The LINNEAN SOCIETY of London BOTANICAL Journal of the Linnean Society

Botanical Journal of the Linnean Society, 2013, **173**, 153–175. With 14 figures

Three modes of heterochrony explain lobule diversity in *Radula* subgenus *Cladoradula* (Porellales: Jungermanniopsida), a small lineage of early land plants today

MATT A. M. RENNER FLS[1]*, NICOLAS DEVOS[2], ELIZABETH A. BROWN[1] and MATT J. VON KONRAT FLS[3]

[1]*Royal Botanic Gardens and Domain Trust, Mrs Macquaries Road, Sydney, NSW 2000, Australia*
[2]*Department of Biology, Duke University, Box 90388, Durham, NC 27708, USA*
[3]*The Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL, USA*

Received 9 December 2012; revised 6 June 2013; accepted for publication 2 July 2013

Changes in lobule morphology in *Radula* subgenus *Cladoradula* show liverworts have the capacity for dramatic, relatively rapid morphological change by heterochrony. In individuals of *R. bipinnata*, *R. boryana* and *R. tenax*, lobules on secondary and tertiary shoots are progenetic with respect to lobules on primary shoots, in that the slope of the relationship between growth duration and shape does not change. However, in *R. campanigera*, lobules on secondary and tertiary branches exhibit different slopes from primary branches, but have the same growth duration, a pattern consistent with neoteny. The trajectory of allometric growth is extended or truncated in different species compared with outgroup and ancestral nodes. Changes in duration of lobule growth explain 85% of variation in lobule shape between species. Species are related by relatively shallow nodes in the crown of the *Radula* subgenus *Cladoradula* clade, suggesting that divergence and associated heterochronic changes have occurred relatively recently. The rapid morphological diversification in the crown contrasts with the relative stasis between the ancestral node and *R. brunnea*, the outgroup used in this analysis. A robust primary axis may be required to hold shoots away from vertical surfaces to maximize light interception, and hypermorphosis in lobule ontogeny could be a by-product of the longer growth durations required to build axes sufficiently large to perform this structural role. Alternatively, the large auriculate lobules could function in external water transport systems by providing continuity of surfaces for solute transport via capillary action. *Botanical Journal of the Linnean Society*, 2013, **173**, 153–175.

ADDITIONAL KEYWORDS: anatomy – geometric morphometrics – liverworts – ontogeny – phylomorphospace.

INTRODUCTION

Heterochrony, defined as 'a change in timing or rate of developmental events relative to the same events in the ancestor' (McKinney & McNamara, 1991), is the most general explanation for evolutionary changes in morphology (Gould, 1977; Raff & Kaufman, 1983; Lord & Hill, 1987; McKinney & McNamara, 1991; McLellan, 1993; Pryer & Hearn, 2009) and may go to the deepest nodes of land plant evolution. Extant land plants exhibit an extraordinary diversity of forms in gametophytic and sporophytic generations, both during growth and development and at maturity (Ligrone, Duckett & Renzaglia, 2012).

Plants belonging to the 'bryophyte' grade (Mishler *et al*., 1994; Lewis, Mishler & Vilgalys, 1997; Qiu *et al*., 1998, 2006) are usually conceptualized as small, with uncomplicated structural organization. Perhaps because of their 'failure' to transition to a sporophyte-dominated life phase, they have been described as 'evolutionary dead ends', evolution of which is

*Corresponding author. E-mail: matt.renner@rbgsyd.nsw.gov.au

characterized by 'fundamental mistakes' (Schuster, 1984). Although this view is now passé, documented evolutionary innovations resulting from changes in timing or rate of growth in liverworts all involve reductions of morphology to increasingly simplified ends, as in the highly derived species *Cololejeunea metzgeriopsis* (K.I.Goebel) Gradst., R.Wilson, Ilk.-Borg. & Heinrichs, *Myriocolea* Spruce (Gradstein, Reiner-Drehwald & Schneider, 2003; Gradstein *et al*., 2006; Gradstein & Wilson, 2008), *Radula aguirrei* R.M.Schust. and *R. yanoella* R.M.Schust. (Schuster, 1991).

Liverworts exhibit life cycle characteristics intermediate between putative green algal ancestors and other land plants (McManus & Qiu, 2008), including matrotrophy of the developing diploid embryo, which, by mitotic division, produces a multicellular sporophyte. Differences in the timing of meiosis and other aspects of sporophyte growth account for some of the morphological differences between liverworts, mosses and hornworts (Mishler & Churchill, 1985; Graham, 1993; Renzaglia *et al*., 2000). It is possible that a constellation of changes, such as hypermorphosis and/or growth-rate acceleration in sporophytes, in combination with neoteny and paedomorphosis in gametophytes, characterize the transition to sporophyte-dominated life phases early in the evolution of some lineages of land plants.

Evolution proceeds through gene mutation and changes in allelic frequencies and, importantly, also through temporal shifts in gene action and expression (Gould, 1977). Variables including timing of growth onset and cessation and growth rate itself regulate the ontogenetic trajectory from juvenile morphologies to final adult form (Alberch *et al*., 1979; McNamara, 1982). Changes in time of initiation, cessation and rate of growth interact in the development of novel structures through modification of ontogeny, i.e. heterochronic changes, and are a major source of morphological innovation in plants (Guerrant, 1982; Li & Johnston, 2000; Bateman, Rudall & James, 2006; Box *et al*., 2008). For example, neoteny in *Cololejeunea* (Spruce) Schiffn., in particular *C. metzgeriopsis*, is thought to be an adaptation to life in short-lived habitats provided by the surfaces of living leaves (Gradstein *et al*., 2006). Accelerated development and early maturation also characterize species of Marsileaceae that inhabit fluctuating and ephemeral aquatic habitats (Pryer & Hearn, 2009).

Like virtually all members of the clade designated 'Leafy 1' by Davis (2004), *Radula* (L.) Dumort. has conduplicately lobed leaves with one (or both) of the postical lobe(s) folded under the antical, forming a sac-like structure (Davis, 2004; Forrest *et al*., 2006). In *Radula*, this sac encloses the ventral face of the incubously inserted leaf, a form usually referred to as a lobule (Schuster, 1966, 1984; Heinrichs *et al*., 2005). Lobules are a character system expressing considerable diversity, the adaptive value of which is unknown. However, water storage and nutrient acquisition through symbiosis with rotifers or other small organisms have both been postulated (Schuster, 1966). Water storage seems unlikely as water is rapidly lost from lobules attributable to phenomena associated with surface tension as the plant dries (Blomquist, 1929). Lobules may also serve as components of an external water transport system via capillary action between external surfaces, or possibly even nutrient acquisition through passive traps, or providing homes and being first by proximity to capitalize on decomposition of animals and animal waste of those that live in bryophyte mats. One genus with conduplicately bilobed leaves (*Colura* (Dumort.) Dumort.) has complicated trapdoor-like structures at the opening, suggesting an active trapping role (Barthlott *et al*., 2000).

Radula subgenus *Cladoradula* Spruce is a small lineage of 13 distinctive species (M. J. von Konrat, L. Soderström, A. Hagborg, unpubl. data) having a circum-equatorial distribution, with species extending northwards into China, Japan and North America. Species of *Radula* subgenus *Cladoradula* are relatively large and have regularly pinnate or bipinnately branched shoot systems (Fig. 1). As circumscribed by Spruce (1884), Jones (1977) and Yamada (1979), but not Castle (1937), the subgenus is a monophyletic lineage sister to the remainder of *Radula* (Devos *et al*., 2011a, b). *Radula* subgenus *Cladoradula* exhibits diversity in lobule shape both in individuals and between species. In individuals, lobule shape appears correlated to branch order and, as a result, disparate morphologies may be exhibited by different branches in a single shoot system (Fig. 1). In extreme instances, 'microphyllous' branches may occur in the same shoot systems as 'normal' shoot systems with strong auriculate lobules.

In this paper, we use geometric morphometric methods to quantify lobule shape and investigate its dependence on size, its allometry (Gould, 1966; Klingenberg, 1998). Evolution of lobule morphology cannot be understood without knowledge of development (Jones, 1993), and we investigate lobule ontogeny to aid our interpretation of ancestral shape states reconstructed onto phylogeny. Our purpose is to examine and compare lobule ontogenies and query how these ontogenies have changed. We demonstrate that, despite being a relatively old and outwardly stenotypic lineage, the lobules of *Radula* subgenus *Cladoradula* exhibit the capacity for dramatic and relatively rapid morphological change via heterochrony. Heterochronic changes explain a substantial portion of variation in lobule morphology in this clade.

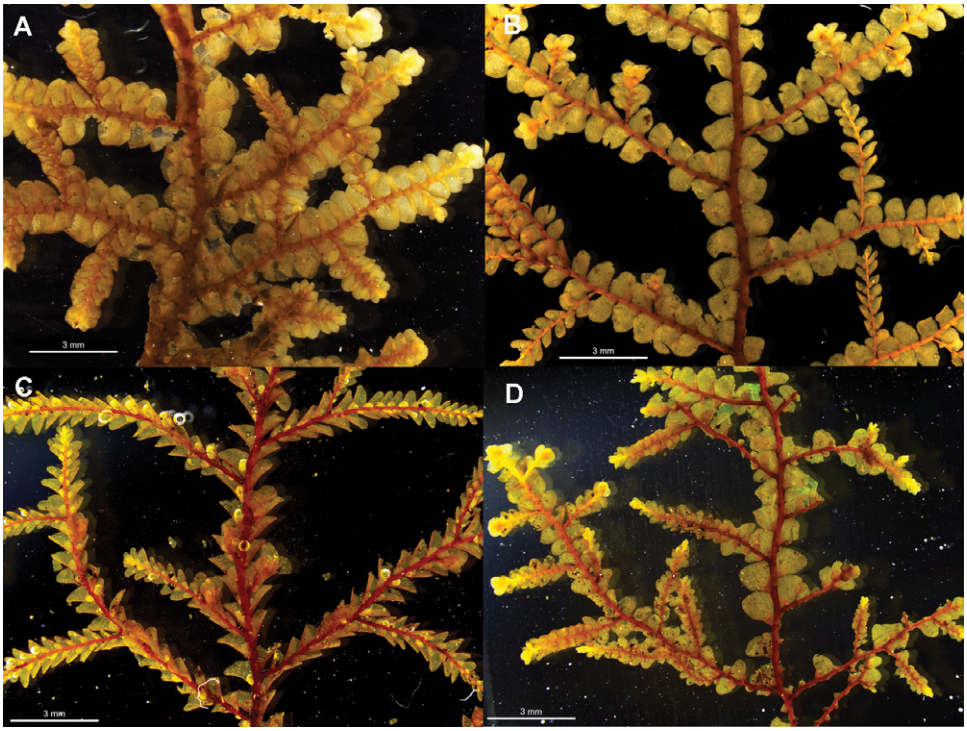

Figure 1. Shoot systems for four species of *Radula* subgenus *Cladoradula* studied in detail in this study, showing regular bipinnate branching. A, *Radula bipinnata* (NY 00877325). B, *Radula boryana* (E 00115207). C, *Radula campanigera* (Hiro225 GOET). D, *Radula tenax* (DUKE 0028925).

MATERIAL AND METHODS

STUDY MATERIAL

With one exception, all voucher specimens of subgenus *Cladoradula* and subgenus *Dactyloradula* Devos, M.A.M.Renner, Gradst., A.J.Shaw & Vanderp. included in the phylogenetic analysis of Devos *et al*. (2011a) were studied. For examination of allometry, four voucher specimens were investigated [*Radula bipinnata* Mitt. (NY 00877325), *Radula boryana* (F.Weber) Nees (E 00115207), *Radula campanigera* Mont. (Hiro225 GOET) and *Radula tenax* Lindb. (DUKE 0028925)] because: (1) they encompass the diversity of lobule morphology exhibited by subgenus *Cladoradula*; (2) they exhibit regular bipinnate branching; and (3) vouchers could withstand examination. For examination of ontogeny and phylogeny, the four species cited above, plus *R. brunnea* Steph. (H 3196644), *R. gottscheana* Taylor (Ingram1765 GOET) and *R. perrottetii* Gottsche ex Steph. (NY 00840810), were studied. We did not include the voucher of *R. paganii* Castle, as we have not yet been able to substantiate the placement of this morphologically distinctive accession in the phylogeny. This exclusion means that our appraisal of morphological change in subgenus *Cladoradula* may be more conservative than reality.

ALLOMETRY

A nested approach was taken to sampling lobules. For each voucher 45 lobules were selected, comprising five each from three first-, three second- and three third-order shoots, making a total of 180 lobules in this component of our study. In each shoot the first five mature lobules back from the shoot apex with undamaged margins, preferably not subtended by a branch, were sampled. Lobules were slide mounted in water with ventral surface uppermost for digitization. Lobules, of which the natural conformation precluded meaningful comparison of shape without compression to achieve a flat conformation, were dissected with their subtending stem sector and slide mounted to achieve as much flattening as possible without tearing. For digitization, a stack comprising four images was compiled showing the lobule apex, lobule–lobe junction, antical end of the stem insertion and ampliate portion of lobule margin in focus. These four focal depths ensured the lobule margin was in focus more or less in its entirety. Digital lobule images were captured with a digital camera and microscope (Leica IM300 with IM1000 software, Photomakroscope M 400). Stem diameter for each lobule was measured at the postical extremity of the stem insertion using ImageJ (Abramoff, Magalhaes & Ram, 2004).

LANDMARKS

The problem of separating the size and shape components of form has been resolved by the advent of geometric morphometric methods that can quantify geometry in multivariate form (Rohlf, Loy & Corti, 1996; Viscosi & Cardini, 2011). Analysing shape as an integrated whole avoids problems of non-independence often encountered in studies employing traditional morphometric methods to quantifying form (Jensen *et al.*, 1993; Jensen, Ciofani & Miramontes, 2002; Lexer *et al.*, 2009; Viscosi *et al.*, 2009). Combining quantification of shape with multivariate analyses renders geometric morphometrics a powerful tool for analysing variation and differences in shape (Adams, Slice & Rohlf, 2004), and investigations of shape allometry have now been completed using geometric morphometric approaches (Cardini & Elton, 2008; Pryer & Hearn, 2009; Viscosi & Cardini, 2011). An excellent botanical primer on geometric morphometrics, including a worked example with explanations of terminology, is presented by Viscosi & Cardini (2011).

Geometric morphometrics can be broadly divided into methods based on landmarks and those based on outlines. The shape of outlines can be represented by Fourier series (Dryden & Mardia, 1998), including elliptical Fourier analysis (Rohlf, 1986), or by open-curve eigenshape analysis (MacLeod, 1999) among other methods. Landmark methods rest on the identification of comparable points across objects and three kinds of landmarks are accommodated in geometric morphometric analyses: (1) anatomical landmarks correspond to homologous points compatible with criteria of homology (Patterson, 1982); (2) mathematical landmarks are points located according to some mathematical or geometrical property of the object (Dryden & Mardia, 1998); and (3) semi-landmarks are points located on outlines or between other landmarks and can be allowed to 'slide' around curves or along lines to improve their mathematical correspondence according to an optimization criterion (Bookstein, 1997; MacLeod, 2002).

Lobules are inherently curved structures, and definition of curves has been historically difficult, as an examination of the variety of shapes routinely described in botanical literature as 'ovate' will illustrate. A range of methods has been developed to quantify shapes using curves when few or no homologous points exist, including open-curve eigenshape analysis (MacLeod, 1999) and elliptical Fourier analysis (McLellan & Endler, 1998). A good example employing elliptical Fourier analysis to quantify curved shapes was presented by Pryer & Hearn (2009) in a study of leaf ontogeny in Marsileales. Unlike fern leaves, in which shape exhibits variation through development such that homologous points on outlines are difficult to identify, the outlines of *Radula* lobules always have four corresponding points where different anatomical structures join. These four points divide the lobule outline into four regions, which we regard as anatomically homologous in their entirety. On each outline we placed semi-landmarks constrained to lie in order on an outline; these are fixed in order but not position. Comparison is achieved because points along curves are topologically correspondent by geometry and order, but topological correspondence does not imply homological correspondence. This may appear problematic for geometric approaches based on homologous correspondence between landmarks. However, using topological correspondence between landmarks 'is justified when it is the only level of correspondence assessment available upon which to base morphological comparisons' (MacLeod, 2002). We adopted an approach combining landmarks and semi-landmarks because, although there is no necessary implication of biological homology between semi-landmarks (MacLeod, 2002), a combination of landmarks and semi-landmarks can capture points of biological homology and the associated subdivision of outline into homologous portions that reflect the homology of different sections of the lobule outline. Elliptical Fourier analysis would describe lobule outlines effectively, but would not reflect homology relations between different parts of the outline explicitly.

Landmarks were digitized in tpsDig2 ver. 2.16 (Rohlf, 2010a, b). Landmarks were: (1) antical end of stem insertion; (2) lobule papilla attachment; (3) lobe–lobule junction; (4) postical end of stem insertion; (5–14) antical lobule margin (semi-landmarks between 1 and 2); (15–24) exterior lobule margin (semi-landmarks between 2 and 3); (25–34) lobule keel; with 25 fixed at keel apex opposite landmark 3; (26–34) between landmarks 25 and 4; and (35–38) stem insertion line (semi-landmarks between landmarks 1 and 4) (Fig. 2). Measurement error was assessed qualitatively by re-digitizing primary shoot lobules for *R. bipinnata* and *R. boryana*, as the repand margin, particularly on the auriculate portion of the outline, presented the greatest challenge to digitization in the whole study. Orthogonal generalized Procrustes superimposition analysis (GPA) was performed on each set of landmarks to remove variation attributable to position, rotation, translation and scale. A full description of these methods is given by Dryden & Mardia (1998). Whether the amount of shape variation was small enough to permit valid statistical analysis of the linear tangent space approximation to the non-linear Kendall's shape space was tested using tpsSmall (Rohlf, 2003a). Calculation of tangent configuration and partial warp scores, extraction of centroid size and principal com-

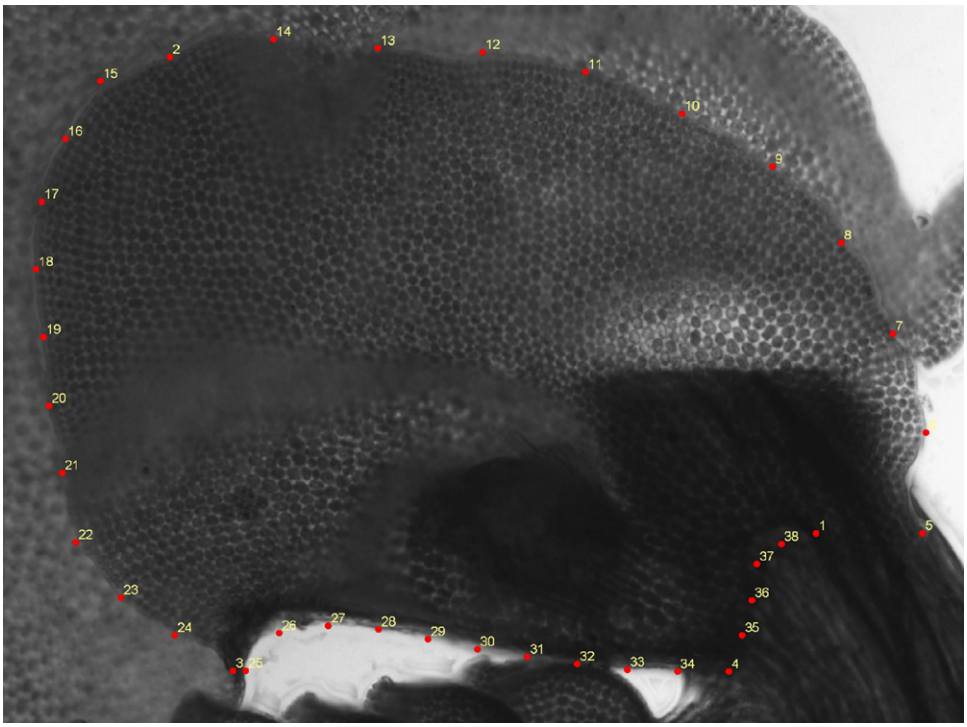

Figure 2. One of the sets of landmarks used in this study, on a lobule of *Radula brunnea*.

ponent analysis (PCA) of partial warp scores were completed using MorphoJ (Klingenberg, 2011), in which the average lobule shape of the data set formed the tangent point for analysis. Principal components analysis provides an effective means of extracting the main modes of variation in shape (Dryden & Mardia, 1998). Relative warps are principal components orientated in the multidimensional shape space defined by partial warps; for details on the calculation and use of relative warps in description of shape variation and interpretation of shape changes see Rohlf *et al*. (1996), Dryden & Mardia (1998) and Viscosi & Cardini (2011). We refer to relative warps as principal components, as this terminology is more familiar. Visualizations of shapes expressed as deformations using the thin-plate spline can be reconstructed at any point along these principal component axes, as a visual aide to description of parameters of variation (Bookstein, 1989).

We asked the following questions about the static relationship between lobule size and shape, branching pattern and shoot size of these four species (*R. bipinnata*, *R. boryana*, *R. campanigera* and *R. tenax*): (1) Are lobule size and stem size dependent? (2) Are lobule shape and lobule size dependent? (3) How do individuals express these relationships (between primary, secondary and tertiary shoots)? (4) Do all species express the same relationship?

A simple way of investigating the relationship between size and shape for lobules is to compare box plots showing centroid size and first principal component score for lobules for each species. Variation in shape at maturity effectively describes how variable lobule ontogeny is. To detect any effect of size from comparisons between species, the regression slopes for each species were compared using a multiple analysis of covariance (MANCOVA) design with species as groups and size as the covariate with a species × size interaction term. This test compared the variance explained by two models, one in which species regression slopes were forced to be parallel, the other in which species regression slopes were unconstrained. A lack of significant difference between the fit of the two models, assessed by comparing the residual sums of squares matrices of each, indicates that the allometric pattern is the same across species. The multiple multivariate regression of partial warp scores onto lobule centroid size required for the MANCOVA was completed using tpsRegr (Rohlf, 2011). Centroid size (Bookstein, 1991), the square root of the sum of squared distances from each landmark to the centroid of each sample configuration (Rohlf *et al*., 1996), was used as a measure of geometric size, because it is the scaling (size) component of uniform variation removed from form during a Procrustes superposition. Centroid size is convenient because in a single value the size of lobules is summarized in a concise, meaningful and directly comparable way. If separate slopes are not required, it is then possible to establish what proportion of shape variation allometry explains and to

compare shapes between species with the size effect removed. If differences in shape result from variation in size alone, there should be no significant differences between species when allometry is controlled. The regression approach and permutations in MorphoJ can be used to examine the effects of allometry on comparisons between species. See Viscosi & Cardini (2011) for an explanation and worked example employing the MANCOVA approach to relationship between size and shape using geometric morphometric methods, and Rohlf (2011) for details of analysis.

Ontogeny

Because there are close connections between heterochrony and changes in allometric growth trajectory (Klingenberg, 1998), allometry is one means through which we understand heterochrony (Blackstone, 1987a, b; McKinney, 1988; Klingenberg & Spence, 1994; Fiorello & German, 1997). Establishing what kind of heterochrony has occurred in a group of organisms has three parts: (1) estimate allometric relationships; (2) time calibrate those relationships by reference to ontogeny; and (3) establish polarity of changes in timing by reference to phylogeny.

The time component distinguishes heterochrony from purely morphological concepts such as allometry (Alberch, 1985; Blackstone, 1987a; Klingenberg, 1998). Studies of heterochrony have tended to compare animals with a sequestered germ line in which sexual maturity is a clearly defined calibration point of comparison between individuals. Calibrating ontogeny in organisms with serially homologous structures such as plants is more challenging, but one approach employed by Jones (1993) and Pryer & Hearn (2009) is to compare the sequence of ontogenetic stages along a stem. This approach uses an intrinsic measure of time in terms of discrete developmental events (Klingenberg, 1998), specifically the sequence of leaves produced above the cotyledons, and fulfils the criteria for studies of heterochrony posited by Reilly, Wiley & Meinhardt (1997). In herbarium material of bryophytes it is difficult to study growth and development of leaves at specific positions forwards along a stem, as the stem origin is usually unavailable as a referent for comparison. However, the shoot apex is available, and the sequence of leaves back from the shoot apex may be used to calibrate mature ontogenies in different shoots (Klingenberg, 1998). This intrinsic measure of time is also based on the number of discrete developmental events, i.e. the number of merophyte cleavage events at the apical cell. Effectively, this is equivalent to measures based on node number made in vascular plants, which allows the duration of ontogeny to be measured against another developmental process, in this case meristematic growth. If we assume that rates of both processes are relatively constant, then comparison within and between individuals is possible.

To investigate lobule ontogeny, apices from two or three shoots of each order were dissected with a pair of Inox no. 5 'Biologie' tweezers and slide mounted in water. Like Pryer & Hearn (2009), we did not measure continuous development of a single leaf (ontogenetic allometry); however, we did not compare a sequence of mature lobules along a stem. Instead, we investigated the sequence of lobules between the apical cell and the first mature lobule on a stem. We take this sequence to be homologous between stems, within and between species, and this assumption allows us to perform what is effectively a space (position)-for-time substitution on lobule growth, where time is calibrated by position of the lobule behind the apical cell. This is not a perfect calibration, as by itself it does not necessarily discriminate between changes in rate or duration, but it is the best calibration available for the non-living material forming the basis of this study. This approach to intrinsic calibration assumes that the sequence of lobule growth is the same in all lobules on mature shoot sectors, and that the relative rates of lobule and apex growth are comparable between shoots and species. Both assumptions could be violated, but without studies of growth we cannot speculate on the lack of dependence of metabolic rates on environment conditions and nutrient status of shoots in different parts of a shoot.

Use of sexual maturity as a referent for growth comparison is problematic because: (1) production of gynoecia terminates shoot growth; (2) an increase in shoot stature usually precedes production of gynoecia; and (3) antheridia are produced on lateral branches.

Mature leaf form was defined as the final form produced in the development of a given shoot or branch, with no further ontogenetic transformation in later leaves following Mishler (1986). Juvenile leaves were defined as those produced on immature shoots associated with sporeling growth. Early developmental stages were referred to as 'young', also following Mishler (1986).

Our approach is entirely phenomenological in that evolution of growth is examined at the scale of the whole organism and characterized by growth trajectories that represent the aggregate dynamics of many unknown developmental processes occurring at cellular, tissue and organismal scales (Pryer & Hearn, 2009). We have made no assessment of the degree of variation expressed by species beyond the level of individual. Plasticity and genetic determinacy probably contribute to variation in ontogeny and morphology, but we have not assessed the relative contribution of either. However, no significant developmental variation between populations of a single species was

observed in the study of species of *Tortula* Hedw. grown under standard conditions by Mishler (1986).

The quantification of lobule morphology employed in this study included the lobule insertion, but we did not specifically investigate changes in stem insertion that occur during lobule ontogeny.

Phylogeny reconstruction

As ancestor–descendant relationships are part of the definition of heterochronic mode (McLellan, 1993), phylogeny must be known in order to identify which kind of changes have occurred. The role of heterochrony in changes in lobule ontogeny was established by reconstructing ancestral states onto a molecular phylogenetic tree using maximum parsimony, following the approach of Pryer & Hearn (2009). Relationships between the 93 *Radula* spp. sampled by Devos *et al*. 2011a, b) were reconstructed on the basis of their data, comprising six plastid markers: the *trnL–trnF* spacer, *trnG* intron, *rps4* (including the *trnS–rps4* spacer and *rps4* gene), the *psbA–trnH* spacer, the *psbT–psbH* region and the first half of the *atpB* gene (Stech & Quandt, 2010). Primers and references are given in Devos *et al*. (2011b). The best fitting substitution model for each marker was identified with the Akaike information criterion by MrModeltest (Nylander, 2004) in conjunction with PAUP* (Swofford, 2002). Ultrametric trees summarizing relationships and relative divergence times were estimated using the Bayesian software BEAST ver. 1.4.8 (Drummond & Raumbaut, 2007). Six partitions, with separate substitution models for each were specified. A GTR + I + Γ substitution model was selected for *atpB, psbA–trnH, rps4, trnG* and *trnL–trnF* and an HKY + I + Γ selected for *psbT*. Base frequencies were estimated from data, four gamma categories were assigned for each substitution model and all substitution models and clock models were unlinked. Substitution model priors followed default settings in BEAUTi ver. 1.7.2. A separate uncorrelated lognormal relaxed-clock modelled substitution rates for each partition, with rates estimated relative to *atpB*. A uniform prior with range 0–100 was applied to each clock, a speciation birth–death model (Gernhard, 2008) with a uniform distribution applied to node heights and an unweighted pair-group mean aggregate (UPGMA) dendrogram was used as the starting tree. The phylogenetic tree was not time-calibrated, but branches in resulting ultrametric trees are proportional to time. The analysis was run for 10 000 000 generations and sampled every 1000. Burn-in length and convergence between the four runs were confirmed by comparing trace files for each run in Tracer ver. 1.5 (Rambaut & Drummond, 2009). After excluding the first 10% of samples as burn-in, the 50% majority rule tree summarizing the sample and 1000 randomly selected trees were used in correlation analysis and phylomorphospace reconstruction. The topology and branch lengths of the clade containing *R. brunnea* and species of *Radula* subgenus *Cladoradula* were used in character state reconstruction and correlation analyses.

Polarity of change in lobule ontogeny

Five lobules from each of three primary shoots were measured for each species and the average shape calculated. The average shape for each species was included in a GPA of 93 of the species included in the phylogenetic tree. The position of each *Cladoradula* sp. on the first six principal components of this GPA was scored in a data matrix with duration of growth. Ancestral states for shape and duration of growth were reconstructed on the topology of the majority-rule ultrametric tree from BEAST, using squared-change parsimony as implemented in Mesquite (Maddison & Maddison, 2011), with *R. brunnea* as the outgroup. First principal component scores and growth duration were reconstructed for ancestral nodes and then used to establish the polarity of changes in both characters. The significance of correlation between first principal component scores and the duration of lobule growth was assessed using a phylogenetic variance–covariance matrix within a standard linear model (Freckleton, Harvy & Pagel, 2002), implemented by the package CAIC (Purvis & Rambaut, 1995) in R 2.12.0 (R Development Core Team, 2010).

RESULTS

Lobule allometry

Stems of primary, secondary and tertiary shoots in all species show differences in stature, as illustrated for stem sections of *R. bipinnata* in Figure 3. The size of mature lobules is strongly positively correlated with stem diameter, and a significant positive relationship between lobule centroid size and stem diameter exists for the whole data set ($R^2 = 0.796$; $F_{1, 178} = 693.5$, $P < 0.0001$).

TpsSmall (Rohlf, 2003b) verified that the tangent plane projection did not significantly distort the distances among the surveyed lobules of four species (Pearson product–moment correlation = 0.9999, slope of relationship between tangent space vs. Procrustes distances = 0.9748). The mean and maximum Procrustes shape distances to the consensus configuration were 0.2898 and 0.7182 units of Procrustes shape distance, respectively, suggesting there was considerable variation between lobules, and > 0.2 as

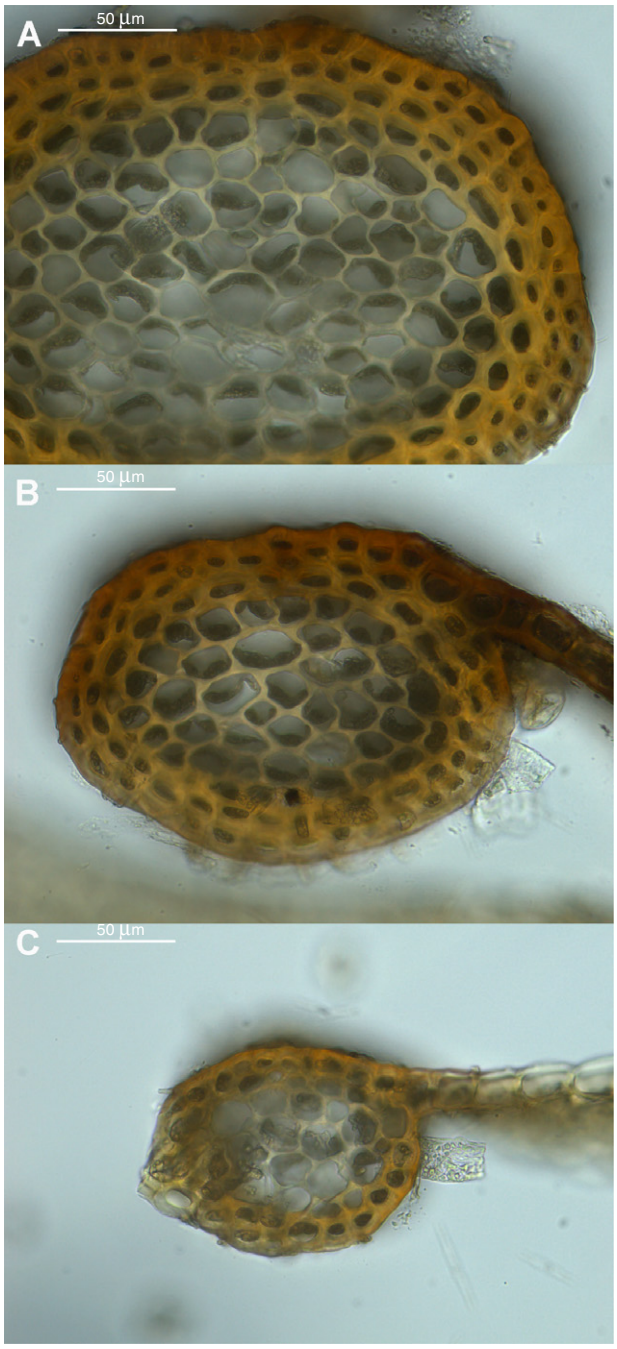

Figure 3. Representative transverse stem sections for *Radula bipinnata* (NY 00877325) showing differences in stature between (A) primary, (B) secondary and (C) tertiary shoots.

recommended by Dryden & Mardia (1998). High distance values were associated with lobules having large, auriculate bases.

Visual assessment of digitization error suggested that this made a modest contribution to the scatter of points. With the exception of two outliers, replicate measurements fell within the same region of shape space as the original measurements, suggesting that the contribution to variation from error was small compared with that existing within the data set (Fig. 4).

The first principal component explains 80.5% of shape variation in Procrustes fitted data and is associated with changes from quadrate lobules with no ampliate base at the positive end to triangular lobules with a broadly and deeply ampliate base at the negative. Lobules at the negative end of this first component have a straight or weakly curved keel and a longitudinal stem insertion, whereas at the opposite end they have an arched keel and a transverse stem insertion. Visualizations of shapes at either end of the first principal component are similar to observed lobules, specifically those of primary shoots in *R. bipinnata* and *R. boryana* at the negative end and those of tertiary shoots of *R. tenax* at the positive end (Fig. 5). The second principal component explains 8.6% of variation and, from the negative to positive ends, is associated with a change from roughly triangular lobules with a broadly ampliate base, an arched keel and transverse stem insertion to ovate lobules with a pronounced, if tiny, auricle at the top of the stem insertion, a curved keel and a longitudinal stem insertion. Triangular lobules at the negative end of this axis are similar to observed lobules, which occupy the same region of space; however, no real lobules occur near the shape space at the positive end of the second component (Fig. 5).

A significant allometric relationship between lobule centroid size and lobule shape exists for the whole data set (Wilks Lambda = 0.3783, $F_{72, 107} = 11.96$, $P < 0.0001$), which explained 50.7% of variation in shape. Visualizations of shapes expressed as deformations using the thin-plate spline show that small lobules are rectangular and do not have a deeply auriculate interior base, whereas large lobules are more triangular and have a deeply auriculate base (Fig. 6).

In each species, lobules of primary, secondary and tertiary branches form clusters in different, although often overlapping, regions of shape space (Fig. 7). The lobules of *R. bipinnata* and *R. boryana* encompass nearly all of the shape space described above, with lobules of primary shoots clustering at the negative end of the first principal component, secondary shoots clustering in the middle and tertiary shoots at the positive end. The spread of lobules is broadest at the negative end of the axis and narrowest at the positive end. In *R. bipinnata* there is a discontinuity in lobule distribution along the first principal component between lobules of third-order shoots and the rest. In *R. campanigera* and *R. tenax* lobules of primary, secondary and tertiary shoots cluster in the same order,

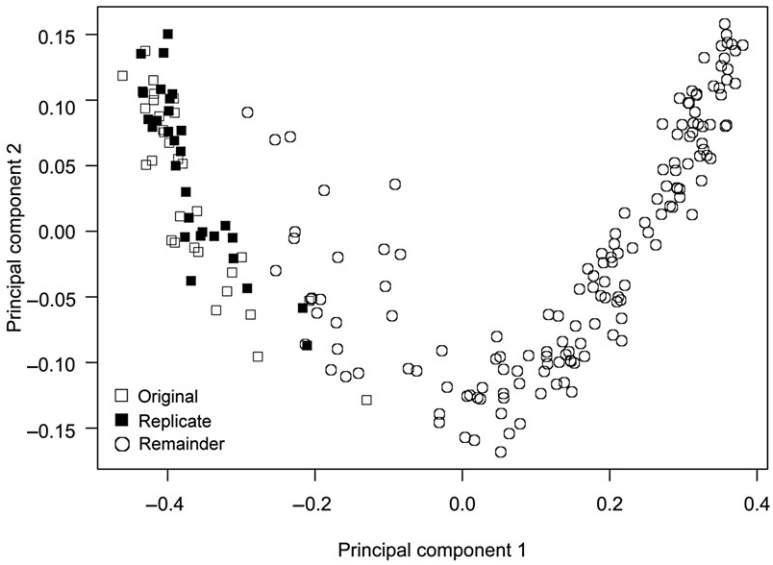

Figure 4. Digitization error. Open circles and open squares represent the original measurements for the whole data set. Closed squares represent replicate measurements for open squares, the lobules on primary shoots of *R. bipinnata* and *R. boryana*. The plot shows that these replicate measurements fall within the same region of shape space as the original measurements, although there is some scatter between replicates.

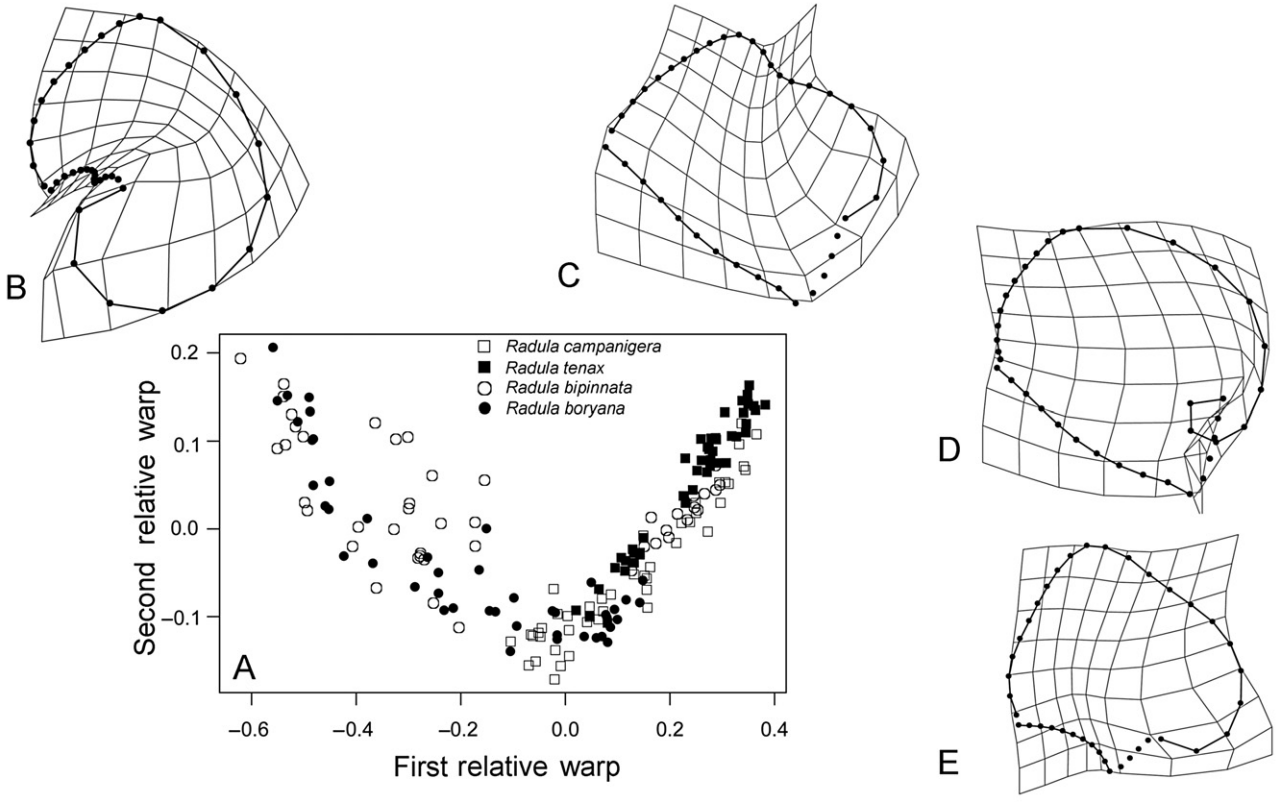

Figure 5. Relative warps plot for all data from four focal species. In this plot, the first principal component explains 80.5% and the second principal component 8.6% of variation in lobule shape. Visualizations of shapes expressed as deformations using the thin-plate spline illustrate shape changes described by the principal components are shown, for first principal component with visualization above (B, C) and the second principal component to the side (D, E). Species are represented by different symbols.

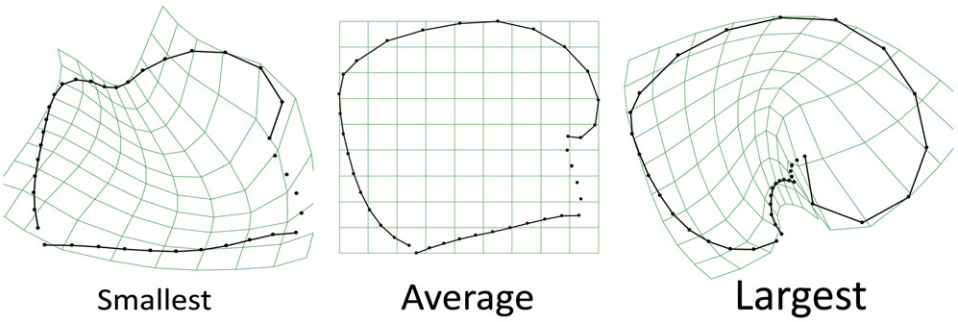

Figure 6. Visualizations of shapes expressed as deformations using the thin-plate spline showing shape changes associated with size for smallest, average and largest measured values of centroid size.

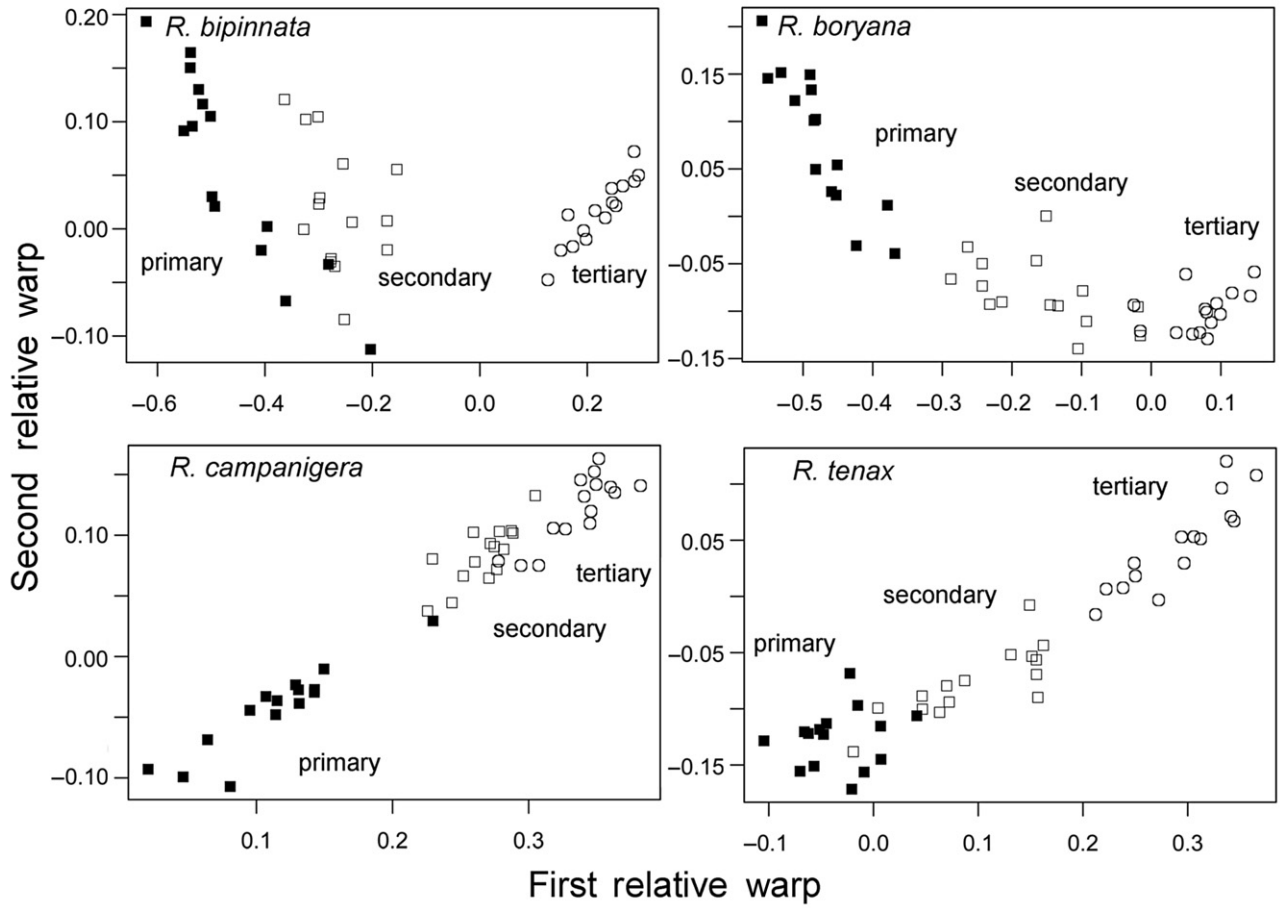

Figure 7. Lobules for *Radula bipinnata*, *R. boryana*, *R. campanigera* and *R. tenax* plotted on separate axes showing differences between lobules from primary, secondary, and tertiary shoots. Principal component scores derived from generalized Procrustes superimposition analysis (GPA) for combined data are shown in Figure 4.

but the lobules are distributed within a different and narrower range of values along the first principal component. The spread of lobules is roughly equal along all values of the first principal component.

Lobules of different species and different shoots all fall within relatively narrow ranges along the first principal component, suggesting a degree of canalization in lobule ontogenies (Fig. 8). For *R. bipinnata*, *R. boryana* and *R. tenax* lobule centroid size decreases with branch order, but first principal component score increases. However, for *R. campanigera*, centroid sizes for lobules from

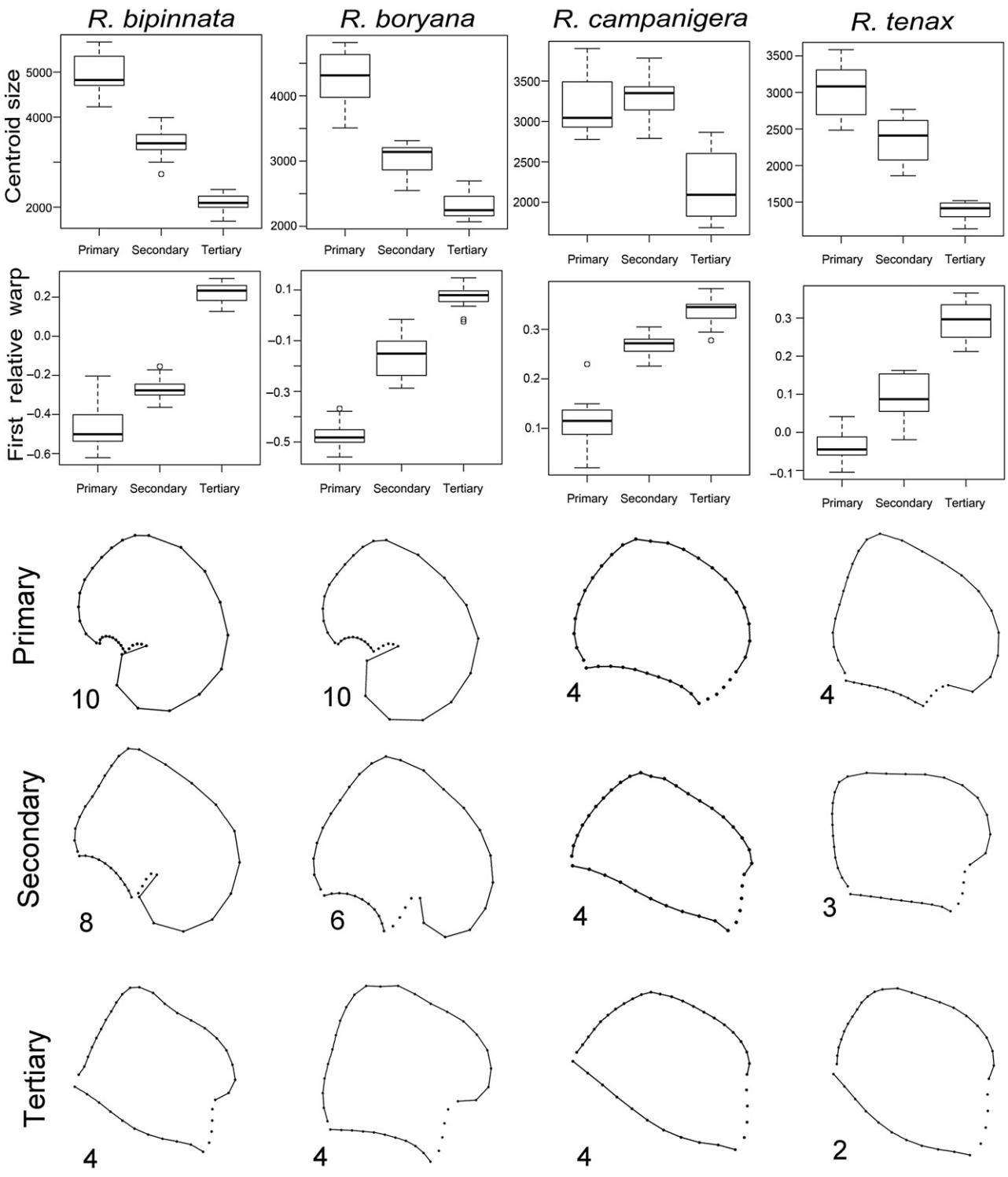

Figure 8. Box plots and average shapes for the four species whose allometry was investigated in this study. Box plots show first principal component scores and centroid size values for lobules on primary, secondary and tertiary shoots for each species. Average lobule shapes are shown for primary, secondary and tertiary shoots for each species.

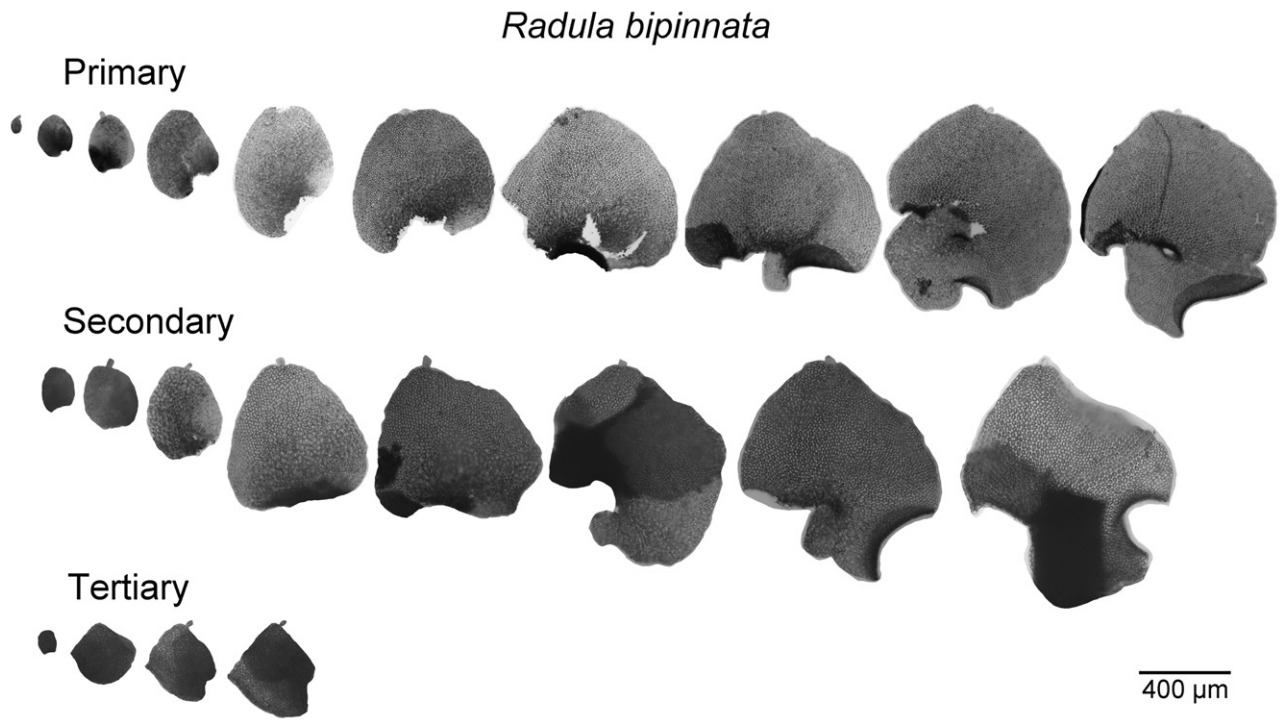

Figure 9. Sequence of lobules behind the shoot apex for different shoot orders in *Radula bipinnata* (NY 00877325), each sequence from a single shoot. Lobule ontogenies were inferred from this sequence.

primary and secondary shoots are more or less the same (Fig. 8).

The same relationship between lobule size and shape is not expressed by all four species. The models with constrained and relaxed allometric slopes produced highly significant relationships between centroid size and lobule shape [model 1 (same slopes): $F_{288,\,12\,600} = 131.5924$, $P < 0.0001$; model 2 (different slopes): $F_{504,\,12\,384} = 104.5401$, $P < 0.0001$]. The per cent variation unexplained was slightly higher for model 1 than model 2 (24.9 and 18.9%, respectively). The test for difference in residual sums of squares matrices from two models returned a significant result (Wilks' Lambda = 0.0136, $F_{216,\,303.8} = 4.491$, $P < 0.0001$), indicating that allometric trajectories of different species pointed in different directions in shape space.

Lobule ontogeny

Patterns of growth in lobules change as lobules mature and those changes follow a clearly defined pattern across species. In *R. bipinnata* the youngest discernable lobules at shoot apices are small buds comprising tens of cells capped by a single, relatively large, papilla. In these youngest multicellular lobules, cell division occurs over the whole lobule (Fig. 9). The zone of cell division, which is visible as a zone of smaller paler thin-walled cells, contracts toward the lobule base as cells at the apex mature, with maturation happening faster in medial than in marginal regions. In the lobule of the fourth leaf back from the apex, cell division is concentrated in the lower interior quarter of the lobule and across the lobule insertion, whereas cells in the other regions are expanding and depositing secondary wall thickenings, or have matured. In the fifth leaf behind the apex, the zone of cell division is further restricted to the region of the lobule base between and including the medial base and the interior margin. Cells in the exterior margin of the lobule base, and other medial and upper cells have all matured. The lobule of the seventh leaf has cells in the medial lobule base that have also matured and the zone of cell division is restricted to the basal part of the interior margin. In the ninth or tenth leaf back from the apex, cell division on the basal part of the interior lobule margin has ceased and the cells have matured. As the lobule has completed growth, expansion and secondary wall deposition, it can be considered mature at this point (Fig. 9).

The ontogeny of lobules on secondary shoots exhibits the same pattern as on primary shoots, but restriction of cell division to the base of the interior margin occurs by the sixth lobule and, by the eighth lobule back from the shoot apex, cell division has ceased (Fig. 9). In lobules on tertiary shoots, cell division appears to contract rapidly downward toward the

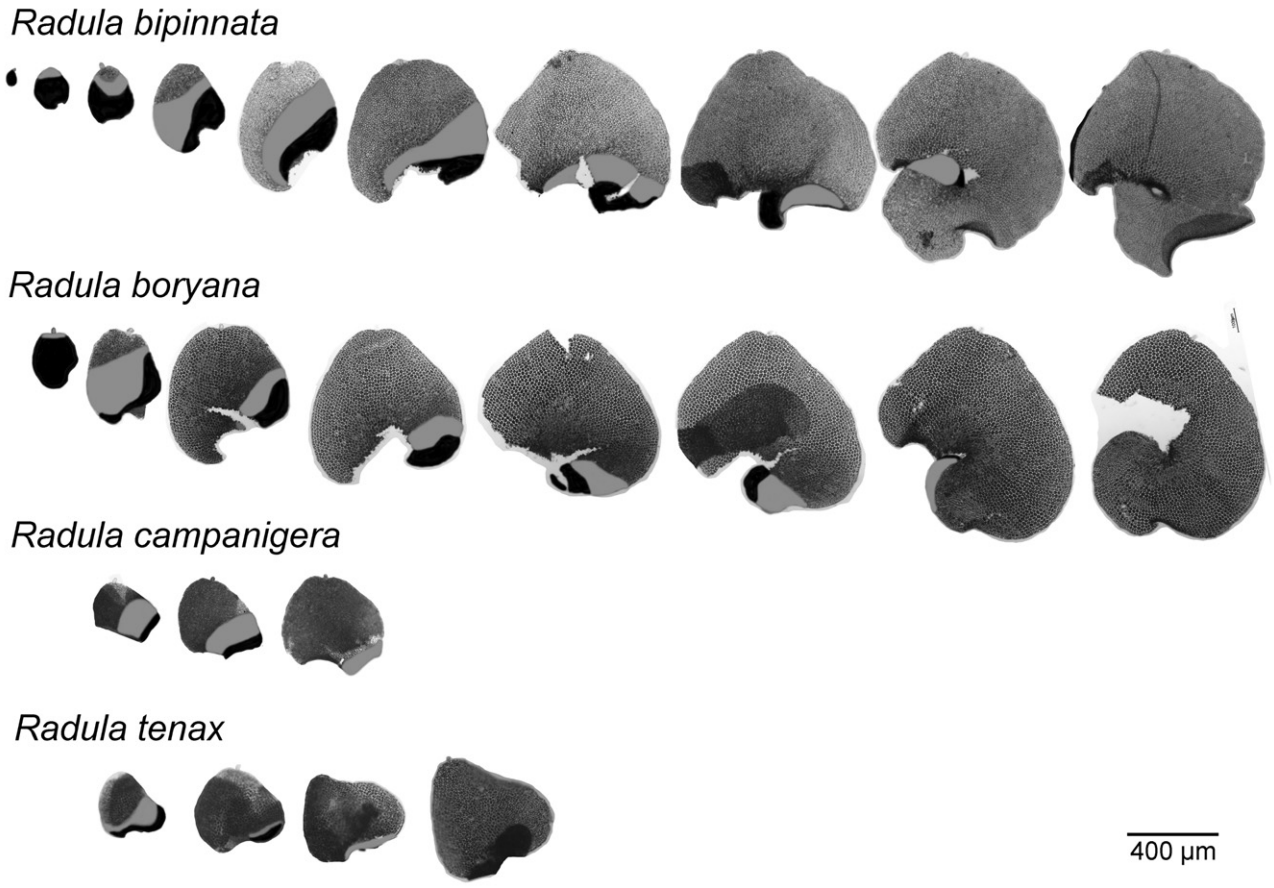

Figure 10. Sequence of lobules behind the shoot apex for primary shoots in four different species of *Radula* subgenus *Cladoradula*, each sequence from a single shoot. Zones of cell division are shown in black and zones of cell expansion and maturation are shown in grey. Mature cells, characterized by large size, dark contents and walls with pronounced secondary thickening are not highlighted. *Radula bipinnata* and *R. boryana* exhibit similar lobule ontogenies, and differ from *R. tenax* in the duration of growth, and *R. campanigera* in both the duration of growth and the pattern of cell division (see text).

lobule base and interior margin, and ceases by the fourth lobule (Fig. 9).

Lobules on primary shoots of *R. boryana* exhibit the same pattern of cell division as those of *R. bipinnata*, but cell division is restricted to the lobule base by the sixth or seventh leaf; by the tenth leaf, the lobule is mature (Fig. 10). On secondary shoots, lobule cell division is restricted to the interior base at the fourth lobule, and lobule maturity is achieved by the sixth leaf. On tertiary shoots of *R. boryana*, cell division is 'further restricted' to the base in the third lobule, and at the fourth the lobule is mature. On primary shoots of *R. tenax*, cell division is restricted to the lobule base in the third leaf, and at the fourth leaf the lobule is mature (Fig. 10). In lobules on secondary and tertiary shoots, lobule maturity is reached at the third leaf.

In *R. campanigera*, cell division occurs in the lobule basal half in the second leaf and is restricted to the entire width of the lobule base in the third, rather than the interior quarter as in other species (Fig. 10). By the fourth leaf, lobule cell division has ceased. Ontogeny is similar in lobules on secondary and tertiary shoots, and in both cell division has ceased in the fourth leaf.

Appendages in *R. brunnea* are the result of localized cell division at the interior base. The duration of growth for *R. brunnea*, *R. gottscheana* and *R. perrottetii* is summarized in Table 1.

Polarity of change in lobule ontogeny

In a GPA and relative warps analysis of primary shoot average lobule shape for the seven species, the first and second principal components describe the same deformations as the components from the whole data set. The first and second principal components describe 88.1 and 7.4% of variation, respectively; in total, 95.5% of lobule shape variation.

Table 1. Duration of growth, in terms of which is the first leaf to have reached maturity, for lobules of *Cladoradula* spp. on different branch orders

	Primary	Secondary	Tertiary
R. bipinnata	10th	8th–9th	4th
R. boryana	10th	6th	4th
R. campanigera	4th	4th	4th
R. tenax	4th	3rd	2nd–3rd
R. perrottetii	6th		
R. gottscheana	9th–10th		
R. brunnea	7th–8th	1st	–

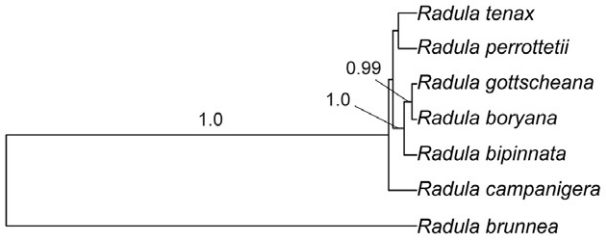

Figure 11. Ultrametric phylogeny for *Radula* subgenus *Cladoradula* reconstructed for this study. Values shown are posterior probability values > 90%.

When ancestral states for duration of lobule ontogeny and lobule shape were reconstructed on the *Cladoradula* phylogenetic tree (Fig. 11), the ancestor was reconstructed as having lobules that matured in 6.33 leaves and had leaf shape similar to *R. brunnea* (Fig. 10). At the basal node in subgenus *Cladoradula*, growth duration diverges in opposite directions in each daughter lineage. *Radula campanigera* has shorter growth duration, of 4.00 leaves, whereas the other daughter lineage is reconstructed as having a duration of 6.65 leaves. Daughter lineages from the next bifurcation also diverge in opposing directions. In the lineage containing *R. bipinnata, R. boryana* and *R. gottscheana*, reconstructed states increase toward the tip values of 10.00 leaves, whereas in the lineage containing *R. perrottetii* and *R. tenax* reconstructed states decrease to the tip value of 4.00 for *R. tenax*, but increase again for *R. perotteii*.

The phylomorphospace with growth duration reconstructed at nodes (Fig. 12) shows growth duration and shape are related, and a significant relationship between growth duration and first principal component scores exists (Table 2; multiple R^2: 0.941, adjusted R^2: 0.929 ($F_{2,5}$ = 79.5; P = 0.00016). Diagnostic regression tests returned non-significant results, indicating the assumption of evolution via Brownian processes was not violated (Garland, Harvey & Ives, 1992).

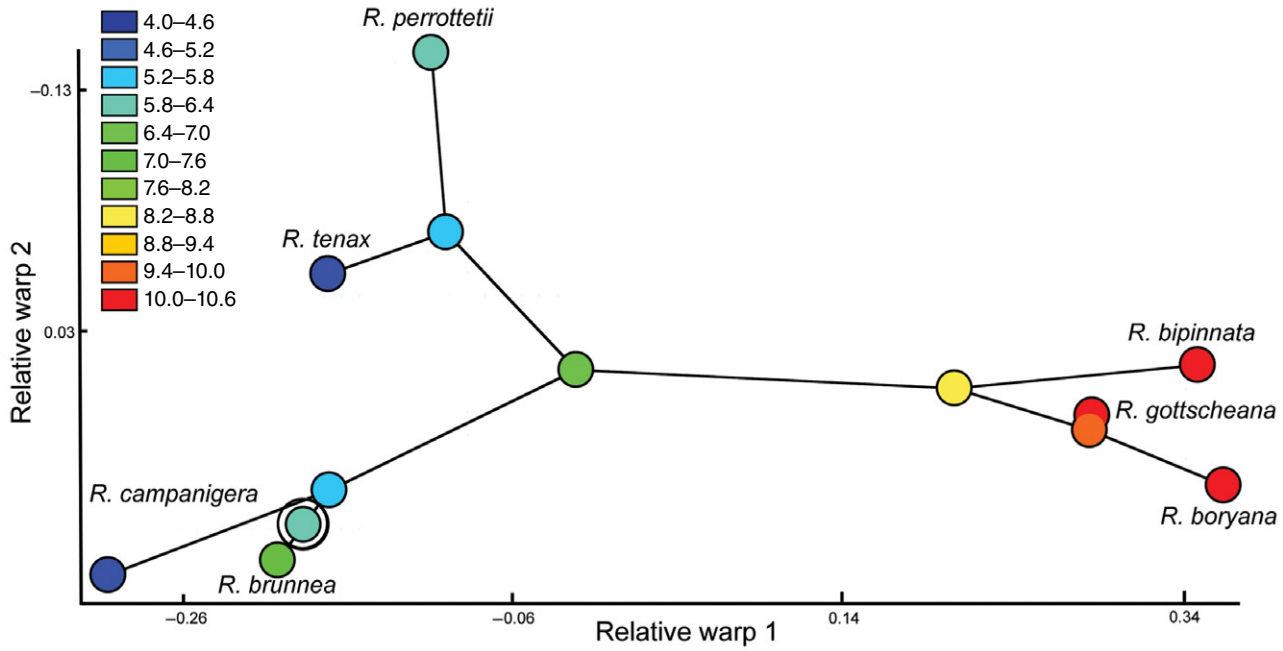

Figure 12. Phylomorphospace, with phylogeny with ancestral states reconstructed using squared-change parsimony mapped onto the first and second principal components derived from generalized Procrustes superimposition analysis (GPA) of averaged data for primary shoot lobules. Circles at nodes show duration of lobule ontogeny measured in terms of number of nodes produced by the apical cell before lobule maturity is reached. In this plot, the first principal component explains 85.5% and the second 7.7% of variation in average lobule shape.

Table 2. Regression coefficients for correlation between first principal component score and duration of lobule growth

	Estimate	Standard error	*t*-value	*P*
(Intercept)	−0.721308	0.112535	−6.4096	0.00137
Duration	0.0396767	0.0070414	5.6348	0.000296

Residual standard error 0.6899 on 5 degrees of freedom.
Multiple R^2: 0.941, adjusted R^2: 0.929 ($F_{2,5} = 79.5$; $P = 0.00016$).

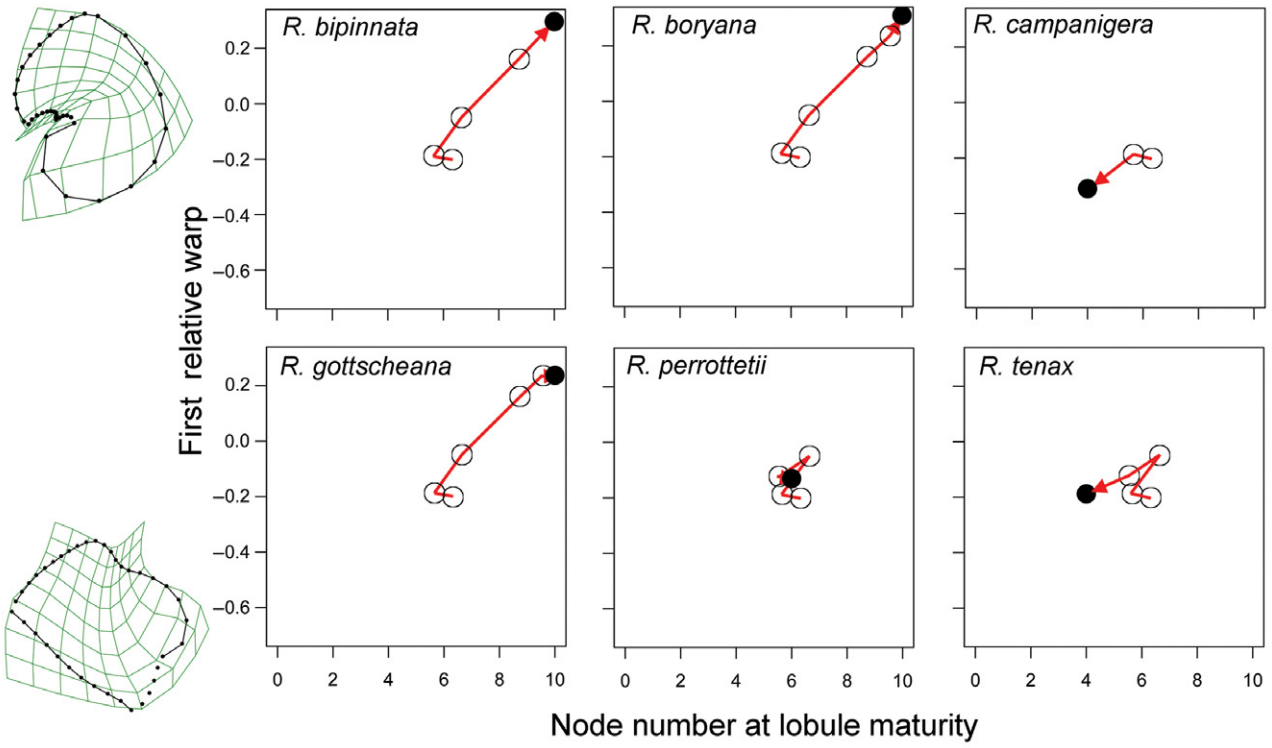

Figure 13. Scores on the first principal component plotted against duration of lobule growth for each species (filled circle) and their ancestral nodes (open circles), with the sequence of nodes linked by an arrow showing the trajectory of change from the base to the respective tip of the tree. Thin-plate splines show deformations associated with the first principal component.

DISCUSSION

INTERSPECIFIC VARIATION

Changes in lobule morphology in *Radula* subgenus *Cladoradula* demonstrate that liverwort lineages have the capacity for morphological change by multiple modes of heterochrony, and that morphological changes via heterochrony in liverwort gametophytes can be dramatic and relatively rapid in a phylogenetic context.

If the nodes from the phylogenetic tree leading to and including each species are plotted for their reconstructed first principal component scores and growth durations, three patterns of change emerge in the six species studied (Fig. 13). In *R. bipinnata*, *R. boryana* and *R. gottscheana*, values for shallower nodes move toward the upper right-hand corner of the plot, indicating increase in both first principal component scores and growth duration up the branches leading to each species (Fig. 13). The increase is approximately linear in all species; there does not appear to be a significant change in slope of the relationship between the two variables. This pattern of linear increase in both growth duration and shape is typical of hypermorphosis, in which existing allometric trajectories are extended by longer growth to achieve novel forms (Alberch *et al*., 1979). Evidence for the extension of allometric growth trajectories is seen in lobule ontogeny for *R. bipinnata*, *R. boryana* and *R. tenax*, in which adult lobules on primary branches

pass through forms expressed by lobules on secondary and tertiary branches as they grow. In contrast to *Cucurbita* L., where differences between leaves in different races are established early in ontogeny (Jones, 1993), differences in lobules of these three *Radula* spp. appear to be established late in ontogeny (see discussion below), indicating conservation of ontogenetic trajectories. Conserved trajectories indicate 'the maintenance of ancestral associations among traits.' (Klingenberg, 1998), but this maintenance does not preclude change in mature form.

In *R. campanigera* and *R. tenax*, changes between terminal and ancestral nodes involve a decrease in growth duration and first principal component score. In *R. tenax*, lobules on primary shoots are more similar in first principal component scores to lobules on secondary branches in *R. boryana*, growth duration of which is six nodes, than to the tertiary branches of *R. bipinnata* that have the same duration of growth. The first principal component scores of *R. tenax* overlap with the secondary and tertiary lobules in *R. boryana*. In part, this may be attributable to their triangular shape, transverse stem insertion and broadly but weakly ampliate lobule base. Character state changes between ancestral and terminal nodes in *R. tenax*, the growth duration and the similarity to lobules on branches of other species (see below) are consistent with progenesis, perhaps with a degree of acceleration to achieve an ampliate lobule base by the fourth lobule. Further evidence for acceleration can be seen in lobule ontogeny, where cell division has contracted to the lobule base by the second or third node, whereas, in lobules of similar age (position) in *R. boryana*, cell division is still broadly distributed in the interior basal quarter and the lobules have not yet developed an ampliate interior base (Fig. 10).

According to our representation, *R. campanigera* is also progenetic with respect to its ancestor. This may be supported by lobule ontogeny, in that cell division is rapidly restricted to the lobule base, where it is evenly distributed across the base before ceasing. Maturation of individual leaves from apex to base has also been observed in *Tortula* (Mishler, 1986).

Radula perrottetii does not exhibit a clear correlation between changes in growth duration and first principal component scores in its ancestral nodes. Ancestral nodes leading to this species, including the basal node of subgenus *Cladoradula*, cluster around growth duration values of six nodes and first principal component scores of 0.0 to –0.2. The lobules of *R. perrottetii* may be similar to those possessed by the ancestor of subgenus *Cladoradula*, given the similarity of first principal component scores and growth duration between it and the ancestral node.

Allometric trajectories can be extended or truncated (Klingenberg, 1998) and shifts in timing of growth typically involving early termination of development are common in descendant species (Li & Johnston, 2000). Heterochrony is known to play a role in the morphological evolution of bryophytes, and a range of heterochronic changes in different leaf structures were documented in the moss genus *Tortula* by Mishler (1986), including hypermorphosis in leaf papillae of *T. papillosissima* (Copp.) Broth., paedomorphosis in leaves of *T. andicola* Mont. and *T. bogotensis* Hampe, in which transitions in leaf characteristics along branches showed the incorporation of juvenile morphology into adults of descendant species (Mishler, 1986, 1988). Although paedomorphosis has been documented for some liverworts (e.g. Gradstein *et al*., 2006), *Radula* subgenus *Cladoradula* appears to be the first example in liverworts in which both paedo- and peramorphosis explain current morphological diversity. By generating larger novel lobule structures in addition to reduced morphologies, heterochrony may contribute to the 85% of variation in *Radula* subgenus *Cladoradula* lobule morphology explained by the first principal component, with which changes in growth duration are significantly correlated. Changes in timing and rate of growth may characterize many morphological innovations exhibited by the gametophyte generation of liverwort species.

Radula subgenus *Cladoradula* illustrates that liverworts have the capacity for relatively rapid morphological change via heterochrony. Although absolute time calibration of the phylogeny for *Radula* is not yet available, reconstruction of topologies with branch lengths proportional to time is, and hence it is possible to speculate in fairly general terms about relative rates of morphological change in these quantitative characters. That species are related by relatively shallow nodes, and short branches, in the crown of *Radula* subgenus *Cladoradula* clade suggests divergences and associated heterochronic changes have occurred relatively recently. The rapid morphological diversification in the crown of *Cladoradula* contrasts with the relative stasis between the ancestral node and *R. brunnea*. Five of the species in the ingroup have undergone heterochronic changes, but it appears from the ancestral state reconstruction that *R. perrottetii* has not. If this reconstruction is accurate, then *R. perrottettii* retains the ancestral condition and heterochronic changes have not occurred in all species of subgenus *Cladoradula*.

Although our majority-rule phylogenetic tree is fully resolved and the relationship between ingroup species and *R. brunnea* is fully supported, some of the nodes in *Cladoradula* are not well supported. Despite potential variance regarding our ancestral state reconstruction resulting from weak phylogenetic signal, conclusions drawn about changes in ontogeny are robust with respect to the outgroup. Character

state reconstructions at ancestral nodes may be influenced by variation in topology of the estimated phylogeny. However, the degree of impact has not been assessed, except that correlation between relative warp (RW1) scores and growth duration is also significant using the topology of Devos *et al*. (2011a), which differs in some relationships from ours.

Squared-change parsimony may reconstruct different values for nodes from other methods of ancestral character state reconstruction. In one study of different state reconstruction methods, most hypothesized values differed minimally among methods, but at some nodes they varied by up to 50% (Cohen, 2012). We also acknowledge that the use of single point values for terminal nodes does not capture variation in quantitative characters (Stevens, 1991; Gift & Stevens, 1997), but we hold that mean shapes can be meaningfully employed to investigate macroevolutionary dynamics, as have other studies (e.g. Sidlauskas, 2008).

Intra-individual variation

Variation in pteridophyte and angiosperm leaf morphology is tied to differences in leaf ontogeny (Gleissberg & Kadereit, 1999; Li & Johnston, 2000; Pryer & Hearn, 2009), and variation in ontogenetic processes supplies static variation upon which natural selection can act to produce evolutionary change. In the ontogeny of *R. bipinnata* primary shoot lobules, parallels are seen with the morphology of mature lobules from secondary and tertiary shoots of the same shoot system. Heterochronic changes along allometric growth trajectories occur in individuals of three species of *Radula* subgenus *Cladoradula*. In *R. bipinnata* the morphology of mature lobules on secondary and tertiary shoots is seen in the ontogeny of lobules on primary shoots, in which there is a transition from triangular lobules expressed early in ontogeny (as in tertiary shoots) to lobules with a small auricle (as in secondary shoots) to the deeply auriculate mature lobules expressed late in ontogeny. This pattern suggests that some form of heterochrony is responsible for differences between shoots in individuals of *R. bipinnata*.

A schematic representation of lobule ontogeny can be conceived to explain variation between shoots. If we locate the origin of each slope at 0 duration, and a first principal component value corresponding approximately to the smallest lobules observed along shoot apex (an approximation sufficient for our purposes) and then draw lines representing ontogenetic trajectories from this origin to each average lobule shape, the resulting scheme suggests that lobules on secondary and tertiary shoots are progenetic with respect to lobules on primary shoots (Fig. 14), in that the slope of the relationship between growth duration and shape does not change. Lobules on secondary and tertiary branches in *R. boryana* and *R. tenax* also exhibit differences consistent with paedomorphic changes by progenesis (early maturation) compared with the primary shoot lobules (Fig. 14). However, in *R. campanigera*, lobules on secondary and tertiary branches exhibit different slopes from the primary branches (given our somewhat subjective origin), as mature shapes differ even although the durations of growth are the same.

Neoteny is change in shape only, and results in different allometry from ancestors (Shea, 1983; Klingenberg, 1998). The slope of the relationship between shape and growth duration changes in secondary and tertiary branches in a pattern consistent with neoteny (Alberch *et al*., 1979). Intraspecific differences attributable to heterochrony are known in other plant groups (Mayers & Lord, 1983; McLellan, 1990, 1993; Clearwater & Gould, 1993; McLellan & Dengler, 1995). However, we are not aware of another group where intraspecific, and indeed intra-individual variation is explained by different heterochronic patterns in relatively closely related species. The pattern of lobule ontogeny is different in *R. campanigera*; i.e. cell division across the entire base. This may explain neoteny of secondary and tertiary branches, and why the four species were found not to exhibit the same relationship between size and shape.

Diversity in scaling relationships is usually phylogenetically correlated, with extreme variation occurring between groups, and limited variation occurring within groups (Eberhard & Gutierrez, 1991; Frankino *et al*., 2005). In contrast to these examples, *Radula* subgenus *Cladoradula* exhibits substantial variation in a small group.

Regularly bipinnate and pinnate shoot systems that exhibit morphological differences between branches of different order are characteristic of many liverwort groups, including Porellaceae, some subgenera of *Frullania* Raddi, Lepidolaenaceae and Lepidoziaceae. Differences in shoot morphology in these groups may also result from intra-individual heterochrony.

Lobule insertion

The insertion of lobules on tertiary branches of *R. bipinnata*, *R. boryana*, *R. campanigera* and *R. tenax* is longitudinal, whereas in other branches they are mostly transverse. This is one aspect of lobule morphology that is not replicated in the ontogeny of lobules on primary shoots. Young lobules of *R. bipinnata* primary shoots have a semi-circular stem insertion, which as a result is neither wholly longitudinal nor transverse (Fig. 8). What seems to happen as lobules grow is the upper end of this

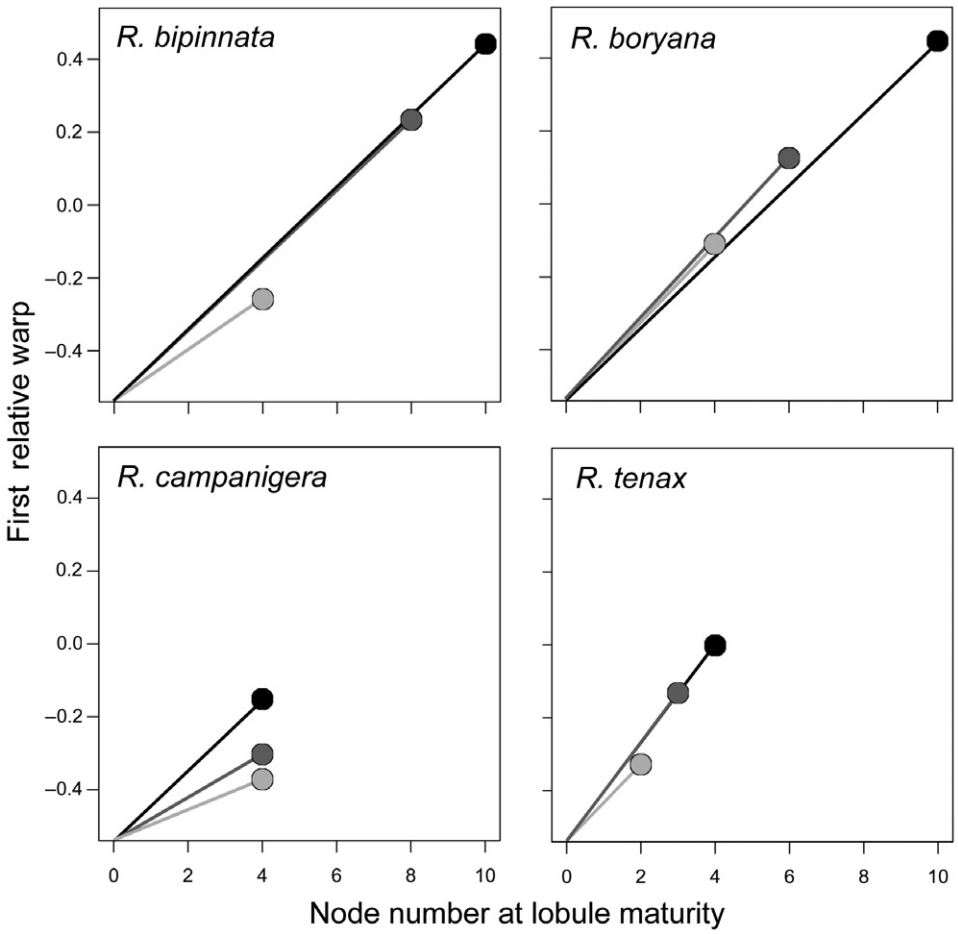

Figure 14. Schematic representation of ontogenetic lobule shape trajectories, *sensu* Alberch *et al*. 1979 and Pryer & Hearn 2009, showing time (measured in terms of the number of nodes between the lobule and the shoot apex at the time cell division in the lobule ceases on the x-axis) and scores on the first principal component describing 85.5% of variation in shape on the y-axis. The origin for each growth trajectory is somewhat arbitrarily located at a growth duration of 0, and a score on the component axis whose visualization using the thin-plate spline corresponds to the shape of the smallest lobules measured in this study.

insertion lengthens, whereas the lower does not, and a transverse insertion, which retains a semi-circular shape, results. In lobules of tertiary shoots the reverse may achieve a longitudinal insertion. Growth in either case would have to be coupled to changes in the underlying stem architecture, as it involves the point of fusion between the lobule and stem. The nature of our investigation precluded observation of this relationship. Schuster (1980) claimed that in *Radula* a transverse lobule insertion was surely primitive. Assessment of this claim will require broader sampling across Porellales.

Ecological considerations

Heterochrony is significant because it can produce novelties simply by changing the timing of developmental events or the rate of developmental processes (Li & Johnston, 2000), and heterochronic changes underpin morphological diversification in the leaves of a wide range of plants. Sequential paedomorphosis in Hawaiian *Cyanea* Gaudich. appears to be a significant source of diversification in leaf morphology (Lammers, 1990). Changes in leaf ontogeny underpinned diversity in leaf morphology in *Tortula*, and suggested juvenile morphology had been incorporated into the adult morphology of descendent species (Mishler, 1988). The non-homology of 'leaves' in the gametophyte generation of bryophytes and sporophyte generation of 'higher' plants has presented opportunities to test the generality of the evolutionary malleability of photosynthetic organs, as suggested by leaf diversity in *Tortula* (Mishler, 1988) and the *Radula* lobules studied here. Photosynthetic

organs are usually more variable than reproductive organs (Li & Johnston, 2000), but morphological diversification in reproductive organs can be attributable to heterochrony, or mixtures of heterochronic modes in different parts (Guerrant, 1982); e.g. in flowers of *Dactylorhiza viridis* (L.) R.M.Bateman, Pridgeon & M.W.Chase (Box *et al*., 2008). Mixtures of heterochronic changes in an organism stress the fact that species cannot be paedomorphic or peramorphic; only parts can, and only with respect to an outgroup (Klingenberg, 1998).

Regularly pinnate branch systems that exhibit large differences between shoots, but little variation within shoots (Fig. 7), suggest that apical cells have some sort of 'positional memory' in the context of shoot architecture; they do not freely transform shoots from one form into another. The correlation between shoot stature and lobule morphology, and the regular branching patterns combine to produce architecturally complex shoot systems in *Radula* subgenus *Cladoradula*. Shoot systems occupy a plane, distributing photosynthetic tissue throughout (see Fig. 1). Regularly produced branches of progressively smaller stature fill gaps between larger branches in *R. bipinnata* and, to a lesser extent, *R. boryana* and *R. gottscheana*.

The form of all photosynthetic plants must perform four functions at the level of individual organism: (1) light interception; (2) gas exchange; (3) water acquisition and transport; and (4) mechanical integrity (Niklas, 2004). The performance of different functions can place antagonistic demands on morphology (Gates, 1965; Nobel, 1983), and different morphologies may be better suited to optimizing one function over others (Niklas, 2004).

A plane-filling morphology was found to optimize light interception (Niklas, 2004), and this form is expressed by species of Hypnodendrales (i.e. Bell, Newton & Hyvönen, 2012) that grow in terrestrial microsites from horizontal surfaces. Large space-filling bryophytes are expected to outcompete small compact forms for space and light (Proctor, 1990), by spreading photosynthetic tissue more diffusely in space (Monsi, Uchijima & Oikawa, 1973). Stable hyper-humid environments may relax functional 'pressures' for water conservation (Niklas 2004) and facilitate longer growth durations, thereby facilitating the development of architectures to optimize light capture. The planar, regularly bipinnately branched shoot architectures exhibited by many species of *Radula* subgenus *Cladoradula* may serve to optimize light interception, but from horizontal rather than vertical substrates. A robust primary axis is required to provide the requisite structural support to hold regularly branched, planar shoot systems horizontally away from tree trunks and the sides of large rocks. The larger primary stems with heavily thickened cortical cell walls may serve this function. However, to attain large size they need to grow for longer durations. Lobule shape changes resulting from hypermorphosis could be a by-product of longer growth durations at shoot apices required to build primary axes sufficiently large to perform a structural role. Phylogenetic trends may be the result of deterministic, developmental causes or may simply be an artefact of increasing the duration of ontogeny (Niklas, 1982). Niklas (1982), in simulation studies of early land plant branching morphology, observed that evolutionary transitions from geometric to binomial branching were achieved simply by increasing shoot size. The phylogenetic trends observed in *Cladoradula* lobules could be an artefact of the increasing duration of ontogeny necessary to meet structural demands placed on stems to support shoot systems held away from the substrate. In this case, auriculate lobules may be considered a 'spandrel' in the context of a larger structure (Gould & Lewontin, 1979). Large, open growth forms are viable only where net radiation, wind speed and saturation deficit are all relatively low, conditions met in sheltered habitats such as forest interiors where bryophytes are often prominent (Proctor, 1990).

Gametophytes of *Radula* subgenus *Cladoradula*, in particular *R. bipinnata*, attain the greatest stature of species in the genus, and are among the largest species of Leafy 1 *sensu* Davis (2004), alongside species of *Frullania* Raddi and *Porella* L. that exhibit similar architecture, habitats and size. Structural strategies enabling development of a robust gametophyte must be employed to overcome pressures associated with a motility-based fertilization system (Renzaglia *et al*., 2000) requiring uninterrupted access to water for reproductive success, and to facilitate continual vegetative growth (Renzaglia *et al*., 2000). The large auriculate lobules could function in external water transport systems by providing continuity of surfaces for solute transport via capillary action. Porous basal cells and mamillose and papillose leaf cells in *Tortula* form capillary channels for the movement of water up the shoot and across the leaf lamina (Dilks & Proctor, 1979; Proctor, 1979, 1981). Incubous leaf insertion in epiphytic species may achieve a balance between water transport via capillary action on the underside of shoots and gas exchange for photosynthesis. Rapid drying is harsher on cellular integrity and metabolism in mosses (Bewley, 1979, cited in Proctor, 1990), and rapid rehydration may also be damaging as a result of solute loss before membrane integrity is re-established, and through more general cell damage (Oliver & Bewley, 1984). Overlapping concave leaves allow both external capillary conduction and free gas exchange over a

wide range of water content of plants (Dilks & Proctor, 1979; Proctor, 1979, 1984), and may buffer against water deficit.

Implications for taxonomy and nomenclature

The relative structural simplicity of liverworts usually means species in genera, and even entire families are outwardly similar, and this may pose challenges for those interested in meaningful classifications and origins of diversity alike (Heinrichs *et al*., 2010; Feldberg *et al*., 2011; Renner, Brown & Wardle, 2011). Qualitative character differences between species may be few (Renner & Braggins, 2004; Renner, 2006; Devos *et al*., 2011a), but *Radula* spp. typically exhibit quantitative differences in size and shape of structures found in sterile gametophytes, and these have been widely employed in alpha-taxonomic studies (Yamada, 1979, 1983, 1984). Typically, differences between species in lobule shape have been communicated to give the impression that these are absolute. This study suggests otherwise, as have a growing number of others (e.g. Pätsch *et al*., 2010).

In *Begonia* L. (McLellan, 1993), the occurrence of leaves of many shapes on a single plant is relevant to species circumscription. Patterns of variation may be relevant to species circumscription, as in the difference between *R. bipinnata* and *R. boryana*, where the most significant difference appears to be the relative stature and shape of the tertiary shoot lobules. Differences characterizing species of *Tortula* appear for the most part in later developmental stages (Mishler, 1986). If leaf shape is used, mature adult leaves should be considered for consistency of difference between species.

Given the frequently fragmentary nature of type specimens for many older *Radula* spp., those comprised of portions of secondary and/or tertiary shoots will not be representative of species morphology. Recognition of hierarchically structured shoot systems and an understanding of morphological variation in different branches of those shoot systems will be necessary before an accurate appraisal of type specimens will be possible.

ACKNOWLEDGEMENTS

This study was completed during an Australian Biological Resources Study (ABRS) Postdoctoral Fellowship awarded to M.A.M.R., in collaboration with E.A.B., N.D. and the Royal Botanic Gardens & Domain Trust (grant RFL210-36B). We thank curators and staff at herbaria BR, E, MO, NY, DUKE for loan of specimens included in this study; Anders Hagborg (Field Museum, Chicago) and Lars Söderström (Trondheim) who, with M.J.v.K. run the Early Land Plants Today (ELPT) project, for their ongoing support; Simon Ho for advice on BEAST analysis; A. Jon Shaw for stimulating the current collaboration; Lindsey J. Gray for constructive feedback on draft manuscripts; three anonymous reviewers for comments; M.A.M.R. thanks participants of the XIth Australian Bryophyte Workshop at Merimbula for tolerating a lengthy oral presentation of this topic and providing feedback on it.

REFERENCES

Abramoff MD, Magalhaes PJ, Ram SJ. 2004. Image processing with ImageJ. *Biophotonics International* **11:** 36–42.

Adams DC, Slice DE, Rohlf FJ. 2004. Geometric morphometrics: 10 years of progress following the 'revolution'. *Italian Journal of Zoology* **71:** 5–16.

Alberch P. 1985. Problems with the interpretation of developmental sequences. *Systematic Zoology* **34:** 46–58.

Alberch P, Gould SJ, Oster F, Wake B. 1979. Size and shape in ontogeny and phylogeny. *Paleobiology* **5:** 296–317.

Barthlott W, Fischer E, Frahm J-P, Seine R. 2000. First experimental evidence for zoophagy in the hepatic *Colura*. *Plant Biology* **2:** 93–97.

Bateman RM, Rudall PJ, James KE. 2006. Phylogenetic context, generic affinities and evolutionary origin of the enigmatic Balkan orchid *Gymnadenia frivaldii* Hampe ex Griseb. *Taxon* **55:** 107–118.

Bell NE, Newton AE, Hyvönen J. 2012. Epiphytism and generic endemism in the Hypnodendrales: *Cyrtopodendron*, *Franciella* and macro-morphological plasticity. *Taxon* **61:** 498–514.

Bewley JD. 1979. Physiological aspects of desiccation tolerance. *Annual Review of Plant Physiology* **30:** 195–238.

Blackstone NW. 1987a. Allometry and relative growth: pattern and process in evolutionary studies. *Systematic Zoology* **36:** 76–78.

Blackstone NW. 1987b. Size and time. *Systematic Zoology* **36:** 211–215.

Blomquist HL. 1929. The relation of capillary cavities in the Jungermanniaceae to water absorption and storage. *Ecology* **10:** 556–557.

Bookstein FL. 1989. Principal warps: thin-plate splines and the decomposition of deformations. *IEEE Transactions on pattern analysis and machine intelligence* **11:** 567–585.

Bookstein FL. 1991. *Morphometric tools for landmark data. Geometry and biology*. Cambridge: Cambridge University Press.

Bookstein FL. 1997. Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. *Medical Image Analysis* **1:** 225–243.

Box MS, Bateman RM, Glover BJ, Rudall PJ. 2008. Floral ontogenetic evidence of repeated speciation via paedomorphosis in subtribe Orchidinae (Orchidaceae). *Botanical Journal of the Linnean Society* **157:** 429–454.

Cardini A, Elton S. 2008. Variation in guenon skulls I: species divergence, ecological and genetic differences. *Journal of Human Evolution* **54:** 615–637.

Castle H. 1937. A revision of the genus *Radula*. Introduction and Part I. Subgenus *Cladoradula*. *Annales Bryologici* **9:** 13–56.

Clearwater MJ, Gould KS. 1993. Comparative leaf development of juvenile and adult *Pseudopanax crassifolius*. *Canadian Journal of Botany* **72:** 658–670.

Cohen JI. 2012. Continuous characters in phylogenetic analyses: patterns of corolla tube length evolution in *Lithospermum* L. (Bignoniaceae). *Biological Journal of the Linnean Society* **107:** 442–457.

Davis EC. 2004. A molecular phylogeny of leafy liverworts (Jungermanniidae: Marchantiophyta). In: Goffinet B, Hollowell V, Magill R, eds. *Molecular systematics of bryophytes*. St Louis: Missouri Botanical Gardens Press, 61–86.

Devos N, Renner MAM, Gradstein SR, Shaw AJ, Vanderpoorten A. 2011a. Molecular data challenge traditional subgeneric divisions in the leafy liverwort genus *Radula*. *Taxon* **60:** 1623–1632.

Devos N, Renner MAM, Gradstein SR, Shaw AJ, Laenen B, Vanderpoorten A. 2011b. Evolution of sexual systems, dispersal strategies and habitat selection in the liverwort genus *Radula*. *New Phytologist* **192:** 225–236.

Dilks TJK, Proctor MCF. 1979. Photosynthesis, respiration and water content in bryophytes. *New Phytologist* **82:** 97–114.

Drummond AJ, Raumbaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214.

Dryden IL, Mardia KV. 1998. *Statistical shape analysis*. Chichester: John Wiley and Sons.

Eberhard WG, Gutierrez EE. 1991. Male dimorphisms in beetles and earwigs and the question of developmental constraints. *Evolution* **45:** 18–28.

Feldberg K, Vána J, Schulze C, Bombosch A, Heinrichs J. 2011. Morphologically similar but genetically distinct: on the differentiation of *Syzygiella concreta* and *S. perfoliata* (Adelanthaceae subfam. Jamesonielloideae). *The Bryologist* **114:** 686–695.

Fiorello CV, German RZ. 1997. Heterochrony within species: craniofacial growth in giant, standard, and dwarf rabbits. *Evolution* **51:** 250–261.

Forrest LL, Davis EC, Long DG, Crandall-Stotler BJ, Clark A, Hollingsworth ML. 2006. Unraveling the evolutionary history of the liverworts (Marchantiophyta): multiple taxa, genomes and analyses. *The Bryologist* **109:** 303–334.

Frankino WA, Zwaan BJ, Stern DL, Brakefield PM. 2005. Natural selection and developmental constrains in the evolution of allometries. *Science* **307:** 718–720.

Freckleton RP, Harvy PH, Pagel M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *American Naturalist* **160:** 712–726.

Garland T, Harvey PH, Ives AR. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology* **41:** 18–32.

Gates DM. 1965. Energy, plants, and ecology. *Ecology* **46:** 1–13.

Gernhard T. 2008. The conditioned reconstructed process. *Journal of Theoretical Biology* **253:** 769–778.

Gift N, Stevens PF. 1997. Vagaries in the delimitation of characters states in quantitative variation – an experimental study. *Systematic Botany* **46:** 112–125.

Gleissberg S, Kadereit JW. 1999. Evolution of leaf morphogenesis: evidence from developmental and phylogenetic data in Papaveraceae. *International Journal of Plant Science* **160:** 787–794.

Gould SJ. 1966. Allometry and size in ontogeny and phylogeny. *Biological Reviews* **41:** 587–640.

Gould SJ. 1977. *Ontogeny and phylogeny*. Cambridge: Harvard University Press.

Gould SJ, Lewontin RC. 1979. Spandrels of San-Marco and the panglossian paradigm – a critique of the adaptationist program. *Proceedings of the Royal Society B – Biological Sciences* **205:** 581–598.

Gradstein SR, Reiner-Drehwald ME, Schneider H. 2003. A phylogenetic analysis of the genera of Lejeuneaceae (Hepaticae). *Botanical Journal of the Linnean Society* **143:** 391–410.

Gradstein SR, Wilson R. 2008. Protonemal neoteny in bryophytes. In: Mohamed H, Baki BB, Nasrulhaq-Boyce A, Lee PKL, eds. *Bryology in the new millennium*. Kuala Lumpur: University of Malaya, 1–11.

Gradstein SR, Wilson R, Ilkiu-Borges AL, Heinrichs J. 2006. Phylogenetic relationships and neotenic evolution of *Metzgeriopsis* (Lejeuneaceae) based on chloroplast DNA sequences and morphology. *Botanical Journal of the Linnean Society* **151:** 293–308.

Graham LE. 1993. *Origin of land plants*. New York: John Wiley.

Guerrant EO. 1982. Neotenic evolution of *Delphinium nudicaule* (Ranunculaceae): a hummingbird pollinated larkspur. *Evolution* **36:** 699–712.

Heinrichs J, Gradstein SR, Wilson R, Schneider H. 2005. Towards a natural classification of liverworts (Marchantiopyta) based on the chloroplast gene *rbcL*. *Cryptogamie Bryologie* **26:** 131–150.

Heinrichs J, Hentschel J, Bombosch A, Fiebig A, Reise J, Edelmann M, Kreier H-P, Schäfer-Verwimp A, Caspari S, Schmidt AR, Zhu R-L, von Konrat MJ, Shaw B, Shaw AJ. 2010. One species or at least eight? Delimitation and distribution of *Frullania tamarisci* (l.) Dumort. *s.l.* (Jungermanniopsida, Porellales) inferred from nuclear and chloroplast DNA markers. *Molecular Phylogenetics and Evolution* **56:** 1105–1114.

Jensen RJ, Ciofani KM, Miramontes LC. 2002. Lines, outlines, and landmarks: morphometric analyses of leaves of *Acer rubrrum, Acer saccharіunum* (Aceraceae) and their hybrid. *Taxon* **51:** 475–492.

Jensen RJ, Hokanson SC, Isebrands JG, Hancock JF. 1993. Morphometric variation in oaks of the Apostle Islands in Wisconsin: evidence of hybridization between *Quercus rubra* and *Q. ellipsoidalis* (Fagaceae). *American Journal of Botany* **80:** 1358–1366.

Jones CS. 1993. Heterochrony and heteroblastic leaf development in two subspecies of *Cucurbita argyrosperma* (Cucurbitaceae). *American Journal of Botany* **80:** 778–795.

Jones EW. 1977. African hepatics XXX. The genus *Radula* Dumortier. *Journal of Bryology* **9:** 461–504.

Klingenberg CP. 1998. Heterochrony and allometry: the analysis of evolutionary change in ontogeny. *Biological Reviews* **73:** 79–123.

Klingenberg CP. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* **11:** 353–357.

Klingenberg CP, Spence JR. 1994. Heterochrony and allometry: lessons from the water strider genus *Limnoporus*. *Evolution* **47:** 1834–1853.

Lammers TG. 1990. Sequential paedomorphosis among the endemic Hawaiian Lobelioideae (Campanulaceae). *Taxon* **39:** 206–211.

Lewis LA, Mishler BD, Vilgalys R. 1997. Phylogenetic relationships of the liverworts (Hepaticae), a basal embrophyte lineage, inferred from nucleotide sequence data of the chloroplast gene *rbcL*. *Molecular Phylogenetics and Evolution* **7:** 377–393.

Lexer C, Joseph J, van Loo M, Prenner G, Heinze B, Chase MW, Kirkup D. 2009. The use of digital image-based morphometrics to study the phenotypic mosaic in taxa with porous genomes. *Taxon* **58:** 349–364.

Li P, Johnston MO. 2000. Heterochrony in plant evolutionary studies through the twentieth century. *The Botanical Review* **66:** 57–88.

Ligrone R, Duckett JG, Renzaglia KS. 2012. Major transitions in the evolution of early land plants: a bryological perspective. *Annals of Botany* **109:** 851–871.

Lord EM, Hill JP. 1987. Evidence for heterochrony in the evolution of plant form. In: Raff RA, Raff E, eds. *Development as an evolutionary process*. New York: Alan R. Liss, 47–70.

MacLeod N. 1999. Generalizing and extending the eigenshape method of shape visualization and analysis. *Paleobiology* **27:** 226–241.

MacLeod N. 2002. Phylogenetic signals in morphometric data. In: MacLeod N, Forey PL, eds. *Morphology, shape, and phylogeny*. London: Taylor & Francis, 100–138.

Maddison WP, Maddison DR. 2011. *Mesquite: a modular system for evolutionary analysis*. *Version 2.75* Available at: http://mesquiteproject.org

Mayers AM, Lord EM. 1983. Comparative flower development in the cleistogamous species *Viola odorata*. I. A growth rate study. *American Journal of Botany* **70:** 1548–1555.

McKinney ML. 1988. Classifying heterochrony: allometry, size and time. In: McKinney ML, ed. *Heterochrony in evolution: a multidisciplinary approach*. New York: Plenum Press, 17–34.

McKinney ML, McNamara KJ. 1991. *Heterochrony: the evolution of ontogeny*. New York: Plenum Press.

McLellan T. 1990. Development of differences in leaf shape in *Begonia dregei* (Begoniaceae). *American Journal of Botany* **77:** 323–337.

McLellan T. 1993. The roles of heterochrony and heteroblasty in the diversification of leaf shapes in *Begonia dregei* (Begoniaceae). *American Journal of Botany* **80:** 796–804.

McLellan T, Dengler NG. 1995. Pattern and form in repeated elements in the development of simple leaves of *Begonia dregei*. *International Journal of Plant Sciences* **156:** 581–589.

McLellan T, Endler JA. 1998. The relative success of some methods for measuring and describing the shape of complex objects. *Systematic Biology* **47:** 264–281.

McManus HA, Qiu YL. 2008. Chapter 4: life cycles in major lineages of photosynthetic eukaryotes, with special reference to the origin of land plants. *Fieldiana Botany* **47:** 17–33.

McNamara KJ. 1982. Heterochrony and phylogenetic trends. *Paleobiology* **8:** 130–142.

Mishler BD. 1986. Ontogeny and phylogeny in *Tortula* (Musci: Pottiaceae). *Systematic Botany* **11:** 189–208.

Mishler BD. 1988. Relationships between ontogeny and phylogeny, with reference to bryophytes. In: Humphries CJ, ed. *Ontogeny and systematics*. New York: Columbia University Press, 117–136.

Mishler BD, Churchill SP. 1985. Transition to a land flora. Phylogenetic relationships of the green algae and bryophytes. *Cladistics* **1:** 305–328.

Mishler BD, Lewis LA, Bucheim MA, Renzaglia KS, Garbary DJ, Delwiche CF, Zechman FW, Kantz TS, Chapman RL. 1994. Phylogenetic relationships of the green-algae and bryophytes. *Annales of the Missouri Botanical Garden* **81:** 451–483.

Monsi M, Uchijima Z, Oikawa T. 1973. Structure of foliage canopies and photosynthesis. *Annual Review of Ecology and Systematics* **4:** 301–327.

Niklas KJ. 1982. Computer simulations of early land plant branching morphologies – canalization of patterns during evolution. *Paleobiology* **8:** 196–210.

Niklas KJ. 2004. Computer models of early land plant evolution. *Annual Review of Earth and Planetary Science* **32:** 47–66.

Nobel PS. 1983. *Biophysical Plant Physiology Ecology*. New York: Freeman.

Nylander JAA. 2004. *MrModeltest v2*. Uppsala: Evolutionary Biology Centre, Uppsala University.

Oliver MJ, Bewley JD. 1984. Plant dessication and protein synthesis. VI. Changes in protein synthesis elicited by desiccation of the moss *Tortula ruralis* are effected at the translational level. *Plant Physiology* **74:** 923–927.

Pätsch P, Hentschel J, Linares-Palomino R, Zhu R-L, Heinrichs J. 2010. Diversification and taxonomy of the liverwort *Jubula* Dumort. (Jungermanniopsida: Porellales) inferred from nuclear and chloroplast DNA sequences. *Systematic Botany* **35:** 6–12.

Patterson C. 1982. Morphological characters and homology. In: Joysey KA, Friday AE, eds. *Problems of phylogenetic reconstruction*. London: Academic Press, 21–74.

Proctor MCF. 1979. Structure and eco-physiological adaptation in bryphytes. In: Clarke GCS, Duckett JG, eds. *Bryophyte systematics*. London: Academic Press, 479–509.

Proctor MCF. 1981. Physiological ecology of bryophytes. *Advances in Bryology* **1:** 79–166.

Proctor MCF. 1984. Structure and ecological adaptation. In: Dyer AF, Duckett JG, eds. *The experimental biology of bryophytes*. London: Academic Press, 9–37.

Proctor MCF. 1990. The physiological basis of bryophyte production. *Botanical Journal of the Linnean Society* **104:** 61–77.

Pryer KM, Hearn DJ. 2009. Evolution of leaf form in marsileaceous ferns: evidence for heterochrony. *Evolution* **63:** 498–513.

Purvis A, Rambaut A. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *Computer Applications in the Biosciences* **11:** 247–251.

Qiu Y-L, Cho Y, Cox CJ, Palmer JD. 1998. The gain of three mitochondrial introns identifies liverworts as the earliest land plants. *Nature* **394:** 671–674.

Qiu Y-L, Li L, Wang B, Chen Z, Knoop V, Groth-Malonek M, Dombrovska O, Lee J, Kent Lm Rest J, Estabrook GF, Hendry TA, Taylor DW, Testa CM, Ambros M, Crandall-Stotler B, Duff JR, Stech M, Frey W, Quandt D, Davis CC. 2006. The deepest divergences in land plants inferred from phylogenomic evidence. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 15511–15516.

R Development Core Team. 2010. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing, Available at: http://www.R-project.org/. ISBN 3-900051-07-0.

Raff RA, Kaufman TC. 1983. *Embryos, genes, and evolution*. New York: Macmillan.

Rambaut A, Drummond AJ. 2009. *Tracer v1.5* Available at: http://beast.bio.ed.ac.uk/Tracer [accessed 31 July 2012].

Reilly SM, Wiley EO, Meinhardt DJ. 1997. An integrative approach to heterochrony: the distinction between interspecific and intraspecific phenomena. *Biological Journal of the Linnean Society* **60:** 119–143.

Renner MAM. 2006. Plants seen and unseen: *Radula pseudoscripta* sp. nov. and *Radula uvifera* (Radulaceae: Jungermanniopsida). *New Zealand Journal of Botany* **44:** 337–348.

Renner MAM, Braggins JE. 2004. The sterile gametophyte as a source of informative characters in the genus *Radula* (Radulaceae: Hepaticae). *Nova Hedwigia* **78:** 243–268.

Renner MAM, Brown EA, Wardle GM. 2011. The *Lejeunea tumida* species group is positively polyphyletic. *Australian Systematic Botany* **24:** 10–18.

Renzaglia KS, Duff RJ, Nickrent DL, Garbary DJ. 2000. Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny. *Philosophical Transactions: Biological Sciences* **355:** 769–793.

Rohlf FJ. 1986. Relationships among eigenshape analysis, Fourier analysis, and analysis of coordinates. *Mathematical Geology* **18:** 845–854.

Rohlf FJ. 2003a. *tpsRelw. Version 1.36*. New York: Stony Brook University, Department of Ecology and Evolution, Available at: http://www.lie.bio.sunysb.edu/morph/ [accessed 20 September 2009].

Rohlf FJ. 2003b. *tpsSmall. Version 1.20*. New York: Stony Brook University, Department of Ecology and Evolution, Available at: http://www.lie.bio.sunysb.edu/morph/ [accessed 10 April 2012].

Rohlf FJ. 2010a. *tpsUTIL Version 1.46*. New York: Stony Brook University, Department of Ecology and Evolution, Available at: http://www.lie.bio.sunysb.edu/morph/ [accessed 12 November 2010].

Rohlf FJ. 2010b. *tpsDIG2. Version 2.16*. New York: Stony Brook University, Department of Ecology and Evolution, Available at: http://www.lie.bio.sunysb.edu/morph/ [accessed 12 November 2010].

Rohlf FJ. 2011. *tpsREGR. Version 2.38*. New York: Stony Brook University, Department of Ecology and Evolution, Available at: http://www.lie.bio.sunysb.edu/morph/ [accessed 14 April 2012].

Rohlf FJ, Loy A, Corti M. 1996. Morphometric analysis of old world Talpidae (Mammalia, Insectivora) using partial-warp scores. *Systematic Biology* **45:** 344–362.

Schuster RM. 1966. *The Hepaticae and Anthocerotae of North America, vol. 1*. Chicago: The Field Museum of Natural History.

Schuster RM. 1980. *The Hepaticae and Anthocerotae of North America, vol. 4*. Chicago: The Field Museum of Natural History.

Schuster RM. 1984. Chapter 15. Evolution, phylogeny and classification of the Hepaticae. In: Schuster RM, ed. *New manual of bryology. Vol. 2*. Miyazaki: The Hattori Botanical Laboratory, 892–1070.

Schuster RM. 1991. On neotenic species of *Radula*. *Journal of the Hattori Botanical Laboratory* **70:** 51–62.

Shea BT. 1983. Allometry and heterochrony in the African Apes. *American Journal of Physical Anthropology* **62:** 275–289.

Sidlauskas B. 2008. Continuous and arrested diversification in sister clade of characiform fishes: a phylomorphospace approach. *Evolution* **62:** 3135–3156.

Spruce R. 1884. Hepaticae Amazonicae et Andinae. *Transactions and Proceedings of the Botanical Society of Edinburgh* **15:** 1–590.

Stech M, Quandt D. 2010. 20,000 species and five key markers: the status of molecular bryophyte phylogenetics. *Phytotaxa* **9:** 196–228.

Stevens PF. 1991. Character states, morphological variation, and phylogenetic analyses: a review. *Systematic Botany* **16:** 553–583.

Swofford DL. 2002. *PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4*. Sunderland: Sinauer.

Viscosi V, Cardini A. 2011. Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for beginners. *PLOS One* **6:** 1–20.

Viscosi V, Lepais O, Gerber S, Fortini P. 2009. Leaf morphological analyses in four European oak species (*Quercus*) and their hybrids: a comparison of traditional and geometric morphometric methods. *Plant Biosystems* **143:** 564–574.

Yamada K. 1979. A revision of Asian taxa of *Radula*, Hepaticae. *Journal of the Hattori Botanical Laboratory* **45:** 201–322.

Yamada K. 1983. Four new species of *Radula* from Cuba. *Journal of the Hattori Botanical Laboratory* **54:** 241–249.

Yamada K. 1984. A *Radula* collection made by Dr M. L. Hicks in Queensland, Australia. *Cryptogamie, Bryologie, Lichenologie* **5:** 191–199.