1 Original Article

2 **Incongruent phylogenies and its implications for the study of diversification, taxonomy and**
 3 **genome size evolution of *Rhododendron* (Ericaceae)**

4

5

6

7 GULZAR KHAN¹, JENNIFER NOLZEN ¹, HARTWIG SCHEPKER² and DIRK C. ALBACH^{1*}

8

9

10

11 ¹*Institute for Biology and Environmental Sciences, Carl von Ossietzky University Oldenburg, Carl*
 12 *von Ossietzky Strasse 9-11, 26111 Oldenburg, Germany*

13 ²*Stiftung Bremer Rhododendronpark, Deliusweg 40, 28359 Bremen, Germany*

14 *Email: schepker@rhododendronpark-bremen.de*

15

16

17

18 ^{*}Email for correspondence: dirk.albach@uni-oldenburg.de

19

20 **Total words: 8294**

PREMISE: *Rhododendron* L. (Ericaceae Juss.), is the most species-rich genus of woody flowering plants with > 1000 species. Despite the interest in the genus and numerous previous phylogenetic analysis, the infrageneric classification for the genus is still debated, partly due to its huge diversity, partly due to homoplasy in key characters and partly due to incongruence between phylogenetic markers. Here, we provide a broad coverage of representative species of all *Rhododendron* subgenera, sections, and most subsections to resolve its infrageneric phylogeny or highlight areas of incongruence, support previous analyses of diversification patterns and establish a relationship between genome size evolution and its diversification.

METHODS: We generated sequences of two plastid (*trnK* and *trnL-F*) and two nuclear (ITS and *rpb2-i*) markers for a total of 259 *Rhododendron* species, and used likelihood and Bayesian statistics to analyze the data. We analyzed the markers separately to discuss and understand incongruence among the data sets and among previous studies.

RESULTS: We found that the larger a subgenus, the more strongly it is supported as monophyletic. However, the smaller subgenera pose several problems, e.g., *R.* subgen. *Azaleastrum* consists of two sections inferred to be polyphyletic. The main shift to higher diversification in the genus occurred in the Himalayan/SE Asian clade of *R.* subgen. *Hymenanthes*. We found that polyploidy occurs in almost all subgenera but most polyploid species are within *R.* subgen. *Rhododendron* sections *Rhododendron* and *Schistanthe*.

CONCLUSION: Whereas previous reports stated that genome sizes of tropical plants are lower than those of colder and temperate regions in angiosperms in general, our study provides evidence for such a shift to small genome-tropical species within a genus. Taken together, we see the merit in the recognition of the five major clades at the sub generic level but given the amount of incongruence a large amount of species cannot be confidently assigned to one of these five clades. Further, genome-wide data will be necessary to assess whether these currently unassignable taxa are independent taxa, assignable to one of the five major clades or whether they are inter-subgeneric hybrids.

KEYWORDS: genome size evolution; incongruence; phylogeny; polyploidy; *Rhododendron*

Rhododendron L. (Ericaceae Juss.) is the most species-rich genus of woody flowering plants, placed among the twenty largest plant genera >1000 species (Frodin, 2004). The genus has a worldwide distribution (except limited distribution in Central & South America and Africa) with a center of diversity in China (GBIF; Fang and Ming, 1995; Wu and Raven, 2005; Brown et al., 2006). However, many of these species have a limited distribution and are, thus, threatened. According to Gibbs et al. (2011), about 70% of the species of *Rhododendron* are classified as vulnerable, threatened, endangered or critically endangered. Many of the species in the genus are used as ornamentals and/or used as medicinal plants, with abundant literature on their ethno-

medicinal use as anti-inflammatory agents, pain killers, in gastro-intestinal disorders, common cold, asthma, skin diseases, and toxic agents (used in the form of insecticide or poison; see Popescu and Kopp, 2013 for a review). Innocenti et al. (2010) suggested that some species of *Rhododendron* have antibacterial activities. Rezk et al. (2015) extended this work and demonstrated that there is a higher susceptibility of Gram-positive bacteria (e.g., *Bacillus subtilis* Ehrenberg) and lesser susceptibility of Gram-negative bacteria (e.g., *Escherichia coli* (Migula) Castellani and Chalmers) towards *Rhododendron* leaf extracts.

This interest in the genus as ornamental and medicinal plant has led to a long list of studies investigating its diversification and classification. Linnaeus (1753) classified the species in two genera, *Azalea* (species with five stamens) and *Rhododendron* (species with ten stamens). Over the next century, as the number and diversity of known species increased, additional genera such as *Rhodora* L. (Linnaeus, 1762), *Vireya* Blume (Blume, 1826) and *Anthodendron* Rchb. (Reichenbach, 1827) were proposed. Especially, in the second half of the nineteenth century *Rhododendron* enjoyed an increase of species number based on the botanical exploration of the Himalayas and China (e.g., Hooker, 1849) and starting a rhododendronmania in Europe (Musgrave et al., 1998). Sleumer (1949, 1966) proposed a detailed classification for the genus that proved highly influential until today. He classified all known *Rhododendron* species at that time into five subgenera and 13 sections. Chamberlain et al. (1996), based on a number of more narrowly focused morphological taxonomic studies, refined previous classifications for the genus grouping the species into eight subgenera and 12 sections (Sleumer, 1966; Cullen, 1980; Chamberlain, 1982; Philipson and Philipson, 1986; Judd and Kron, 1995; Chamberlain et al., 1996). One notable difference between both classifications is the inclusion of *R. sect. Therorhodion* Maxim. In Chamberlain et al. (1996) as a subgenus of *Rhododendron*, whereas Sleumer (1966), following Small (1914) considered it separate from *Rhododendron*. The classification of Chamberlain et al. (1996) is still widely used by specialists and gardeners (Cox and Cox 1997; Goetsch et al., 2005). Phylogenetic analyses started to have an influence on the classification of *Rhododendron* in the last 30 years. The most important changes are the inclusion of three genera, *Ledum* (Kron and Judd, 1990), *Diplarche* Hook.f. & Thomson and *Menziesia* Sm. (Craven, 2011). With respect to the intrageneric classification, Goetsch et al. (2005), based on DNA-based phylogenetic analyses, suggested a reduction to five subgenera, *R. subgen. Therorodion*, *R. subgen. Rhododendron*, *R. subgen. Hymenanthes* (including *R. subgen. Pentanthera* sect. *Pentanthera*), *R. subgen. Choniastrum* (= *R. subgen. Azaleastrum* sect. *Choniastrum*) and *R. subgen. Azaleastrum* (incl. *R. subgen. Pentanthera* sect. *Sciadorhodion*, *R. subgen. Mumeazalea*, *R. subgen. Candidastrum*, *R. subgen. Tsutsusi*; see details in Table S1).

Establishing relationships in *Rhododendron* based on morphological characteristics but also molecular markers has been difficult because of frequent convergence and hybridization between species. Hybridization is considered to have played an important role in the evolution and

93 speciation of *Rhododendron* through homoploid or allopolyploid speciation (Milne et al., 1999 and
 94 2003; Milne and Abbott, 2008). This is reflected clearly by the large number of horticultural hybrids
 95 in *Rhododendron* (over 28,000; Leslie, 2004) as well as the occurrence of natural hybridization
 96 indicating weak reproductive barriers (Kron et al., 1993; Milne and Abbott, 2008; Milne et al., 1999
 97 and 2003; Zha et al., 2008 and 2010; Zhang et al., 2007). Besides homoploid hybridization,
 98 polyploidy, the occurrence of three or more sets of homologous chromosomes in the genome also
 99 occurs naturally in *Rhododendron*, ranging from triploids to dodecaploids (Ammal, 1950) and even
 100 more extensively exploited in horticulture (Jones et al., 2007). In *Rhododendron* the basic
 101 chromosome number is 13 (with exception of *R. camtschaticum* Pall. with $n = 12$) with more than
 102 70% of the species counted (~15% total) being diploid ($2n = 26$; Ammal et al., 1950; Väinölä, 2000;
 103 and Rice et al., 2015), but little is known about the importance of polyploidization in the
 104 diversification of the genus. Though hybridization can play diverse roles in promoting speciation
 105 (Abbott et al., 2013; and Milne et al., 2010), in *Rhododendron*, hybrid populations have often been
 106 found to show higher fitness than their parents only in mosaic habitats created along altitudinal,
 107 radiation (Milne et al., 2003) or soil pH (Milne and Abbott, 2008) gradients.

108 The large number of species has stirred interest in investigating the underlying reasons for its
 109 diversification. Milne et al. (2010) demonstrated that *R.* subgen. *Hymenanthes* (Blume) K. Koch
 110 within South East (SE) Asia has been the clade to diversify fastest. Similarly, Schwery et al. (2015)
 111 supported that the greatest diversity within *Rhododendron* occurs in the Himalayas and Malesia,
 112 detected a nested Himalayan *Rhododendron* radiation of species of *R.* subgen. *Hymenanthes*, and
 113 a separate diversification of *R.* section *Schistanthe* Schltr. (= *Vireya*) accompanied by an
 114 eastwards dispersal, as predicted by (Brown et al., 2006) and Goetsch et al. (2011). However,
 115 hybridization or polyploidy have not been considered by these studies are, until now, insufficiently
 116 considered in the analyses trying to understand the diversification of the genus, in contrast to
 117 biogeographic processes (Shrestha et al., 2018). A phylogenetic study analyzing the importance of
 118 hybridization and establishing a robust infrageneric classification requires as many species as
 119 possible representing all subgenera, sections, and subsections. Till to date about 400 species of
 120 the more than 1000 *Rhododendron* species known to data have been studied in different
 121 phylogenetic studies. However, these studies mostly focused on some specific subgenera and/or
 122 sections. Only few studies considered the phylogeny of the whole genus *Rhododendron* and
 123 mostly using a single DNA region, e.g., plastid *matK* - 51 species (Kurashige et al., 2001); nuclear
 124 ITS - 21 species (Gao et al., 2002); nuclear *rpb2* - 88 species (Goetsch et al., 2005) or *trnK*, *trnL-F*
 125 & ITS – 87 species (Grimbs et al., 2017). Shrestha et al. (2018) investigated the global distribution
 126 and molecular phylogeny of *Rhododendron* in a biogeographical context, using 423 species with a
 127 concatenated dataset of nine plastid genes, nuclear ribosomal ITS data and six introns of one
 128 nuclear gene (RPB). However, neither did they report how much missing data was included nor
 129 did they discuss whether and where incongruence between different data sets occurred. In
 130 addition, Shrestha et al. (2018) depicted relationships without support values, which does not allow

evaluation of the robustness of relationships. Differences between these phylogenies and previous classifications have raised doubts regarding the validity of the morphology-based *Rhododendron* classification and prompted Goetsch et al. (2005) to propose a new classification. However, the differences between analyses based on different DNA regions at the subgeneric and sectional level prevented widespread support for this alternative classification for *Rhododendron*. To this end, here our objective is to reconstruct the phylogeny of *Rhododendron* using representative species of all subgenera, sections, and subsections for discussing support for alternative classifications, especially the DNA-based classification of Goetsch et al. (2005). We used both plastid and nuclear markers for phylogeny reconstruction. Based on this phylogeny, we analyzed the importance of hybridization and polyploidization in diversification of *Rhododendron* in a phylogenetic context. We provide here a critical evaluation of phylogenetic studies and results from different markers in the genus. We, further, use the results to infer patterns of diversification in the genus focusing on the importance of polyploidy and genome size evolution. Our main questions were: (1) Does expansion of the sampling resolve the phylogenetic relationships within *Rhododendron* and support the monophyly of major clades; (2) Do different DNA markers similarly show and support previous diversification patterns? And (3): Are polyploidy and genome size evolution related to the diversification of the genus? To follow these questions, we generated sequences of two plastid (*trnK* and *trnL-F*) and two nuclear (ITS and *rpb2-i*) markers for a total of 307 individuals from 259 *Rhododendron* species. We analyzed all four datasets separately and discuss the incongruences among these markers and previous studies. In addition, we used BAMM (Bayesian Analyses of Macroevolutionary Mixtures; Rabosky, 2014) to investigate the pattern of diversification in *Rhododendron*.

MATERIAL AND METHODS

Sampling

We based our sampling strategy on two criteria, first to include as many species as possible and second to represent all subgenera, sections, and subsections of *Rhododendron* following Chamberlain et al. 1996 (Table S1), which we managed with the exception of two subsections of *R.* subgen. *Hymenanthes* (subsectt. *Barbata*, *Lanata*), the monotypic *R.* subgen. *Pentanthera* subsect. *Sinensia*, and four monotypic subsections in *R.* subgen. *Rhododendron* (subsect. *Afghanica*, *Campylopogon*, *Camelliflorum*, *Virgata*). We collected fresh leaves of 307 individuals from 259 species at Rhododendron-Park Bremen and downloaded the available sequences of other species from GenBank (Table S2). To root the phylogeny of *Rhododendron*, we used *Calluna vulgaris* (L.) Hull, *Empetrum nigrum* L., *Kalmia angustifolia* L., *Kalmia procumbens* (L.) Desvaux, *Vaccinium x intermedium* Ruthe, and *Vaccinium myrtilloides* Michx. as outgroups. The outgroup species belong to the same family, covering the main lineages of family Ericaceae and cultivated in the Botanical Garden of the Carl von Ossietzky-University (Oldenburg, Germany)

167 except *Phyllodoce empetriformis* (Sm.) D.D, collected in Botanical Garden Bochum (Bochum,
168 Germany) with permission. For DNA extraction the leaves were silica gel dried, while for flow
169 cytometry, we used fresh leaves. Vouchers for all species are stored in the herbarium of Carl von
170 Ossietzky-University (OLD).

171 **Genome size estimation, DNA extraction and PCR amplification**

172 To estimate nuclear genome sizes of *Rhododendron* species, we used flow cytometry following
173 the basic protocol of Galbraith et al. (1983). We used *Zea mays* L. 'CE-777' (2C = 5.430 pg),
174 *Hedychium gardnerianum* (2C = 4.02 pg) or *Solanum pseudocapsium* (2C = 2.60) as an internal
175 standard (Temsch et al., 2010, Meudt et al., 2015; Table S2). Briefly, we prepared intact nuclei
176 suspensions by chopping 0.5 - 1 cm² of fresh leaf tissue of *Rhododendron* and the internal
177 standard together with 1100µl of nuclei extraction buffer (OTTO I buffer: 100mM citric acid;
178 0.5 % (v/v) Tween 20 (pH approx. 2.3) after Otto (1990)). The intact nuclei suspension was filtered
179 through a 30µm CellTrics[®] nylon mesh filter (Partec). The filtered solution was then incubated at
180 37 °C for 30 min and stained with 2 ml propidium iodide buffer for 1 hour at 4 °C. This staining step
181 also involved a treatment with RNase. Lastly, we ran the suspension on the flow cytometer
182 (CyFlow SL, Partec, Munster, Germany), measuring 5000 particles and at least 1000 nuclei of
183 sample and standard. We repeated this process three times on different days. The genome sizes
184 (2C-value in pg DNA) were determined by comparing the mean relative fluorescence of each
185 sample with the standard. The relationship between ploidy levels and genome sizes (monoploid
186 1Cx- value in pg DNA) was determined with documented chromosome numbers and ploidy levels
187 in the literature.

188 We extracted total genomic DNA from silica gel-dried leaves (Chase and Hills, 1991), with
189 minor modifications (100 µl elution buffer, centrifuge at 6.000 x g for 5 min) using the innuPREP
190 Plant DNA Kit (Analytik Jena, Jena, Germany). Following upon the completion of DNA extraction,
191 we amplified two plastid gene regions, *trnK* (*matK* and the 3' end *trnK*) & *trnL-F* (*trnL* intron, *trnL* 3'
192 exon, *trnL-trnF* spacer); and two nuclear gene regions, the *rpb2-i* (segment 2, 3, and 5) and the
193 ribosomal Internal Transcribed Spacer (ITS) through polymerase chain reaction (PCR, Table S3).
194 To amplify *trnK*, we designed two new internal primers based on the existing *trnK* of
195 *Rhododendron setosum* D.Don (OLD00775) using the program PRIMER3 (Rozen and Skaletsky,
196 1999), i.e. MK1538F (TAT GGG TGT TTA AAG AGC) and MK1785R (TCT ATC ATT TGA CTC
197 CGT ACC A). For other regions, we used the available primers (White et al., 1990; Taberlet et al.,
198 1991; Liu et al., 1999). All amplification reactions of target regions were carried out in 25 µl with
199 2.5 µl 10x PCR buffer, 1 µl MgCl₂ (50 mM), 0.5 µl dNTPs (10 mM), 1 µl of each primer
200 (10 pmol/µl), 1 µl DMSO (only in case of nuclear regions), 1 µl BSA, 0.2 µl *Taq* polymerase
201 (5 units), and 10-20 ng genomic DNA. The amplification of ITS and *trnL-F* regions were performed
202 on a TProfessional Standard Thermocycler (Biometra GmbH, Goettingen, Germany) and those of

the *trnK* and *rpb2-i* on a Mastercycler® gradient (Eppendorf AG, Hamburg, Germany; details of PCR reactions profile in Table S3). Sequencing was conducted by GATC Biotech (Konstanz, Germany) on an ABI 3730xl (PE Applied Biosystem) automated sequencers. To check the quality of all sequences, we used GENEIOUS PRO v5.4.6 (Kearse et al., 2012). Lastly, we used MAFFT algorithm (Kato et al., 2002) to align the sequences and visually inspected the alignment.

Phylogenetic reconstruction and divergence time

We analyzed the data considering them as four separate data sets (*trnK*, *trnL-F*, ITS, and *rpb2-i*) using the Maximum Likelihood and Bayesian inference approaches. Maximum Likelihood (ML) analyses were conducted in RAXML v.7.9.5 (Stamatakis, 2006) with the substitution model (GTR+ Γ with four rate categories for ITS, *rpb2* and *trnK*; and HKY for *trnL-F*). The selection of best substitution models was implemented in jMODELTEST v.3.7 (Posada, 2008) using the Akaike Information Criterion (AIC). We used non-parametric bootstraps (1000 replicates) to determine support for each node (BS up to 70 was considered as weak, BS 70 – 90 as medium, and BS > 90 as strong). Similarly, the Bayesian trees were analyzed in MRBAYES v.3.2.2 (Ronquist and Huelsenbeck, 2003) using the same substitution model with two independent runs, each consisted of four Markov chains. All runs were allowed to proceed for ten million generations, sampled every 1000 generations. A consensus tree was generated with a 50 percent majority rule consensus after discarding the first 10 percent generations as burn-in.

Similarly, we estimated time-calibrated phylogenetic trees in BEAST v.2.3 (Bouckaert et al., 2014) for which, the input file was generated in BEAUTi v.2.3 (Bouckaert et al., 2014) using an uncorrelated lognormal relaxed clock (Drummond et al., 2006) with a birth-death prior and GTR substitution model. Since fossils of *Rhododendron* are scarce and difficult to use for each branch, we used the oldest *Rhododendron* fossil as a first calibration point. According to Schwery et al., 2015, the estimated fossil age of *Rhododendron* (without *R. camtschaticum*) is 58 mya with a standard deviation of 2 my, based on the oldest fossil of *Rhododendron* and 17 other fossils from Ericaceae. As second calibration point, we used 28.10 mya as leaf fossil age of *R.* subgen. *Hymenanthes* from the late Oligocene (Axelrod, 1998) with normally distributed prior and the corresponding standard deviation.. The actual analysis was run for 100 million MCMC each, sampling the results after every 10.000 chains. We used the program Tracer v.1.5 (Rambaut and Drummond, 2009) to check upon the convergence of the chains and estimated sample sizes (ESS > 200). To compute the maximum clade credibility tree, we used TreeAnnotator v.2.3 (Drummond et al., 2012) with node heights being the median of the age estimates deleting the first 10 % generations as burn-in. Finally, we investigated if there were any influence of the priors on the analyses and information content of the data by repeating the analyses with the same settings without data.

Genome size evolution and diversification

To investigate whether the continuous traits related to genome size (2C genome size, 1Cx monoploid genome size, ploidy level) have any significant phylogenetic signal, we estimated the phylogenetic signal of these characters with the function `phylosig` in the R-package ‘`phytools`’ (Revell, 2012) using two different methods: Blomberg’s K statistic (Blomberg et al., 2003) and Pagel’s λ (Pagel, 1999). A bar plot of the trait ‘ploidy level’ was visualized using ‘`phytools`’ by mapping it on the side of the BEAST tree. To calculate and map the ancestral character states for continuous characters 2C- and 1Cx-values on the BEAST tree for the data sets, we used the function `contMap` in ‘`phytools`’ by estimating the maximum likelihood ancestral character states for continuous traits with `fastAnc`.

The net diversification rate and the number and location of monoploid genome size rate shifts in *Rhododendron* were determined using BAMM (Rabosky, 2014) as employed in the R-package ‘`BAMMtools`’ (Rabosky et al., 2014). Though BAMM has been criticized by Moore et al. (2016), simulation studies suggested robustness of diversification analyses by BAMM (Rabosky et al., 2017; see also in Mitchel et al., 2019). The most important critique of BAMM is an error in the rate of extinction in the absence of fossil records (Rabosky, 2010; Marshall, 2017; and Rabosky, 2018). However, we here used BAMM only for diversification/speciation analysis. Additionally, since *Rhododendron* is monophyletic (details in results section), we only accounted for incomplete sampling to improve the robustness of BAMM based results. In *Rhododendron*, we estimated the `globalSamplingFraction` as number of species used in the dataset/total number of *Rhododendron* species (for example 0.235 for *trnK* as 259/1100 total *Rhododendron* species) following Igea and Tanentzap (2020; also see in Spriggs et al., 2015). For the actual analysis, we used the BEAST trees as input for running both the trait (1Cx-value) and speciation rate. Three replicates were run in BAMM for 30 million generations and saved after every 5,000th generation. ‘`BAMMtools`’ was used to plot the likelihoods of sampled generations after discarding the first 10% of chains. To assess convergence, effective sample size was checked for each prior to be >200. Furthermore, we calculated Bayes factors (BFs), plotted the best shift rate configurations and estimated rates of speciation (diversification analysis) and evolution of monoploid genome size (trait analysis).

RESULTS

Molecular phylogenetic reconstruction

Details of variables sites, number of informative sites, and species used in each dataset are given in Table 1. The results from phylogenetic analyses of the four DNA regions (Figs. 1-2, S1-6) agree in several aspects. These are the monophyly of *Rhododendron*, the sister-group relationship of *R.* subgen. *Therorhodon* to the rest of the genus, the monophyly of *R.* subgen. *Rhododendron* (the former *Ledum* excluded) and *R.* subgen. *Tsutsusi* (except in *trnLF*), as well as the polyphyly of *R.* subgen. *Azaleastrum* and *R.* subgen. *Pentanthera*, within the latter, *R.* sect. *Pentanthera* is monophyletic if *R. canadense* is included (except in *rpb2*). In *R.* subgen. *Azaleastrum*, the two

275 sections *R. sect. Azaleastrum* and *R. sect. Choniastrum* are monophyletic. Finally, the analyses
276 agree that in *R. subgen. Tsutsusi*, *R. sect. Tsutsusi* (excl. *R. tashiroi*) and *R. sect. Brachycalyx*
277 (including *R. tashiroi*) are monophyletic. In the following, we will only mention the points, in which
278 the single region analyses differ from the rest.

279 Our results based on the plastid markers, the *trnK* phylogeny (259 species, CI = 0.736, RI =
280 0.917; Table 1; Fig. 1 and S1) and *trnL-F* region (169 species, CI = 0.697, RI = 0.883; Table 1; Fig.
281 S2-3), are highly congruent. Results from the *trnK* dataset suggest that within *R. subgen.*
282 *Hymenanthus*, species of subsections *Argyrophylla* (*R. insigne*, *R. riei*), *Fortunea* (*R. calophyllum*,
283 *R. praevenum*), *Irrorata* (*R. annae*), and all included species of *Pontica* (except *R. degonianum*
284 and *R. smirnowii*) form a group, which includes almost all species from outside SE Asia (South-
285 West Eurasia = Turkey and Caucasus; North-East Asia = Japan, Korea and Manchuria to East
286 Siberia; western North America and eastern North America) and is sister to the remaining
287 subsections of *R. subgen. Hymenanthus* (SE Asian clade = mainly southern China, Himalaya
288 Mountains and Taiwan). *Rhododendron* subgen. *Rhododendron* is divided into two clades of which
289 one encompasses the sections *Rhododendron* (excluding subsection *Glaucum*) and *Pogonanthum*,
290 and the second contains the vireyas (section *Schistanthe*). The monotypic *R. subgen.*
291 *Candidastrum* is sister to the combined *R. subgen. Tsutsusi* and *R. section Azaleastrum*, but the
292 clade was weakly supported (BS/PP $\leq$ 70). The monotypic *R. subgen. Mumeazalea* is sister to *R.*
293 *sect. Choniastrum*.

294 Our results based on the *trnL-F* region showed only some, slight differences and was generally
295 less well resolved. The *trnL-F*-based tree recovered *R. subgen. Azaleastrum* section *Choniastrum*
296 (with *Mumeazalea*) as sister to *Tsutsusi* section *Tsutsusi*. In addition, *R. section Azaleastrum* is
297 sister to the clade of *R. subgen. Choniastrum* and *R. subgen. Tsutsusi* with strong support (Fig.
298 S2). *Rhododendron vaseyi* (*R. section Rhodora*) and *R. pilosum* (*R. section Sciadorhodion*, former
299 *Menziesia*) clustered closer to *R. subgen. Rhododendron* (Fig. S2). *Rhododendron* subgen.
300 *Hymenanthus* is divided into the same two clades as above and within *R. subgen. Rhododendron*
301 section *Schistanthe* (including *R. section Rhododendron* subsections *Genestieriana*, *Glaucum*, and
302 *Micrantha*) is sister to section *Pogonanthum* and the remaining subsections of section
303 *Rhododendron*.

304 In the phylogeny based on the nuclear ITS region (197 species, CI = 0.662, RI = 0.908; Table
305 1; Fig. 2 and S4) reveals *R. subgen. Mumeazalea* clusters with section *Azaleastrum* and with *R.*
306 section *Choniastrum*. In addition, *R. sections Rhodora* and *Sciadorhodion* show a sister group
307 relationship to the clade of *R. section Azaleastrum*, *Mumeazalea*, and *Tsutsusi*, but this
308 relationship is weakly supported. Within *R. section Rhododendron* the subsections *Ledum*,
309 *Micrantha*, and *Rhododendron* form a clade (Fig. 2) which is sister to the clade including *R. section*

310 *Pogonanthum*, the remaining subsections of *R.* section *Rhododendron*, and *R.* section
311 *Schistanthe*.

312 In the phylogeny based on *rpb2-i* sequences (170 species, CI = 0.674, RI = 0.864; Table 1;
313 Fig. S5-6) all *Rhododendron* species (except *R. camtschaticum*) fall into three large clades (Fig.
314 S6). The first clade comprises *R.* subgenera *Hymenanthus* and *Pentanthera*, in which *R.* subgen.
315 *Pentanthera* is not monophyletic. The second clade contains *R.* subgenera *Azaleastrum* section
316 *Azaleastrum*, *Candidastrum*, *Mumeazalea*, *Pentanthera* section *Sciadorhodion*, *R. nipponicum*
317 (section *Viscidula*) and *R. vaseyi* (section *Rhodora*), and *R.* subgen. *Tsutsusi*. The remaining clade
318 encompasses *R.* subgen. *Azaleastrum* section *Choniastrum* and *R.* subgen. *Rhododendron*.

319 **Diversification regime shifts**

320 All three runs of diversification analysis in BAMM showed similar results (including log likelihoods
321 and number of shifts; data not shown). For the *trnK* species tree the frequent shift configuration of
322 the 95 % credible set of shift configurations ($f = 0.15$) shows two 'core shifts' to higher
323 diversification rates (red and orange circles and branches) and one 'core shift' to slower
324 diversification rate (light blue circle and branches; Fig. 3). The first shift to diversification rate
325 acceleration (red clade) includes species of *R.* subgen. *Hymenanthus* from the SE Asian clade.
326 The second clade with higher diversification rate (orange clade) is within *R.* subgen.
327 *Rhododendron*. This clade contains species from section *Rhododendron* in part (excluding species
328 from *R.* subsect. *Baileya*, *Boothia*, *Edgeworthia*, *Lapponica*, *Maddenia*, and *Tephropepla*) and the
329 two species included from *R.* section *Pogonanthum*. This diversification shift with rate acceleration
330 is also found in the diversification analysis with only 105 taxa matching those included in the trait
331 analysis ($f = 0.35$; Supplementary Material, Fig. S7A). The second shift within *R.* subgen.
332 *Rhododendron* shows a diversification rate slowdown (light blue clade). The mean speciation rate
333 in *Rhododendron* as calculated by BAMM was 0.429 speciation events per million years (Myr) and
334 has increased over time (Fig. 3, inset).

335 However, the diversification analysis of the *rpb2-i* species tree ($f = 0.14$) shows one 'core shift'
336 to a higher diversification rate in *R.* subgen. *Hymenanthus* for the SE Asian clade (Fig. S8A) and a
337 second 'core shift' with diversification rate slowdown for *R.* subgen. *Rhododendron* (excluding
338 subsection *Ledum* (L.) K.A.Kron & W.S.Judd). Both shifts are not seen in the diversification
339 analysis with only 56 taxa matching those for the trait analysis, despite a generally similar pattern
340 (Fig. S8B). The diversification analysis of the ITS species tree shows only one 'core shift' from *R.*
341 *camtschaticum* ssp. *camtschaticum* Pall. to a higher diversification rate for all remaining species of
342 *Rhododendron* (Fig. S9A). In the diversification analysis with only 90 taxa a 'core shift' to a slower
343 diversification rate for deciduous species (*R.* subgen. *Azaleastrum* section *Azaleastrum*, *R.*
344 subgen. *Mumeazalea*, *R.* subgen. *Pentanthera* section *Sciadorhodion*, and *R.* subgen. *Tsutsusi*
345 (Fig. S9B) has been revealed.

346 **Genome size evolution**

347 The genome sizes for 125 *Rhododendron* species are listed in Table S2. The 1C-values range
348 from 0.677 pg to 2.182 pg for *R. subgen. Hymenanthes*, from 0.543 pg to 1.914 pg for *R. subgen.*
349 *Pentanthera*, from 0.483 pg to 2.777 pg for *R. subgen. Rhododendron* and from 0.571 pg to 0.776
350 pg for *R. subgen. Tsutsusi*. For *R. subgen. Azaleastrum* the genomes size is 0.583 pg and 1.406
351 pg, for *R. subgen. Mumeazalea* 0.540 pg and for *R. subgen. Therorhodon* it is 0.583 pg. Most
352 (87%) species of *R. subgenera Hymenanthes, Pentanthera, and Tsutsusi* are diploid. In contrast,
353 polyploid species (tetra-, hexa- and octoploids) constitute roughly half (53%) of all investigated
354 species of *R. subgen. Rhododendron* sections *Rhododendron* and *Schistanthe*.

355 Among the three continuous traits (2C-value, 1Cx-value, and ploidy level), only the 1Cx-value
356 had a significant phylogenetic signal (Blomberg's $K = 0.248$, $P < 0.005$; Pagel's $\lambda = 0.929$, $P <$
357 0.005). Similarly, the ancestral character state reconstruction analysis using continuous color
358 gradients with 2C-values (Fig. 4A) indicates that the ancestors of *Rhododendron* had small
359 genomes but there have been several increases (yellow to green/blue) of 2C-value along the tree
360 (Fig. 4A). The species with larger genome sizes (green to blue) are mainly species of *R. subgen.*
361 *Rhododendron* sections *Rhododendron* (in part) plus section *Pogonanthum* and section
362 *Schistanthe*. In these two groups almost all polyploid species (87%) are included. The ancestral
363 character state estimation of 1Cx-values indicates genome upsizing for species of *R. subgen.*
364 *Pentanthera* section *Pentanthera*, *R. subgen. Hymenanthes*, and *R. subgen. Rhododendron*
365 section *Rhododendron* subsect. *Baileya*, *Boothia*, *Edgeworthia*, *Maddenia*, and *Tephropepla*
366 (green to blue). In contrast, species of *R. subgen. Rhododendron* section *Schistanthe* show a
367 smaller monoploid genome size (red to yellow) which indicates genome downsizing for this group.

368 In the BAMM trait analysis of monoploid genome size (1Cx-value) the mean rate of monoploid
369 genome size evolution in *Rhododendron* is 0.0005 and has not varied much over time (Fig. S10B,
370 inset). Based on the *trnK* BEAST phylogeny three 'core shifts' are indicated in the trait analysis
371 (Fig. S7B). Two of them are shifts to increased rates, i.e. in *R. subgen. Tsutsusi* section *Tsutsusi*
372 and *R. subgen. Rhododendron* section *Malayovireya*, whereas a third shift to a decreased rate is
373 shown for *R. subgen. Rhododendron* sections *Rhododendron* and *Pogonanthum*. Species of
374 section *Rhododendron* subsect. *Baileya*, *Boothia*, *Edgeworthia*, *Maddenia*, and *Tephropepla* are
375 excluded from this decrease.

376 The trait analysis based on the phylogeny of *rpb2-i* shows only one shift to an increased rate
377 for *R. subgen. Tsutsusi* section *Tsutsusi* (Supplementary Material, Fig. S8C) and based on ITS
378 only one 'core shift' to an increased rate for species of *R. subgen. Rhododendron* sections
379 *Rhododendron* and *Pogonanthum* (except species of subsections *Micrantha* and *Rhododendron*;
380 Fig. S9C) are indicated.

381 DISCUSSION

382 Our results agree in a well-supported monophyly of *Rhododendron* (Figs. 1-2), which is in line with
 383 previous studies e.g., Shrestha et al., 2018; and Schwery et al., 2015), and when species of
 384 *Ledum* and *Menziesia* are included (Craven (2011), Goetsch et al. (2005), Kron and Judd (1990)
 385 and Kurashige et al. (2001). *Rhododendron camtschaticum*, representing *R.* subgen. *Therorhodon*
 386 is sister to the rest of the genus in all analyses. This agrees with other analyses and allows
 387 recognition of *R. camtschaticum* and *R. redowskianum* as separate genus (e.g., Judd and Kron,
 388 2009). However, we continue recognizing them within *Rhododendron*.

389 In contrast, we found a marked difference concerning the internal relationships beyond the
 390 sectional level. Overall, the results revealed the larger a subgenus, the stronger is the support for
 391 its monophyly. The smaller subgenera pose several problems, for example *R.* subgen.
 392 *Azaleastrum* is inferred to be polyphyletic, as previously indicated (Goetsch et al., 2005; Kurashige
 393 et al., 2001; Schwery et al., 2015; and Yan et al., 2015). However, both sections are monophyletic
 394 in all analyses (inclusion of *R. charitopes* in the ITS analysis is dubious). However, several
 395 incongruent placements of species among analyses complicate a new classification of the genus.
 396 Shrestha et al. (2018) provided the most comprehensive phylogenetic analysis to date with
 397 multiple plastid and nuclear markers, but did not reflect incongruent placements among datasets
 398 (Table 2).

399 Phylogeny of *Rhododendron*

400 Goetsch et al. (2005) grouped the species in five subgenera, *R.* subgen. *Therorhodon* with two
 401 species and *R.* subgen. *Rhododendron*, *Hymenantes*, *Azaleastrum* and *Choniastrum* (Table 2).
 402 The largest of these subgenera is *R.* subgen. *Rhododendron* with more than 500 species (Table
 403 S1). There have been debates on whether to recognize the vireyas as separate subgenus or
 404 section (Craven et al., 2008; Argent and Twyford, 2012). Recognition of a separate subgenus in
 405 the traditional sense would lead to recognition of a diphyletic clade since *R.* subsection
 406 *Discovireya* is separate from the rest in most analyses as found earlier by Goetsch et al. (2011).
 407 The phylogeny presented by Shrestha et al. (2018) would allow recognition of three subclades,
 408 one containing most of the members of *R.* sect. *Schistanthe*. However, these clades are
 409 inconsistent among studies and markers and we refrain from suggesting a new sectional
 410 classification. The only question regarding the circumscription of this subgenus is the inclusion of
 411 the former genus *Ledum*, which appears as sister to *R.* subgen. *Rhododendron* in all nuclear DNA-
 412 based analyses (Fig. 2 and S1; Gao et al., 2002; and Goetsch et al., 2005) but as sister to *R.*
 413 *albrechtii* (Fig. 1 and S5; Kurashige et al., 2001) or sister to *R.* subgen. *Hymenantes* and *R.* sect.
 414 *Pentanthera* (Schwery et al., 2015) and distant to *R.* subgen. *Rhododendron* in the plastid DNA-
 415 based analyses. Shrestha et al. (2018) has some species as sister to *R.* subgen. *Rhododendron*
 416 and some as sister to *R. albrechtii* in his combined analysis of plastid and nuclear DNA, which
 417 suggests that missing data in their dataset causes different accessions of species from this group

418 to end up in different positions. In Grimbs et al. (2017) the plastid DNA signal apparently overruled
419 the nuclear signal.

420 The second largest group in *Rhododendron* is *R.* subgen. *Hymenanthes*. Whereas there is
421 mostly strong support for the morphologically well-circumscribed subgenus in the traditional sense
422 (except in *rpb2*; Fig. S5-6; Goetsch et al., 2005), Shrestha et al. (2018) enlarged the subgenus to
423 include *R.* sect. *Pentanthera*. This relationship is strongly supported by *rpb2* (Fig. S5-6; Goetsch et
424 al., 2005) and shown without support by *trnK* (Fig. 1; Kurashige et al., 2001; Schwery et al., 2015).
425 The relationship is not shown but also not strongly refuted by analyses of ITS (Fig. 2 and S4; Gao
426 et al., 2002). The two groups differ in leaves being either deciduous or evergreen, a character
427 traditionally in taxonomy and horticulture important in the distinction between rhododendrons and
428 azaleas. We, therefore, prefer to keep *R.* sect. *Pentanthera* separate from *R.* subg. *Hymenanthes*.

429 The second-smallest subgenus in the classification of Goetsch et al. (2005) is *R.* subg.
430 *Choniastrum* with 15 species. The signal in Shrestha et al. (2018) depicting it as sister to *R.*
431 subgen. *Rhododendron* is largely derived from the *rpb2*-dataset (Fig. S5-6; Goetsch et al. 2005).
432 However, ITS puts the group in a position as sister to *R.* subg. *Azaleastrum* sensu Goetsch et al.
433 (2005; Fig. 2) or in unresolved position in one clade with this subgenus (Gao et al., 2002).
434 Schwery et al. (2015) resolves the section including former genus *Diplarche* based on *matK* and
435 *rbcL* as sister to this clade, as well. Other studies based on plastid DNA markers even group the
436 section among the members of *R.* subgen. *Azaleastrum* sensu Goetsch et al. (2005). We,
437 therefore, consider it premature to recognize this clade at the subgeneric level.

438 The morphologically most heterogeneous subgenus of Goetsch et al. (2005) is *R.* subgen.
439 *Azaleastrum*, which includes *R.* sect. *Azaleastrum*, three sections formerly assigned to *R.* subgen.
440 *Pentanthera* (*R.* sect. *Rhodora*, *R.* sect. *Sciadorhodion* (incl. *Menziesia*), *R.* sect. *Viscidula*), the
441 monotypic *R.* subgen. *Mumeazalea* (*R.* *semibarbatum*) and *R.* subgen. *Candidastrum* (*R.*
442 *albiflorum*) and the large (66 species) *R.* subgen. *Tsutsusi*. The signal for the monophyly of this
443 clade is derived mostly from *rpb2* (Fig. S5-6; Goetsch et al., 2005) with ITS supporting it with
444 inclusion of *R.* sect. *Choniastrum* (Fig. 2 and S4; Gao et al., 2002). Plastid DNA does not support
445 the group as monophyletic. The core of the group is constituted by *R.* subgen. *Azaleastrum* and *R.*
446 subgen. *Tsutsusi*. The monotypic *R.* subgen. *Candidastrum* (*R.* *albiflorum*) clusters with these two
447 based on *trnK* (Fig. 1 and S1; Kurashige et al., 2001), is unresolved together with these based on
448 ITS (Gao et al., 2002) and strongly supported sister to *R.* *albrechtii* based on *rpb2* (Goetsch et al.,
449 2005) and in Shrestha et al. (2018). The case is the other way around for the monotypic *R.*
450 subgen. *Mumeazalea* (*R.* *semibarbatum*), which is sister to *R.* subgen. *Tsutsusi* in Shrestha et al.
451 (2018) or *R.* subgen. *Azaleastrum* with ITS (Fig. 2 and S4; Gao et al., 2002) or in one clade with
452 these two and *R.* sect. *Viscidula* in *rpb2* (Fig. S2-3; Goetsch et al., 2005) but distantly to those and
453 strongly supported sister to *R.* sect. *Choniastrum* in the plastid DNA-based phylogenies (Fig. 1 and

454 S2-3; Kurashige et al., 2001). A third species (group) related to this core group is mono- or ditypic
 455 *R. sect. Viscidula* (*R. nipponicum*), which clusters with this core in *rpb2* (Fig. S5-6; Goetsch et al.,
 456 2005) and in Shrestha et al. (2018) but not using *trnK* (Fig. 1; Kurashige et al., 2001) with weak
 457 support. A fourth species changing positions in different analyses is *R. vaseyi*, the second species
 458 of *R. sect. Rhodora* apart from *R. canadense*, which clusters with *R. sect. Pentanthera*.
 459 *Rhododendron vaseyi* is related to *R. schlippenbachii* of *R. sect. Sciadorhodion* and the species of
 460 former genus *Menziesia* with medium to strong support based on *rpb2* (Fig. S5-6; Goetsch et al.,
 461 2005) and is found there in Shrestha et al. (2018), too. However, ITS and the plastid DNA markers
 462 retrieve it in different positions (Fig. 1-2 and S1-6; Kurashige et al., 2001; Grimbs et al., 2017). The
 463 former genus *Menziesia* has been found to be nested in *Rhododendron* since the studies of Kron
 464 (1997) and Kurashige et al. (2001). Goetsch et al. (2005) found strong support for a clade
 465 consisting of *Menziesia* with *R. schlippenbachii* and *R. vaseyi* and a relationship with either holds
 466 in all analyses but not always with both but almost always with *R. schlippenbachii*. In turn, *R.*
 467 *schlippenbachii* (and *Menziesia*) have different positions, but always close to the Azaleastrum-
 468 Tsutsusi-group. It is, however, noteworthy that other members of *R. sect. Sciadorhodion* rarely
 469 form a monophyletic group with these. For example, *R. albrechtii*, which takes only a distant
 470 relationship with *R. schlippenbachii* in the plastid DNA-based analyses (Fig. 1 and S1-3; Kurashige
 471 et al., 2001; Grimbs et al., 2017).

472 Taken together, we consider it premature to group all species of *Rhododendron* in five
 473 subgenera. We see the merit in the recognition of the five major clades at the subgeneric level but
 474 given the amount of incongruence a large number of species cannot be confidently assigned to
 475 one of these five clades. Further, genome-wide data will be necessary to assess whether these
 476 currently unassignable taxa are independent taxa, assignable to one of the five major clades or
 477 whether they are inter-subgeneric hybrids.

478 Diversification regime shifts

479 Based on this incongruence, we considered it necessary to conduct diversification analyses
 480 separate for each DNA marker, although the *trnLF*-region did not have enough variation to allow a
 481 reliable analysis. Diversification analyses demonstrated nearly the same speciation shifts for the
 482 plastid *trnK* and nuclear *rpb2-i* regions (Fig. 3 and Fig. S8). The main shift to higher diversification
 483 was found for species of *R. subgen. Hymenanthus* from the Himalayan/SE Asian clade, although
 484 the exact species included is not consistent. Most species of subsection *Pontica* with a distribution
 485 outside SE Asia (e.g., SW Eurasia, NE Asia, and N America) show no shift in diversification rate.
 486 This pattern is consistent with the findings of other analyses. For example, Milne et al. (2010)
 487 hypothesized, based on divergence time estimations, a slow diversification outside SE Asia
 488 followed by more rapid diversification of one lineage within SE Asia, followed by immigration of at
 489 least one additional lineage to the region. Additional support for a nested Himalayan radiation is

490 given by Schwery et al. (2015) using a BAMM analysis similar to ours but with much smaller taxon
 491 sampling. The Himalayan-Southwest China region is known as a species-rich area of
 492 *Rhododendron* since Joseph D. Hooker famous travels to India and the Himalayas (1847 – 1851)
 493 but the region is also well known for other highly diverse groups of plants (Qiu et al., 2011). Most
 494 intriguing is the parallel increase in diversification in shrubby *Viburnum* of the region, which has
 495 been dated to the Eocene (Spriggs et al., 2015), similar to *Rhododendron*. Suggested reasons for
 496 the high diversity that may apply to *Rhododendron* are the climatic and physiographic
 497 heterogeneity, a complex geological history and the absence of major Quaternary glaciations (Qiu
 498 et al., 2011) coupled with an increase in precipitation (Wang et al., 2012). These factors may have
 499 spurred a diversification by causing barriers to plant migration (Zhao et al., 2013) and providing
 500 opportunities for frequent niche shifts between temperate and tropical biomes with limited vertical
 501 migration (Spriggs et al., 2015).

502 Similar to *R.* subgen. *Hymenanthus*, a significant rate increase was found in most
 503 diversification analyses in *R.* subgen. *Rhododendron* (Figs. 3 and Fig. S7) and comprises most
 504 species of sections *Pogonanthum* and *Rhododendron*. In contrast, a clade within section
 505 *Rhododendron* containing species of section *Rhododendron* subsections *Baileya*, *Boothia*,
 506 *Edgeworthia*, *Maddenia*, and *Tephropepla* shows no change in diversification rate. All members of
 507 this clade without rate shift occur in the Himalayan Mountains above 1500 meters with many at
 508 least facultative epiphytic species and in an ecological diverse array of habitats such as conifer
 509 forests, grassy hillsides, among rocks, steep slopes, or cliffs. In contrast to our expectations, no
 510 shift in diversification rate was found within the species-rich group of tropical *Rhododendron*
 511 (vireyas, section *Schistanthe*) for subsection *Euvireya* (Fig. 3; Figs. S7-8) which includes almost all
 512 species endemic to the Philippines, Borneo, New Guinea, Sulawesi, and the Solomon Islands.
 513 Nevertheless, here we only included 22% species of *Rhododendron* out of their total 587 species
 514 (Table S1). *Euvireya* species exhibit considerable variation in their ecology, being epiphytic or
 515 terrestrial and occurring from sea level to over 4000 m. Within *Euvireya*, the molecular-
 516 phylogenetic clustering follows geography more closely than traditional taxonomy based upon
 517 morphology, which is similar to the results from (Goetsch et al., 2011). The geographical pattern of
 518 the group was intensively discussed by Brown et al. (2006b). It is considered a classic example of
 519 Malayan radiation (Brown et al., 2006b; and Goetsch et al., 2011). An adaptive radiation can be
 520 accompanied by a diversification rate slowdown, which may sound counter-intuitive at first.
 521 However, since speciation rates usually decrease after an initial rapid diversification either due to
 522 increased competition for resources or niche filling, diversity- or time-dependent factors must be
 523 considered as potential drivers for observed decreases in the rates of diversification (Soulebeau et
 524 al., 2015). Our results are in contradiction with Shrestha et al. (2018), who suggested that tropical
 525 and subtropical mountains are not only the biodiversity and endemism hotspots for the genus
 526 *Rhododendron*, but also function as cradles of *Rhododendron* diversification.

While support for a diversification increase in *R.* subgen. *Hymenanthus* is more or less unambiguous, we have found here a discordance of results from different DNA regions but cannot really distinguish between diversification rate shifts depending on species sampling, evolutionary rate or topology (Fig. 3; and Figs. S7 and S9). Based on the similarity of our analysis using *trnK* with 260 taxa and that of Schwery et al. (2015) with 60 taxa but also using *trnK* (and *rbcL*), topology seems to be the most important. Therefore, more complete taxon sampling and, especially, resolving incongruences between markers in the future seems to be required for more conclusive diversification analyses.

Genome size evolution

Our analysis is the first to analyze genome sizes in a larger number of species of *Rhododendron* (Table S2). Previous genome size estimations had been generated using Feulgen densitometry (Ammal, 1950; Ammal et al., 1950) or flow cytometry using DAPI staining (Jones et al., 2007). Ammal et al. (1950) completed an extensive survey of chromosome numbers and ploidy levels in *Rhododendron* and found the elepidote rhododendrons (*R.* subgen. *Hymenanthus*), evergreen azaleas (*R.* subgen. *Tsutsusi*), and the deciduous azaleas (*R.* subgen. *Pentanthera*) to be predominantly diploid. However, they also demonstrated the occurrence of triploids, hexaploids, octoploids, and dodecaploids ($2n = 12x = 156$) within *R.* subgen. *Rhododendron* and natural tetraploids in other subgenera as well, e.g. in *R.* subgen. *Pentanthera* (*R. canadense* and *R. calendulaceum*; Ammal, 1950; Ammal et al., 1950; and Jones et al., 2007). Our results confirm and expand the pattern that polyploidy occurs in almost all subgenera but most polyploid species are within *R.* subgen. *Rhododendron* sections *Rhododendron* and *Schistanthe* (Fig. 4A). This is rather surprising given the high frequency of polyploids among garden cultivars belonging to *R.* subgen. *Hymenanthus* (Perkins et al., 2012). Within *R.* section *Schistanthe*, the polyploid species are restricted to *R.* subsections *Euvireya* and *Malayovireya*, which occur in Indonesia and New Guinea and most of them are endemic to these islands while the species of the Asian mainland are diploid based on our data. There seems to be no correlation of genome size with habit (epiphytic, terrestrial or both) in this group of vireyas but the monoploid genome sizes are smaller ($1Cx$ -values = 0.483 up to 0.618 pg; light green/yellow to red; Fig. 4B). This genome downsizing has been inferred to be accompanied by a slow-down in the evolution of genome size. Thus, the group seems to have stabilized on a lower level of genome size. Such a pattern has also been shown in the New Zealand radiation of *Veronica*, in which genome downsizing and a slow-down of rate in genome size evolution are associated with a radiation on the polyploid level (Meudt et al., 2015). While previous studies have shown that genome sizes of tropical angiosperms in general are lower than those of colder, temperate regions (Levin and Funderburg, 1979; Ohri, 2005), our study seems to be the first to indicate such a shift to small genome-tropical clade within a specific genus. It remains to be studied whether genome downsizing in this clade is functionally related with the higher frequency of polyploidy and/or its species richness.

Within section *Rhododendron*, almost all polyploid species form a monophyletic clade and seem to be restricted to subsections *Heliolepidia* and *Triflora*. In contrast to the former group, here polyploidy is not associated with genome downsizing. Diploid species from the latter subsection do not cluster with the monophyletic clade of polyploids but with other diploid species from different subsections based on plastid DNA markers (Fig. 4A; *trnK* gene region). In the same analysis using ITS (Fig. S10) diploids are nested among polyploids suggesting an origin of at least four polyploid clades involving the same unknown diploid species. Hybridization and polyploidization, often referred to as whole genome duplication, are both potential speciation mechanism. Genome doubling creates instantaneously lineages reproductively isolated from its diploid progenitors and must overcome competition with their parents for abiotic resources (Soltis et al., 2010). Diversification analyses have demonstrated that polyploids have higher extinction rate and lower diversification rate than diploid lineages (Mayrose et al., 2011) but nevertheless have given rise to major radiations in angiosperms (Tank et al., 2015). In line with this pattern, we did not detect a change in diversification in polyploids. Thus, polyploidy did not increase speciation rate in *Rhododendron* but may however be associated with evolutionary novelties. Our hypothesis is that in this *Heliolepidia*/*Triflora*-group species have a higher chance to produce unreduced gametes, thus the repeated origins of polyploids from similar ancestors, but the lack of genome downsizing prevents diversification on the polyploid level.

Hybridization facilitates the transfer of traits between species, which has been shown to promote adaptive evolutionary change in *Rhododendron* (Milne and Abbott, 2000) and other species. Such introgressed traits may affect various stages of life history including resistance to herbivores and pathogens (Whitney et al., 2015). Recent reviews suggest that resistance is an important component of hybrid survival (Orians, 2000) and that hybridization and polyploidy may be important evolutionary mechanisms for generating novel secondary chemicals important in the diversification of plant-animal interactions (Soltis et al., 2014). Oswald and Nuismer (2007) explored the possibility that new polyploids are initially more resistant to pathogens than their diploid progenitors by using mathematical models and confirmed that polyploids are significantly more resistant. *Rhododendron* may prove to be a living example for the evolution of novel chemicals in polyploids since four of the ten species shown to exhibit the highest antibacterial effects against Gram-positive bacteria by Rezk et al. (2015) are shown here to be polyploid (*R. ambiguum*, *R. cinnabarinum*, *R. concinnum* and *R. rubiginosum*). It remains to be shown that the high antimicrobial activity is due to the origin of novel gene combinations in polyploids.

CONCLUSIONS

Given the large numbers of species, including many rare species with restricted range sizes, there are inherent difficulties in the analyses of phylogenetic relationships, diversification, and trait evolution. Here, we included only 25-30% of the total species from the genus in the study and analyzed all data sets separately. However, by using state of the art analytical tools, relying on

strong support in terms of bootstraps and posterior probabilities, and discussing thoroughly and critically the results, we provide the most up-to-date knowledge of *Rhododendron* phylogeny, diversification and genome size evolution. We compared our results with the DNA-based classification by Goetsch et al. (2005) and warn of adopting this classification uncritically (Table 2). Our results agree in a well-supported monophyly of *Rhododendron*, with *R.* subgen. *Therorhodon* sister to all other taxa of *Rhododendron*. The results recovered marked incongruences between markers in retrieval of internal relationships but also finding many results common across DNA markers and morphology-based classifications. Our results demonstrate that the definition of enlarged *R.* subgen. *Azaleastrum* and *R.* section *Sciadorhodon* by Goetsch et al. (2005) including *R.* subgen. *Candidastrum*, *R.* subgen. *Mumeazalea*, *R. vaseyi* and other species of *R.* sect. *Sciadorhodon* may be premature. Similarly, our results do not consistently support the placement of *R.* subsect. *Ledum* in *R.* subgen. *Rhododendron* and the inclusion of *R.* sect. *Pentanthera* in *R.* subgen. *Hymenanthes*. The diversification analysis revealed that a major rate shift in *Rhododendron* occurred in the Himalayan Mountains above 1500 meters with many at least facultative epiphytic species and in an ecologically diverse array of habitats such as conifer forests, grassy hillsides, among rocks, steep slopes, or cliffs. These results are in contradiction to Shrestha et al. (2018), who suggested tropical and subtropical mountains to be not just the biodiversity and endemism hotspot for the genus but also a cradle of its diversification. However, the topology of the phylogeny is indicated to influence largely the results. Therefore, more complete taxon sampling, especially, resolving incongruences between markers in the future seems to be required for more conclusive diversification analyses. Lastly, we confirm and expand that polyploidy occurs in almost all subgenera but most polyploid species are within *R.* subgen. *Rhododendron* sections *Rhododendron* and *Schistanthe*. The two groups differ, however, in their pattern with the polyploids in *R.* sect. *Rhododendron* originating frequently but not diversifying on the polyploid level and not exhibiting genome downsizing. In contrast, *R.* sect. *Schistanthe* seems to be another example for a polyploid radiating after genome downsizing. It further can serve as an example for the frequently cited reduction in genome size of tropical plants versus their temperate relatives.

ACKNOWLEDGEMENTS AND FUNDING INFORMATION

This study was financially supported by the Stiftung Bremer Rhododendronpark, Germany. The authors would like to thank Silvia Kempen and Eike Mayland-Quellhorst for assistance in the lab, Nikolai Kuhnert, Klaudia Brix and Matthias Ullrich for support of this project.

DATA ACCESSIBILITY

Postprocess data will be available on Dryad.

636 AUTHOR CONTRIBUTIONS

637 Conceived the idea: DCA, JN, HS and KG; Performed experiments: JN; Contributed
638 reagents/materials/analysis tools: DCA, and HS; Analyzed data and authored the drafts of the
639 paper: KG, DCA. All authors contributed, made multiple revisions, and approved the final draft.

640 LITERATURE CITED

- 641 Abbott, R., D. Albach, S. Ansell, J. W. Arntzen, S. J. E. Baird, N. Bierne, J. Boughman, A. Brelsford, C. A. Buerkle, R.
642 Buggs, R. K. Butlin, U. Dieckmann, F. Eroukmanoff, A. Grill, S. H. Cahan, J. S. Hermansen, G. Hewitt, A. G.
643 Hudson, C. Jiggins, J. Jones, B. Keller, T. Marczewski, J. Mallet, P. Martinez-Rodriguez, M. Möst, S. Mullen, R.
644 Nichols, A. W. Nolte, C. Parisod, K. Pfennig, A. M. Rice, M. G. Ritchie, B. Seifert, C. M. Smadja, R. Stelkens, J. M.
645 Szymura, R. Väinölä, J. B. W. Wolf, D. Zinner. 2013. Hybridization and speciation. *Journal of Evolutionary Biology*
646 26: 229–246.
- 647 Ammal, E. 1950. Polyploidy in the genus *Rhododendron*. *Rhododendron Year B* 5: 92–98.
- 648 Ammal, E., I. Enoch, and M. Bridgwater. 1950. Chromosome numbers in species of *Rhododendron*. *Rhododendron Year*
649 *B* 5: 78–91.
- 650 Argent, G. C. G., and A. D. Twyford. 2012. In defense of *Rhododendron* subgenus *Vireya*. *Journal of the American*
651 *Rhododendron Society* 142–143.
- 652 Argent, G. C. G., and D. Mitchell. 2006. *Rhododendrons of subgenus Vireya*. *Royal Horticultural Society London*.
- 653 Axelrod, D. 1998. The Oligocene Haynes creek flora of eastern Idaho.
- 654 Blomberg, S. P., T. Garland, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: Behavioral traits
655 are more labile. *Evolution* 57: 717–745.
- 656 Blume, C. L. 1826. *Bijdragen tot de flora van Nederlandsch Indië*. Batavia: Ter Lands drukkerij.
- 657 Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C. H. Wu, D. Xie, M. A. Suchard, A. Rambaut, A. J. Drummond. 2014.
658 BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 10: e1003537.
- 659 Brown, G. K., G. Nelson, and P. Y. Ladiges. 2006b. Historical biogeography of *Rhododendron* section *Vireya* and the
660 Malesian Archipelago. *Journal of Biogeography* 33: 1929–1944.
- 661 Brown, G. K., L. Craven, F. Udovicic, and P. Landiges. 2006. Phylogeny of *Rhododendron* section *Vireya* (Ericaceae)
662 based on two non-coding regions of cpDNA. *Plant Systematics and Evolution* 257: 57–93.
- 663 Chamberlain, D. F. 1982. A revision of *Rhododendron*: II. Subgenus *Hymenanthes*. *Notes from Royal Botanical Garden*
664 *Edinburgh* 39: 209–486.
- 665 Chamberlain, D. F., R. Hyam, G. Argent, G. Fairweather, and K. S. Walter. 1996. The genus *Rhododendron*: its
666 classification and synonymy. *Royal Botanical Garden, Edinburgh*.
- 667 Chase, M. W. and H. H. Hills. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies.
668 *Taxon* 40: 215–220.
- 669 Cox, P., and K. Cox. 1997. *Encyclopedia of rhododendron species*. Glendoick Publishing, Perth.
- 670 Craven, L. A. 2011. *Diplarche* and *Menziesia* transferred to *Rhododendron* (Ericaceae). *Blumea* 56: 33–35.
- 671 Craven, L. A., L. A. Goetsch, B. D. Hall, and G. K. Brown. 2008. Classification of the *Vireya* group of *Rhododendron*
672 (Ericaceae). *Blumea - Biodiversity, Evolution and Biogeography of Plants* 53:435–442.
- 673 Cullen, J. 1980. Revision of *Rhododendron*. 1. Subgenus *Rhododendron* sections *Rhododendron* and *Pogonanthum*.
674 *Notes from Royal Botanical Garden Edinburgh* 39: 1–207.
- 675 Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST
676 1.7. *Molecular Biology and Evolution* 29: 1969–1973.
- 677 Drummond, A. J., S. Y. W. Ho, M. J. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence.
678 *PLoS Biology* 4: e88.
- 679 Fang, R., and T. Ming. 1995. The floristic study on the genus *Rhododendron*. *Acta Botanica Yunnanica* 17: 359–379.
- 680 Frodin, D. 2004. History and Concepts of Big Plant Genera. *Taxon* 53(3): 753–776.
- 681 Galbraith, D., K. Harkins, J. Maddox, N. Ayres, D. Sharma, and E. Firoozabady. 1983. Rapid flow cytometric analysis of
682 the cell cycle in intact plant tissues. *Science* 220: 1049–1051.
- 683 Gao, L. M., D. Z. Li, C. Q. Zhang, and J. B. Yang. 2002. Infrageneric and sectional relationships in the genus
684 *Rhododendron* (Ericaceae) inferred from ITS sequence data. *Acta Botanica Sinica* 44: 1351–1356.
- 685 Gibbs D, Chamberlain D, Argent G. 2011. The Red List of *Rhododendrons*. *Richmond, UK: Botanic Gardens*
686 *Conservation International*.
- 687 Goetsch, L. A., A. J. Eckert, and B. D. Hall. 2005. The molecular systematics of *Rhododendron* (Ericaceae): a phylogeny
688 based upon RPB2 gene sequences. *Systematic Botany* 30: 616–626.
- 689 Goetsch, L. A., L. A. Craven, and B. D. Hall. 2011. Major speciation accompanied the dispersal of *Vireya*
690 *Rhododendrons* (Ericaceae, *Rhododendron* sect. *Schistanthe*) through the Malayan archipelago: evidence from
691 nuclear gene sequences. *Taxon* 60: 1015–1028.
- 692 Grimbs, A., A. Shrestha, A. Rezk, S. Grimbs, S. I. Hakeem, H. Schepker, M. T. Hütt, D. C. Albach, K. Brix, N. Kuhnert,
693 and M. S. Ullrich. 2017. Bioactivity in *Rhododendron*: A Systemic Analysis of Antimicrobial and Cytotoxic Activities
694 and Their Phylogenetic and Phytochemical Origins. *Frontiers in plant science* 8: 551.
- 695 Hooker, J. D. 1849. The *Rhododendrons* of Sikkim-Himalaya. Dehra Dun: Bishen Singh Mahendra Pal Singh.
- 696 Igea, J., and A. J. Tanentzap. 2020. Angiosperm speciation cools down in the tropics. *Ecology Letters* 23: 692–700.

Innocenti, G., S. Dall'Acqua, G. Scialino, E. Banfi, S. Sosa, K. Gurung, M. Barbera, M. Carrara. 2010. Chemical composition and biological properties of *Rhododendron anthopogon* essential oil. *Molecules* 15: 2326–2338.

Jones, J. R., T. G. Ranney, N. P. Lynch, and S. L. Krebs. 2007. Ploidy levels and relative genome sizes of diverse species, hybrids, and cultivars of *Rhododendron*. *Journal of American Rhododendron Society* 61: 220–227.

Judd, W., and K. Kron. 1995. A revision of *Rhododendron* VI. subgenus *Pentanthera* (sections *Sciadorhodion*, *Rhodora* and *Viscidula*). *Edinburgh Journal of Botany* 52: 1–54.

Judd, W. S. & Kron, K. A. 2009. *Rhododendron* in: Flora of North America North of Mexico Vol. 8 (ed Flora of North American Editorial Committee), Pp. 455–473, Oxford University Press.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12): 1647–1649.

Kron, K. A. 1997. Phylogenetic relationships of Rhododendroideae (Ericaceae). *American Journal of Botany* 84: 973–980.

Kron, K. A., and W. S. Judd. 1990. Phylogenetic relationships within the Rhodoreae (Ericaceae) with specific comments on the placement of *Ledum*. *Systematics Botany* 15: 57–68.

Kron, K. A., L. M. Gawen, and M. W. Chase. 1993. Evidence for introgression in Azaleas (*Rhododendron*; Ericaceae): Chloroplast DNA and morphological variation in a hybrid swarm on Stone Mountain, Georgia. *American Journal of Botany* 80: 1095–1099.

Kurashige, Y., J. I. Etoh, T. Handa, K. Takayanagi, and T. Yukawa. 2001. Sectional relationships in the genus *Rhododendron* (Ericaceae): evidence from matK and trnK intron sequences. *Plant Systematics and Evolution* 228: 1–14.

Leslie, A. 2004. The International Rhododendron Register and Checklist, 2nd ed. *Royal Horticultural Society, London*.

Levin, A. D., and S. W. Funderburg. 1979. Genome Size in Angiosperms: Temperate Versus Tropical Species. *The American naturalist* 114: 784–795.

Linnaeus, C. 1753. Species Plantarum, 1st ed. *Salvius, L, Stockholm*.

Linnaeus, C. 1762. Vol. 1. Species Plantarum. ed. 2. *Stockholm*: i-xvi, 1-784.

Liu, Y. J., S. Whelen, and B. D. Hall. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 17: 1799–1808.

Marshall, C. R. 2017. Five palaeobiological laws needed to understand the evolution of the living biota. *Nature Ecology and Evolution* 1: 0165.

Mayrose, I., S. H. Zhan, C. J. Rothfels, K. Magnuson-Ford, M. S. Barker, L. H. Rieseberg, S. P. Otto. 2011. Recently Formed Polyploid Plants Diversify at Lower Rates. *Science* 333: 1257–1257.

Meudt, H. M., B. M. Rojas-Andrés, J. M. Prebble, E. Low, P. J. Garnock-Jones, and D. C. Albach. 2015. Is genome downsizing associated with diversification in polyploid lineages of *Veronica*? *Botanical Journal of the Linnean Society* 178: 243–266.

Milne, R. I., and R. J. Abbott. 2000. Origin and evolution of invasive naturalized material of *Rhododendron ponticum* L. in the British Isles. *Molecular Ecology* 9: 541–556.

Milne, R. I., and R. J. Abbott. 2008. Reproductive isolation among two interfertile *Rhododendron* species: low frequency of post-F1 hybrid genotypes in alpine hybrid zones. *Molecular Ecology* 17: 1108–1121.

Milne, R. I., C. Davies, R. Prickett, L. H. Inns, and D. F. Chamberlain. 2010. Phylogeny of *Rhododendron* subgenus *Hymenanthes* based on chloroplast DNA markers: between-lineage hybridisation during adaptive radiation? *Plant Systematics and Evolution* 285: 233–244.

Milne, R. I., R. J. Abbott, K. Wolff, and D. F. Chamberlain. 1999. Hybridization among sympatric species of *Rhododendron* (Ericaceae) in Turkey: morphological and molecular evidence. *American Journal of Botany* 86: 1776–1785.

Milne, R. I., S. Terzioglu, and R. J. Abbott. 2003. A hybrid zone dominated by fertile F1s: maintenance of species barriers in *Rhododendron*. *Molecular Ecology* 12: 2719–2729.

Mitchell, J.S., R. S. Etienne, and D. L. Rabosky. 2019. Inferring diversification rate variation from phylogenies with fossils. *Systematic Biology* 68: 1–18.

Moore, B.R., S. Höhna, M. R. May, B. Rannala, and J. P. Huelsenbeck. 2016. Critically evaluating the theory and performance of Bayesian analysis of macroevolutionary mixtures. *Proceeding of national academy of sciences* 113: 9569–9574.

Musgrave, T., C. Gardner, and W. Musgrave. 1998. The plant hunters: two hundred years of adventure and discovery around the world. *Cassell Illustrated*.

Ohri, D. 2005. Climate and growth form: The consequences for genome size in plants. *Plant Biology* 7: 449–458.

Orians, C. 2000. The effects of hybridization in plants on secondary chemistry: implications for the ecology and evolution of plant–herbivore interactions. *American Journal of Botany* 87: 1749–1756.

Oswald, B. P., and S. L. Nuismer. 2007. Neopolyploidy and pathogen resistance. *Proceeding of Biological Sciences* 274: 2393–2397.

Otto, F. J. 1990. DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA In: Z Darzynkiewicz, HA Crissman, eds. *Methods in cell biology* 33: 105–110.

Pagel, M. 1999. The Maximum Likelihood Approach to Reconstructing Ancestral Character States of Discrete Characters on Phylogenies. *Systematics Biology* 48: 612–622.

Perkins, S., J. Perkins, J. M. Oliveira, M. de Castro, S. Castro, J. Loureiro. 2012. Weighing in: Discovering the ploidy of hybrid elepidote rhododendrons, in: Maughan, S. (Ed.), *Rhododendrons, Camellias and Magnolias*. *Royal Horticultural Society* 34–48.

Philipson, W., and M. Philipson. 1986. A revision of *Rhododendron*: 3. Subgenera *Azaleastrum*, *Mumeazalea*, *Candidastrum* and *Therorhodion*. *Notes from Royal Botanical Garden Edinburgh* 44: 1–23.

- Popescu, R., and B. Kopp. 2013. The genus *Rhododendron*: An ethnopharmacological and toxicological review. *Journal of Ethnopharmacology* 147: 42–62.
- Posada, D. 2008. JModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256.
- Qiu, Y. X., C. X. Fu, and H. P. Comes. 2011. Plant molecular phylogeography in China and adjacent regions: Tracing the genetic imprints of Quaternary climate and environmental change in the world's most diverse temperate flora. *Molecular Phylogenetics and Evolution* 59: 225–244.
- Rabosky, D. L. 2010. Extinction rates should not be estimated from molecular phylogenies. *Evolution* 64: 1816–1824.
- Rabosky, D. L. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS One* 9: e89543.
- Rabosky, D. L., J. Chang, P. O. Title, P. F. Cowman, L. Sallan, and M. Friedman, K. Kaschner, C. Garilao, T. J. Near, M. Coll, and M. E. Alfaro. 2018. An inverse latitudinal gradient in speciation rate for marine fishes. *Nature* 559: 392–395.
- Rabosky, D. L., M. Grundler, C. Anderson, P. Title, J. J. Shi, J. W. Brown, H. Huang, and J. G. Larson. 2014. BAMMtools: An R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods in Ecology and Evolution* 5: 701–707.
- Rabosky, D.L., J.S. Mitchell, and J. Chang. 2017. Is BAMM flawed? Theoretical and practical concerns in the analysis of multi-rate diversification models. *Systematic Biology* 66: 477–498.
- Rambaut, A., and A. Drummond. 2009. Tracer version 1.5. 0. <http://tree.bio.ed.ac.uk/software/tracer/>.
- Revell, L. J. 2012. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3: 217–223.
- Rezk, A., J. Nolzen, H. Schepker, D. C. Albach, K. Brix, and M. S. Ullrich. 2015. Phylogenetic spectrum and analysis of antibacterial activities of leaf extracts from plants of the genus *Rhododendron*. *BMC complementary and alternative medicine* 15: 67–76.
- Rice, A., L. Glick, S. Abadi, M. Einhorn, and N. M. Kopelman, et al. 2015. The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. *New Phytologist* 206: 19–26.
- Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Rozen, S., and H. Skaletsky. 1999. Primer3 on the WWW for general users and for biologist programmers. *Bioinformatics Methods and Protocols*.
- Schwery, O., R. E. Onstein, Y. Bouchenak-Khelladi, Y. Xing, R. J. Carter, and H. P. Linder. 2015. As old as the mountains: the radiations of the Ericaceae. *New Phytologist* 207: 355–367.
- Shrestha, N., Z. Wang, X. Su, X. Xu, L. Lyu, Y. Liu, D. Dimitrov, J. D. Kennedy, Q. Wang, Z. Tang, and X. Feng. 2018. Global patterns of *Rhododendron* diversity: The role of evolutionary time and diversification rates. *Global Ecology and Biogeography* 27: 913–924.
- Sleumer, H. 1949. Ein system der gattung *Rhododendron* L. *Botanische Jahrbücher für Systematik* 74: 511–553.
- Sleumer, H. 1966. *Rhododendron*, in: Flora Malesiana. Ser. I, Seed Plants. Noordhoff, Groningen, pp. 474–668.
- Small, J. 1914. Ericaceae. *North American Flora* 29:33–102.
- Soltis, D. E., R. Buggs, J. Doyle, P. Soltis. 2010. What we still don't know about polyploidy. *Taxon* 59: 1387–1403.
- Soltis, P. S., X. Liu, D. B. Marchant, C. J. Visger, and D. E. Soltis. 2014. Polyploidy and novelty: Gottlieb's legacy. *Philosophical Transactions of the Royal Society B: Biological Sciences* 369: 20130351.
- Soulebeau, A., X. Aubriot, M. Gaudeul, G. Rouhan, S. Hennequin, T. Haeuermans, J. Y. Dubuisson, and F. Jabbour. 2015. The hypothesis of adaptive radiation in evolutionary biology: hard facts about a hazy concept. *Organisms Diversity and Evolution* 15: 747–761.
- Spriggs, E. L., W. L. Clement, P. W. Sweeney, S. Madrin, E. J. Edwards, and M. J. Donoghue. 2015. Temperate radiations and dying embers of a tropical past: The diversification of *Viburnum*. *New Phytologist* 207: 340–354.
- Stamatakis, A. 2006. RAxML-VI-HP: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
- Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- Tank, D. C., J. M. Eastman, M. W. Pennell, P. S. Soltis, D. E. Soltis, C. E. Hinchliff, J. W. Brown, E. B. Sessa, and L. J. Harmon. 2015. Nested radiations and the pulse of angiosperm diversification: Increased diversification rates often follow whole genome duplications. *New Phytologist* 207: 454–467.
- Temsch, E. M., J. Greilhuber, and R. Krisai. 2010. Genome size in liverworts. *Preslia* 82: 63–80.
- Väinölä, A. 2000. Polyploidization and early screening of *Rhododendron hybrids*. *Euphytica* 112: 239–244.
- Wang, L., H. Schneider, X. C. Zhang, and Q. P. Xiang. 2012. The rise of the Himalaya enforced the diversification of SE Asian ferns by altering the monsoon regimes. *BMC Plant Biology* 12: 210.
- White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T (eds) PCR protocols. Academic Press, San Diego 315–322.
- Whitney, K. D., K. W. Broman, N. C. Kane, S. M. Hovick, R. A. Randell, and L. H. Rieseberg. 2015. Quantitative trait locus mapping identifies candidate alleles involved in adaptive introgression and range expansion in a wild sunflower. *Molecular Ecology* 24: 2194–2211.
- Wu, Z., and P. Raven. 2005. Apiaceae through Ericaceae, in: Flora of China. Science Press, Beijing.
- Yan, L. J., J. Liu, M. Möller, L. Zhang, X. M. Zhang, D. Z. Li, and L. M. Gao. 2015. DNA barcoding of *Rhododendron* (Ericaceae), the largest Chinese plant genus in biodiversity hotspots of the Himalaya-Hengduan Mountains. *Molecular Ecology Resources* 15: 932–944.
- Zha, H. G., R. I. Milne, and H. Sun. 2008. Morphological and molecular evidence of natural hybridization between two distantly related *Rhododendron* species from the Sino-Himalaya. *Botanical Journal of Linnean Society* 156: 119–129.

- Zha, H. G., R. I. Milne, and H. Sun. 2010. Asymmetric hybridization in *Rhododendron agastum*: a hybrid taxon comprising mainly F1s in Yunnan, China. *Annals of Botany* 105: 89–100.
- Zhang, J. L., C. Q. Zhang, L. M. Gao, J. B. Yang, H. A. T. Li. 2007. Natural hybridization origin of *Rhododendron agastum* (Ericaceae) in Yunnan, China: inferred from morphological and molecular evidence. *Journal of Plant Research* 120: 457–463.
- Zhao, J. L., L. Zhang, S. Dayanandan, S. Nagaraju, D. M. Liu, and Q. M. Li. 2013. Tertiary Origin and Pleistocene Diversification of Dragon Blood Tree (*Dracaena cambodiana*-Asparagaceae) Populations in the Asian Tropical Forests. *PLoS One* 8: 0060102.

TABLE 1. Numerical attributes of the four likelihood phylogenetic trees.

Data set	<i>trnK</i>	<i>trnL-F</i>	ITS	<i>rpb2-i</i>
No. individuals (Species)	307(259)	199(169)	237(197)	170(148)
Newly generated sequences	204	119	162	82
Aligned sequence length (bps)	1750	983	660	3306
No. of variable sites	285 (16.30%)	130 (13.20%)	173 (26.20%)	574 (17.30%)
No. of informative sites	399 (22.80%)	277 (28.10%)	252 (38.10%)	572 17.30%)
Consistency Index (CI)	0.736	0.697	0.662	0.674
Retention Index (RI)	0.917	0.883	0.908	0.864

TABLE 2. Comparison of different molecular phylogenetic analyses with the results of this particular study. The abbreviated letters showed in the table represents, R – R. subgen. Rhododendron; A – R. sect. Azaleastrum; T – R. subgen. Tsutsusi; M – R. subgen. Mumazalea; C – R. sect. Choniastrum; S – R. sect. Sciadorhodion and P – R. sect. Pentanthera; H – R. subgen. Hymenanthus; L – R. sect. Ledum; V – R. sect. Viscidula.

	Kurashige et al. (2001) – <i>matK</i>	This study – <i>trnK</i>	This study – <i>trnL</i>	Grimbs et al. (2017) – <i>trnK</i> , <i>trnL</i> , ITS	Gao et al. (2002) – ITS	This study – ITS	Goetsch et al. (2005) – <i>rpB2</i>	This study – <i>rbB2</i>	Shrestha et al. (2018) – multiple regions
Sect. Schistanthe	Triphyletic (subsect. <i>Discovireya</i> /R. <i>santapaui</i> /rest) nested in R	unresolved within R	unresolved in R	-	single species nested in R	monophyletic nested in R	monophyletic <i>Discovireya</i> not sampled	diphyletic (Subsect. <i>Discovireya</i> /rest) nested in R	Diphyletic (subsect. <i>Discovireya</i> /rest) nested in R
Ledum	R. <i>hypoleucum</i> sister to R. <i>albrechtii</i>	R. <i>tomentosum</i> , R. <i>diversipilosum</i> sister to R. <i>albrechtii</i>	R. <i>tomentosum</i> close to R. <i>albrechtii</i> but sister to P	R. <i>tomentosum</i> sister to R. <i>albrechtii</i>	R. <i>tomentosum</i> sister to R	R. <i>tomentosum</i> sister to R	R. <i>hypoleucum</i> , R. <i>tomentosum</i> sister to R	R. <i>hypoleucum</i> , R. <i>tomentosum</i> sister to R	R. <i>hypoleucum</i> , R. <i>tomentosum</i> sister to R except R. <i>diversipilosum</i> sister to R. <i>albrechtii</i>
Sect. Pentanthera	sister to R. <i>canadense</i> , together sister to H	Sister to R. <i>canadense</i> , together sister to V, M, C, R. <i>vaseyi</i>	sister to R. <i>canadense</i> , together sister to L	sister to H (R. <i>canadense</i> not sampled)	sister to R. <i>canadense</i> in unresolved position	basally branching (R. <i>canadense</i> not sampled)	incl. R. <i>canadense</i> sister to H	together with R. <i>canadense</i> in H	incl. R. <i>canadense</i> ; sister to H
Sect. Choniastrum	sister to M	sister to M	sister to M	sister to T, A	unresolved with A, T, R. <i>albiflorum</i> , R. <i>schlippenbachii</i>	sister to A, T, S, M, R. <i>vaseyi</i>	sister to R, L	sister to R, L	sister to R, L
R. subgen. Tsutsusi	sister to A	sister to A	paraphyletic sister to A+M	sister to A	sister to A+M	sister to A+M	unresolved with A, M, V	sister to V, together sister to M, together sister to A	sister to A+M
R. sect. Azaleastrum	sister to T	sister to T	sister to T, C, M	sister to T	Sister to M	sister to M	unresolved with T, M, V	unresolved with T, M, V	sister to T, M
R. <i>albiflorum</i> (R. subgen. <i>Candidastrum</i>)	unresolved with T, A, R. sect. <i>Sciadorhodion</i>	sister to T, A	-	-	Unresolved with T, A, C, R. sect. <i>Sciadorhodion</i>	-	sister to R. <i>albrechtii</i>	-	sister to R. <i>albrechtii</i> + R. <i>diversipilosum</i>
R. <i>semibarbatum</i> (R. subgen. <i>Mumeazalea</i>)	sister to C	sister to C	sister to C	-	sister to A	sister to A	sister to V	sister to V, T, together sister to A	sister to T
R. <i>nipponicum</i> (R. sect. <i>Viscidula</i>)	sister to M, C	sister to R. <i>vaseyi</i>	-	-	-	-	sister to M	sister to T	Sister to R. <i>quinquefolium</i> (sect. <i>Sciadorhodion</i>), together sister to A, T, M
R. <i>vaseyi</i> (R. sect. <i>Rhodora</i>)	-	sister to V	sister to R	sister to R, H	-	sister to R. <i>albrechtii</i>	sister to R. <i>schlippenbachii</i> , together sister to R. <i>schlippenbachii</i>	sister to R. <i>schlippenbachii</i> , together sister to <i>Menziesia</i>	with R. <i>pentaphyllum</i> sister to <i>Menziesia</i> , together sister to R. <i>schlippenbachii</i>
<i>Menziesia</i>	Unresolved with A, T, C, R. sect. <i>Sciadorhodion</i>	R. <i>pilosum</i> incl. in R. sect. <i>Sciadorhodion</i>	R. <i>pilosum</i> with R. <i>vaseyi</i> sister to R	-	-	5 spp. sister to R. <i>schlippenbachii</i>	2 spp. sister to R. <i>vaseyi</i> , together sister to R. <i>schlippenbachii</i>	-	9 spp. sister to R. <i>vaseyi</i> and R. <i>pentaphyllum</i> , together sister to R. <i>schlippenbachii</i>
R. <i>schlippenbachii</i> (R. sect. <i>Sciadorhodion</i>)	unresolved with <i>Menziesia</i> , A, T, R. <i>pentaphyllum</i> , R. <i>quinquefolium</i>	together with <i>Menziesia</i> and R. <i>pentaphyllum</i> , R. <i>quinquefolium</i> , close to A, T, V. <i>albiflorum</i>	unresolved	basally branching	unresolved with T, A, R. <i>albiflorum</i>	sister to <i>Menziesia</i> , together sister to T, A, M	sister to R. <i>vaseyi</i> + <i>Menziesia</i> , then to R. <i>albiflorum</i> , then to A, T, M, V	sister to R. <i>vaseyi</i> , together sister to <i>Menziesia</i> , sister to A, T, M, V	sister to <i>Menziesia</i> + R. <i>vaseyi</i>
R. <i>albrechtii</i> (R. sect. <i>Sciadorhodion</i>)	sister to R. <i>hypoleucum</i> (L), together unresolved	R. <i>albrechtii</i> sister to L, together sister to H	unresolved	sister to L, together sister to P, H	-	sister to R. <i>vaseyi</i> , together sister to C	R. <i>albrechtii</i> sister to R. <i>albiflorum</i>	sister to R. <i>vaseyi</i> , R. <i>schlippenbachii</i> + <i>Menziesia</i>	sister to R. <i>diversipilosum</i> (L), together sister to R. <i>albiflorum</i>

trnK collapsed sections

Figure 1. Phylogenetic tree of *Rhododendron* inferred from the plastid *trnK* gene region, showing species arranged by subgenus and section. Numbers above branches are bootstrap values, numbers below branches are posterior probabilities (in percent). Numbers in triangles are the number of the included individuals (species list in Table S2). Newly generated sequences are marked with a star (*).

ITS collapsed sections

Figure 2. Phylogenetic tree of *Rhododendron* inferred from the nuclear Internal Transcribed Spacer (ITS) gene region, showing species arranged by subgenus and section. *Calluna vulgaris* (Ericaceae) was used as root. Numbers above branches are bootstrap values, numbers below branches are posterior probabilities (in percent). Numbers in triangles are the number of the represented individuals (species list in Table S2). Newly generated sequences are marked with a star (*).

Figure 3. Result from the Bayesian Analysis of Macroevolutionary Mixtures (BAMM) diversification analysis of *Rhododendron tnK*. Colors of phylogeny refer to decrease (blue) or increase (red) in diversification rates. The size of the circles shows rate of diversification shift i.e., larger circle higher rate shift and smaller circle lowers rate shift. Inset shows the rate vs. time plot from the diversification analysis, where the 'speciation rate' (speciation events) is given per million years, and 'time before present' is in millions of years.

Figure 4. Ancestral genome size values mapped using a continuous color gradient on a BEAST tree of 105 *Rhododendron* species based on the trnK gene region. A, 2C-values and ploidy levels indicated as bar plot; B, 1Cx-values.