Floral Character Evolution in Response to an Aquatic Environment in *Podostemaceae:* A Phylogenetic Approach

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Abstract

Habitat transition is a common driving force for change in morphological characters. One of the most dramatic habitat transitions is that between terrestrial and aquatic ecosystems, which has occurred numerous times in both directions throughout the evolutionary history of flowering plants. Podostemaceae, more commonly known as the riverweeds, evolved from terrestrial to freshwater ecosystems and subsequently experienced an overall reduction in number of floral characters. Taking a phylogenetic approach, this study served as a preliminary investigation into evolutionary trends of four floral characters: 1) stamen number, 2) tepal number, 3) stigma number and 4) locule number. Mapped on a phylogeny based upon Maximum Likelihood, all four characters show overall reduction but characters became reduced at different rates. Stamen and tepal numbers showed a rapid initial decrease followed by a gradual increase, stigma number showed a rapid initial decrease then stabilization, and locule number showed a gradual persistent decrease. The difference in trends among the four floral characters is likely due to differences in habitat, but further research is needed.

It may seem surprising that organisms so well adapted to survive and reproduce in their environments evolve into new habitats, yet habitat transitions have occurred numerous times throughout the history of life. Transitions into new habitats are often accompanied by changes in species morphology, as natural selection acts to favor new and oftentimes novel characters as species adapt (Darwin, 1859). This can explain the transition of green algae onto land that gave rise to land plants and the novel root-shoot system (Graham, 1993). As the environment becomes increasingly dissimilar to its former state, the probability of organisms transitioning without developing major morphological changes decreases. A repeatedly observed habitat change is the return to water from a terrestrial environment, with resulting morphological reductions such as loss of appendages in marine mammals or the reduction of leaves and petals in some aquatic plants (Jefferson et al., 1993). This major habitat transition affects the availability and uptake of water and nutrients by the root system as well as drag on the plant and pollination methods (which may shift from largely insect reliant to a combination of dispersal methods; Vermeij and Dudley, 2000).

Podostemaceae, commonly known as the riverweed family, are the largest strictly aquatic flowering plant family (approximately 280 species, 49 genera) and one of the best examples of reduced morphological features associated with colonization into aquatic habitats (Cook and Rutishauser, 2007). Many species in this family have a small number of stamens and stigma lobes as well as a few, often filamentous, tepals (a term applied to either the petal or the sepal whorl in a flower when these cannot be differentiated) and few leaves, sometimes reduced to scale-like structures (Cusset, 1974, Schenk et al., in press; Fig. 1). Leaves are dichotomously branched as in early land plants and the root-shoot-leaf system has been reduced to such a degree that the homology of these structures is debated (Schenk et al., in press). Some species have flowers that are covered in a spathella (a specialized sac unique to the family) that protects the flower from moving water during development (Cook and Rutishauser, 2007; Fig. 1). These flowers elongate and emerge from the spathella during dry seasons so that pollination can occur (Schenk et al., in press).

Although morphological reduction in this family is

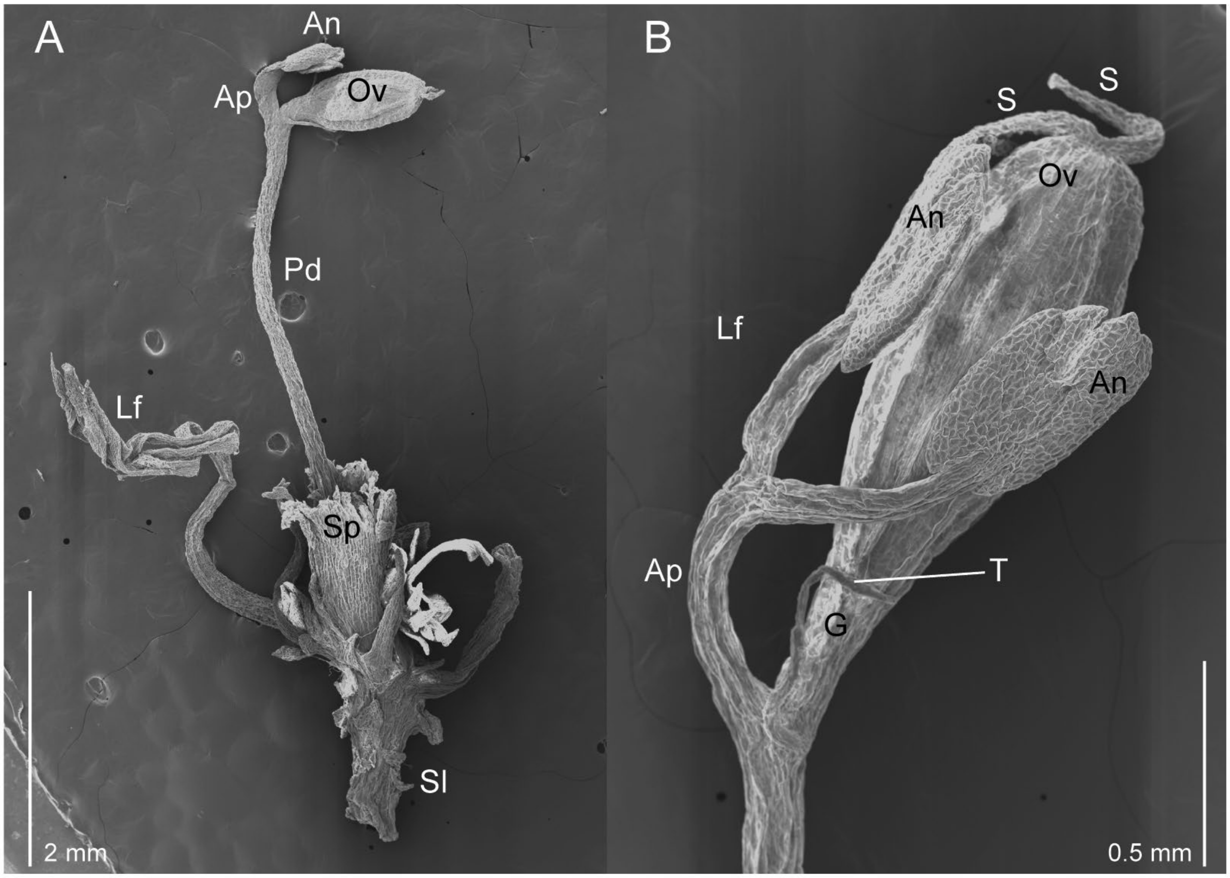

Figure 1. Reduced morphological characters in Podostemaceae based on scanning electron micrographs of *Inversodicraea achoundongii* (Schenk et al., in press). **A**. Shoot terminated with a spathellum, in which the flower has already emerged. **B**. Close-up of mature flower. Abbreviations: Anther (An), andropodium (Ap), gynophore (G), upper elongated leaf (Lf), ovary (Ov), pedicel (Pd), stigma (S), scale leaf (Sl), spathellum (Sp), and tepal (T).

well documented (Moline et al., 2007), scientists have not tracked morphological changes over evolutionary history to see how quickly reduction has occurred, what clades have become the most reduced, and what factors may have led to reduction in some groups but reversals in other others. In addition, the evolution of more complex structures is also often observed in angiosperms, although such transitions are said to follow a relatively indirect path, which may create opposing evolutionary forces between selection for reduced and for complex morphologies (Cronquist, 1968). Here, using a phylogenetic approach, I investigate the consequences of evolution from terrestrial to aquatic environments for changes in the morphology of stamen, style, locule, and tepal number in the family Podostemaceae.

MATERIALS AND METHODS

A phylogeny of Podostemaceae was generated from sequences obtained through GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Four genes were sampled, three from the chloroplast (*matK*, *rbcL* and *trnL*) and one from the nuclear genome (ITS). Sequences were aligned for each marker individually in SATé (Liu et al., 2012), and then manually adjusted. Marker alignments were concatenated and the best-fit DNA substitution model was inferred in JModelTest 2 (Darriba et al., 2012). The final alignment was analyzed in PAUP* v 4b10 (Swofford, 2012). One hundred randomly-replicated searchers were conducted, each optimized with maximum likelihood, and tree-space was explored with bisection reconnection.

For morphological data, a matrix was created with 181 species, 172 from Podostemaceae and nine outgroup species from Hypericaceae, a well-supported sister group (Ruhfel et al., 2011). Characters recorded included tepal number, stamen number, locule number and stigma lobe number. Morphological data were obtained from the literature (e.g., Cusset, 1987; Cook and Rutishauser, 2007; Thiv et al., 2009) using original species descriptions if available or later descriptions by authorities on the family. Ancestral states for the four characters were estimated with Mesquite 2.75 (Maddison and Maddison, 2009). Each of the four characters was optimized with parsimony and traced separately along the tree, which included Podostemaceae and the outgroup. The four resulting character traces were then used to assess general patterns in the family to determine how characters evolved across Podostemaceae following its colonization of aquatic ecosystems.

RESULTS

Tepal Evolution—Tepal number underwent character state transitions 14 times (Fig. 2). The outgroup mostly had ten tepals (five petals, five sepals). The base of Podostemaceae was equivocal (the method was unable to definitively assign a single ancestral state to the root of the tree), followed by a clade with three tepals (*Malaccotristicha*, *Terniopsis*, *Dalzellia*, *Indotristicha* and *Tristicha*), five tepals (*Weddellina squamulosa*) and then two tepals (all remaining Podostemaceae). Following the reduction to two, there were further reductions to one and to zero tepals, as well as reversals towards increasing complexity (Fig. 2). Notable reductions were observed in *Castelnavia* (from two to zero) and *Inversodicraea cristata* (from two to one; Fig. 2).

Reversals to more tepals included the clade containing *Marathrum* (from two to three, and then from three to six), *Podostemum* (from two to three), *Ceratolacis pedunculatum* (possibly from two to three, although the transition may have occurred deeper in the tree and include *Podostemum*), *Apinagia surumuensis* (from two to six) and *Stonesia* sp. (from two to three). Overall, tepals were reduced to two early in the Podostemaceae clade then underwent numerous reversals to higher tepal numbers in some clades, while maintaining two in many clades.

Stamen Evolution—Stamen number underwent character state transitions across the phylogeny 33 times and had many more reductions and reversals than tepals (Fig. 2). The outgroup had numerous stamens, but Podostemaceae were inferred to have an ancestral state of two stamens (Fig. 2). Further reductions to one stamen were observed in *Hydrobryum takakioides*, *Hydrobryum australaoticum*, *Hydrobryum khaoyaiense*, *Cladopus*, the *Polyplerum* + *Farmeria* clade, *Polyplerum schmidtianum*, *Stonesia* sp., *Letestuella tisserantii*, *Ledermanniella pellucida*, *Djinga felicis*, *Ledermanniella bowlingii*, *Saxicolella nana*, *Ledermanniella ledermanii*, *Monandriella linearifolia*, *Saxicolella amicorum* + *S. agumatsa*, *Monostylis capillaceae* and *Castelnavia monandra*.

Reversals in stamen number were seen in *Marathrum* (from two to five, with one reversal back to two in *Marathrum plumosum*), *Apinagia richardiana* (from two or five to five), *Apinagia fluitans* (from two to three), *Apinagia staheliana* (from two or five to eight), *Apinagia surumuensis* (from two or five to 13), *Marathrum oxycarpum* (from two to five; the state in the most recent ancestor is uncertain) and the *Mourera* clade (from two to about 13 stamens; although the number of species in this clade showing reversal is uncertain). The overall trend was a high level of reversals in the less divergent species, with stamen number being maintained around two with additional reductions to one in clades near the base of the tree.

Locule Evolution—Tracing locule number resulted in a tree with 14 steps. Pistils in the outgroup have three or five locules, with an early diverged clade (*Malaccotristicha, Terniopsis, Dalzellia, Indotristicha, and Tristicha*) and root of Podostemaceae having three locules (Fig. 3).

Reduction to one locule was observed in the *Hydrobryum takakioides* + *H. taeniatum* + *H. somranii* clade, *Hydrobryum micrantherum*, *Polyplerum longistylosum*, the *Leiothylax* + *Ledermanniella* + *Winklerella* + *Macropodiella* + *Stonesia* + *Letestuella* (with one reversal to two in *Ledermanniella pellucida*) clade, the *Inversodicraea* + *Ledermanniella* +*Monandriella* clade, the *Marathrum* + *Oserya* clade (with a reversal back to two in *Marathrum schiedeanum*), *Apinagia longifolia,* and *Castelnavia* (Fig. 3).

Reversals in locule numbers from unilocular to bilocular included *Ledermanniella pellucida*, *Saxicolella nana and Marathrum schniedeanum*, with a few others having uncertain ancestral states. In general, locule number in the family was reduced from three to two then either maintained at two or reduced to one with minimal reversals.

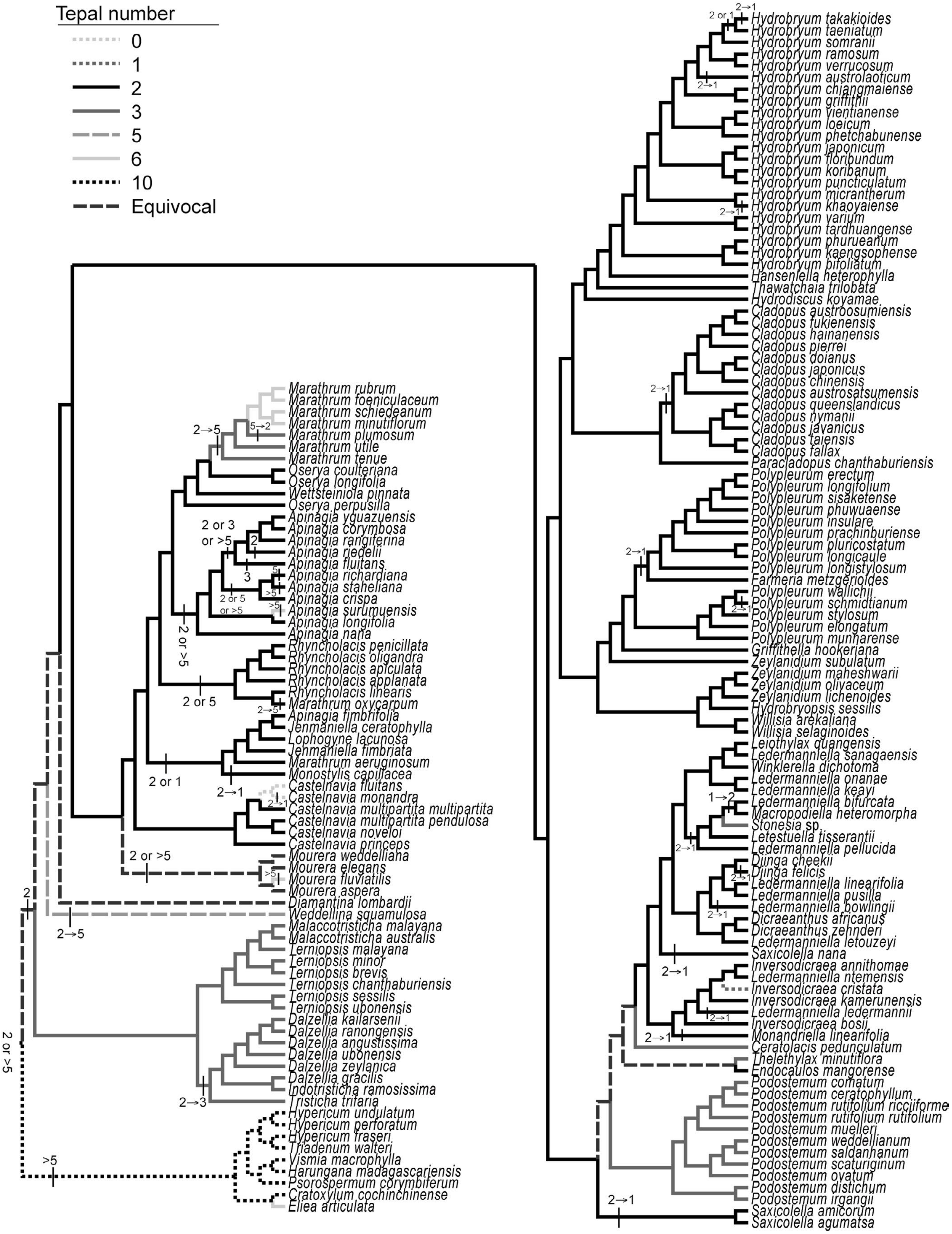

Figure 2. Phylogenetic tracing of tepal number and stamen number in Podostemaceae optimized with parsimony. Tepal number is indicated by shading and coloring (see key). Stamen character states are indicated by number at nodes, with an arrow representing a transition.

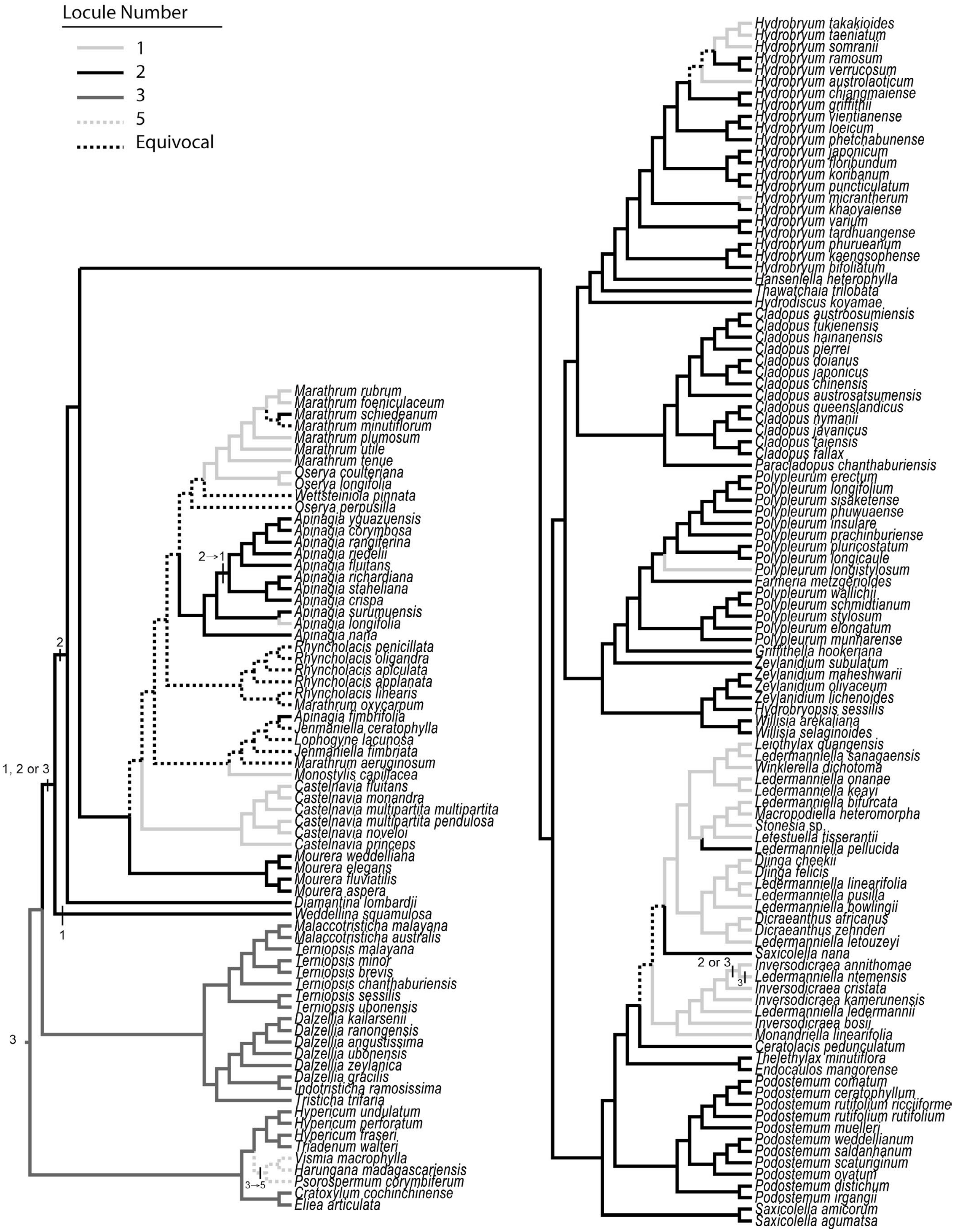

Figure 3. Phylogenetic tracing of locule number and stigma number optimized with parsimony. Locule number is indicated by shading and coloring (see key). Stigma character states are indicated by number at nodes, with an arrow representing a transition.

Stigma Evolution—Tracing stigma evolution resulted in a tree with five steps (Fig. 3). The outgroup had three to five stigmata and an early clade of Podostemaceae (*Malaccotristicha*, *Terniopsis Dalzellia*, *Indotristicha* and *Tristicha*) had three (Fig. 3). Within the sister clade, *Weddellina squamulosa*, which shares the more recent common ancestor to the clade containing the remaining Podostemaceae species, number of stigmata reduced further to one, with the remaining Podostemaceae species reducing to two, although stigma number of the most recent common ancestor is uncertain. Further reduction occurred in part of the *Apinagia* clade, and the only reversal observed was in *Ledermanniella ntemensis*, which possibly went from two to three stigma. Overall, stigma number is fairly stable at two, with only one further reduction and one reversal event.

DISCUSSION

Although a response to habitat change in Podostemaceae has been well documented (Moline et al., 2007), patterns of character evolution in specific clades had not been previously analyzed. The four ancestral state estimations all showed a general trend towards reduction with some exceptions, but reduction occurred at different positions along the phylogeny. Tepal and stamen number showed a rapid initial decrease followed by a gradual increase, locule number showed a gradual persistent decrease and stigma number showed a rapid initial decrease only, suggesting that natural selection is acting differently upon each character.

Many factors may be contributing to the differences in the character patterns, including the effect of water drag on the fitness of the plant (Puijalon et al., 2004). As many species in Podostemaceae are submerged in fast-moving water or in a mist zone for part of the year, then flower in the dry season, it is possible that each number of structures has a cost and benefit in different habitats at different times of the year. If a flower has many more stamen and stigma lobes, it may have more successful pollination and we would expect natural selection to favor increased stamen and stigma numbers (Eugénio et al., 1990). However, more structures also mean increased drag on the plant—most likely having a greater effect on species lacking a spathella, but possibly still causing increased drag on those with a spathella due to a larger, less streamlined shape. These competing selection pressures likely end up creating a type of compromise in the plant characters.

Tepal number may be influenced by pollination more than drag. Most tepals in the family are small, therefore not adding much bulk to the flower. However, tepals are suspected not to play a major role in pollination due to their lack of vibrant color and small size (see the thin tepals in Fig. 1b), although some insects have been observed visiting flowers, potentially for pollination (Rutishauser, 1997). It is therefore possible that further down the backbone of the tree (representing early in Podostemaceae's exploitation of an aquatic habitat), tepals were reduced to two because showy petals were not necessary for pollination.

Of the four characters examined, stigma number seemed to be the most invariable. With only one reversal and one reduction event after two stigmata appear on the tree, it seems as if stigma has the most straightforward path of character change. Such a straightforward paths suggests fewer stigmata are advantageous throughout Podostemaceae, and that minor differences in habitat are not significantly influencing stigma number after the initial reduction.

Although locule number has also been generally reduced (more rapidly than stigma, however), the fluctuation between unilocular and bilocular forms may be due to the fact that the locules are internal. They may be less affected by wind and water drag in the rapidly-moving rivers and waterfalls where they commonly occur, and therefore less heavily selected for a specific locule number. The overall reduction of locule number follows the generalized reduction in number of flowers and is possibly linked to stigma number (Rutishauser and Grubert, 1998), but other potential explanations for the many reversals are uncertain.

One of the reasons why tepal and stamen number seem to have the most transition and fluctuation is because morphological structures are often reduced or modified to the extent that determining their homology is difficult (Khanduri et al., 2014). What was once thought to be a leaf could be a shoot or scale, and a third "tepal" on the andropodium is more likely to be staminodes (Khanduri et al., 2014). By using the most widely accepted data for these characters it was possible to analyze the data with less ambiguity and more consistency, but further investigation into homologous structures, including universal consensus on what constitutes a tepal, stamen, or leaf, would need to be reached in order to further analyze reduction in the family.

Although the patterns of reduction for each character may be due to different selection regimes, the results show that there is a pattern of morphological reduction in Podostemaceae, with some characters showing an increase in complexity after initial reduction. Further analyses of the overall patterns as well as exceptions to the trend would likely be a worthwhile endeavor.

Podostemaceae, with its exhibited reduction over time, shows the effect of introduction into novel habitat, including examples of evolutionary solutions to life in an aquatic ecosystem. Its unique structures represent adaptation to its aquatic environment and suggest that more research is needed to better understand what is causing the change in number and form of its structures. The cause of character transition patterns may be uncertain, but it is clear that reduction is occurring and certain character states are being selected for over others across many species in the family.

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