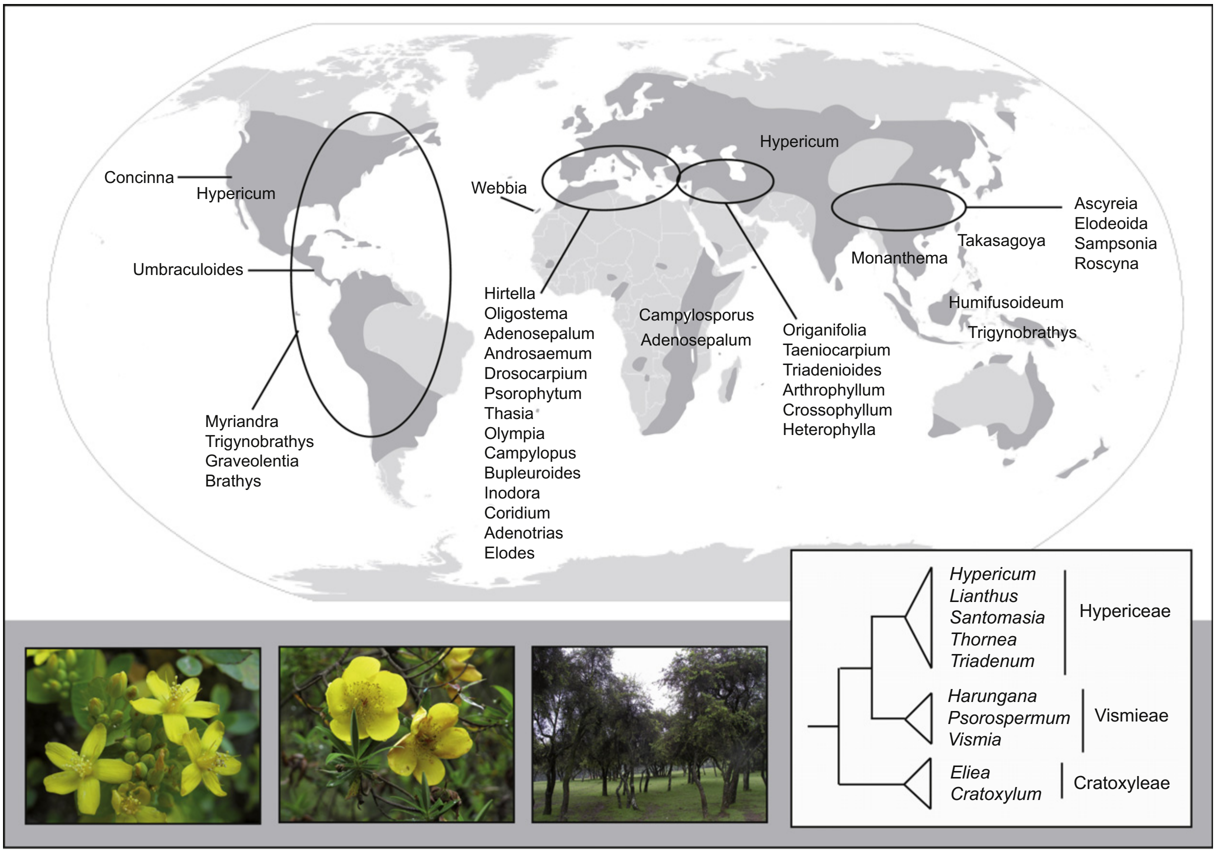

Fig. 1. Present distribution of Hypericum species. Map showing the current distribution of Hypericum species (modified from Robson, 1977); for each section the regions harboring the highest number of species are given. Inset: Schematic representation of phylogenetic relationships among the genera of family Hypericaceae, showing division into tribes. Below, from left to right pictures of *H. tortuosum* flowers (section *Triadenioides*), leaves and flowers of *H. revolutum* (*Campylosporus*) and habit of *H. revolutum*.

Current Angiosperm classification (APGIII 2009, Stevens, 2007) includes the genus *Hypericum* in the family Hypericaceae, belonging to the large clade of mostly tropical plants known as the "clusioid clade" (Davis et al., 2005; Gustafsson and Persson, 2002; Ruhfel et al., 2011; Wurdack and Davis, 2009). Three tribes are recognized within Hypericaceae: the tropical tribes Vismieae Choisy (*Vismia* Vand., *Harungana* Lamarck and *Psorospermum* Spach) and Cratoxyleae Bentham & J.D. Hooker (*Cratoxylum* Blume, *Eliea* Cambess.), and the widespread tribe Hypericeae Choisy, including the genera *Triadenum* Raf., *Thornea* Breedlove & McClintock, *Santomasia* N. Robson, *Lianthus* N. Robson, and *Hypericum* (Fig. 1, inset). Yet, relationships among genera remain unclear (see below).

Hypericum is one of few large genera with an almost complete taxonomic treatment. Robson (Robson, 1977, 1981, 1985, 1987, 1990, 1996, 2001, 2002, 2006, 2010a, 2010b, 2012) published a series of monographs in which he described numerous species and defined the main diagnostic characters for the taxonomy of the genus. Robson divided the genus into 36 sections (see Nürk and Blattner (2010) for a synthesis of Robson's classification), and proposed relationships between sections based on the evolutionary direction of certain traits, such as the habit form, presence of dark glands, corolla shape, or the number of stamen fascicles. Based on Robson's study, Nürk and Blattner (2010) carried out the first morphological cladistic analysis of the genus, and concluded that some of these diagnostic characters were under convergent evolution. They also found discrepancies with Robson's sectional classification, and suggested the inclusion of the monotypic genus *Santomasia* within *Hypericum.*

In contrast to morphological studies, work at the molecular level has been slower in *Hypericum*, probably due to the difficulty to work with such a large and cosmopolitan genus. Ruhfel et al. (2011) analyzed relationships beyond the genus level in the clusioid clade and concluded that *Hypericum* is not monophyletic, with genera *Santomasia*, *Triadenum*, and *Thornea* embedded within. However, their study included only 21 *Hypericum* species, so little could be inferred in terms of infra-generic relationships. Other molecular studies focusing on interspecific relationships in *Hypericum* were too limited in both taxonomic and geographic coverage (Crockett et al., 2004; Heenan, 2008; Park and Kim, 2004; Pilepić et al., 2011). Just recently, Nürk et al. (2012) published the first deep-sampled molecular phylogeny for the genus including ca. 40% of the species diversity. They confirmed the inclusion of *Triadenum* within *Hypericum*, but, contrary to Ruhfel et al. (2011), recovered *Thornea* as the sister group of *Hypericum*. They also reconstructed ancestral states for some diagnostic characters, confirming many of Nürk and Blattner's (2010) conclusions. All the above-mentioned species-level phylogenies were based solely on ribosomal nuclear internal transcribed spacer. It is well known, that phylogenies based on ITS alone can be problematic because this marker displays a complex evolutionary behavior owing to concerted evolution among its multiple copies (Álvarez and Wendel, 2003). Also, biological processes such as hybridization, duplication, introgression, or incomplete lineage sorting may obscure the correlation between gene trees and the species tree. Thus, additional inclusion of plastid genes is desirable when reconstructing evolutionary relationships among species (Doyle, 1992).

Hypericum is unique within the clusioid clade in its variable habit form and mainly temperate distribution (most of the other genera are woody elements of tropical forests). The largest diversity of the genus is found in temperate areas of the Northern Hemisphere,