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Evolution of flower morphology, pollen dimorphism, and nectar composition in *Arcytophyllum*, a distylous genus of Rubiaceae

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Abstract

A phylogenetic study of *Arcytophyllum* based on ITS was conducted and compared with an earlier study based on cpDNA. The position of the widespread *A. thymifolium* as sister to all other species was confirmed and several well-supported clades could be retrieved. The Central American *A. lavarum* is well embedded between exclusively or predominantly South American species. To understand the expression of heterostyly in the genus, we analyzed inter- and intraspecific variation in floral morphology, nectar, pollen–ovule (P/O) ratio and seed set of ten species in 11 populations. Stigma and anther levels differed significantly between the morphs in the species/populations investigated, except for *A. filiforme*, in which anther levels did not differ significantly between the two morphs. Different expressions of heterostyly in *Arcytophyllum* seem independent of phylogenetic relationships. Nectar sugar composition was similar between the morphs. Nectar of most species presented a larger proportion of hexoses than of sucrose; only the most derived species, *A. macbridei* and *A. vernicosum*, have higher sucrose proportions. There is a significant positive correlation between corolla tube length and the proportion of sucrose. Pollen dimorphism, with regard to both number (long-styled > short-styled) and size (short-styled > long-styled), was observed in all taxa investigated except *A. filiforme*. According to the P/O ratios, the breeding systems range from facultative autogamy to facultative xenogamy. The lowest P/O ratios were found in *A. filiforme*, and the highest in *A. rivetii*. Hymenoptera, Diptera and Coleoptera were observed as flower visitors. Seed production did not differ significantly between the morphs in eight of the 11 species/populations investigated. There is, however, a tendency in all species/populations (except in *A. macbridei* Peru) for the short-styled morph to have a higher percentage of seeds per ovule, indicating that the short-styled morphs display higher female reproductive success.

Keywords: Heterostyly; ITS; P/O ratio; Pollination; Seed set; Spermacoceae

Introduction

The South American genus *Arcytophyllum* Willd. ex Roem. & Schult. was one of the many insufficiently known South American Rubiaceae genera until Mena (1990) produced a thorough herbarium-based revision, identifying 15 species occurring from Costa Rica to Bolivia. It was never seriously disputed that *Arcytophyllum* is closely related to *Hedyotis* L., a speciose genus (ca. 400 spp.) of herbs and shrubs distributed worldwide in tropical, subtropical and temperate regions; most *Arcytophyllum* species were originally described in *Hedyotis* (Mena 1990). Terrell (1999)

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transferred another *Hedyotis* species, *H. serpyllacea* Schltd., to *Arcytophyllum,* based on overall similarity. Traditionally, *Hedyotis* and its relatives had been placed into a tribe of their own, Hedyotideae. Andersson and Rova (1999), analyzing the *rps*16 intron of members of subfamily Rubioideae, and Bremer (1996) based on *rbc*L came to the conclusion that Spermacoceae is nested in Hedyotideae (Oldenlandieae), so that the correct tribal affiliation for *Arcytophyllum* (and *Hedyotis*) is therefore Spermacoceae. In a further analysis of the *rps*16 intron comprising more taxa, Andersson et al. (2002) were able to show that *A. serpyllaceum* is not a member of the *Arcytophyllum–Houstonia* clade, but more closely related to *Bouvardia* Salisb., while the remainder of *Arcytophyllum* is indeed monophyletic. These relationships were upheld in a larger sampling of Spermacoceae, again using *rps*16 (Dessein et al. 2005). Unaware of Andersson's efforts, we collected *Arcytophyllum* samples for an ITS analysis, and for an analysis of morphology and nectar in the long-styled (ls) and short-styled (ss) flowers of the always distylous genus.

The flowers of *Arcytophyllum* are distylous. Individual plants produce either ls morphs with the stigma positioned above the anthers, or ss morphs with reciprocal placement of anthers and stigmas (reciprocal herkogamy). Usually, the two distinct hermaphrodite floral morphs coexist in a population at roughly equal frequencies. In general, the style–stamen polymorphism is accompanied by a sporophytically controlled, diallelic self-incompatibility system that prevents self- and intramorph fertilization (Barrett 1992). We are not aware of a comparative study of nectar composition in heterostylous species and its variation within a genus. This paper intends to contribute to the knowledge of *Arcytophyllum* by adding a nuclear marker to the analyses of Andersson et al. (2002) and by interpreting the results of the floral biology studies for 10 out of the 15 known *Arcytophyllum* species in the light of their phylogeny.

Material and methods

Phylogeny

The original matrix of analysis 2 in Andersson et al. (2002), comprising 1783 positions of the *rps*16 intron (871 positions) and the *trn*L-F region (912 positions; comprising the *trn*L intron, part of the *trn*L gene and the *trn*L-F intergenic spacer; flanked by the primers "c" and "f" of Taberlet et al. 1991) of cpDNA of 11 *Arcytophyllum* species, one *Houstonia* (*H. longifolia* Gaertn.) and two *Hedyotis* species [*H. nigricans* (Lam.) Fosb. & *H. serpens* Kunth] was generously provided by the late L. Andersson. From GenBank, ITS sequences of *H. longifolia* (as "*Hedyotis longifolia*") and *H. nigricans* could be retrieved. We originally sequenced 16 accessions (Table 1, including taxonomic authorship data) of *Arcytophyllum* for ITS, using the primers P17F and 26S-82R and the sequencing primers P16F and P25R. Sequence comparison showed that there were no sequence differences between the ls and ss morphs of *A. capitatum* and *A. setosum*; therefore, only one of these sequences was used. The two populations of *A. filiforme* differed in one site, and those of *A. macbridei* in two sites; however, the two populations of *A. thymifolium* differed in as many as 25 sites, even though there is no doubt about their correct identification. Analysis 1 comprises these 14 samples; the two sequences from GenBank were used as outgroups.

To combine the matrices, consensus sequences for the three species with two accessions each were computed using Sequence Navigator (Applied Biosystems). As the species sampled for this study are not identical with the ones used in the study of Andersson et al. (2002), two methods of matrix combination were applied: (1) only the nine species available in both datasets were analyzed; (2) the missing partial sequences were coded as "*n*", and all 13 *Arcytophyllum* species were analyzed. For statistical support, bootstrap values (1000 replicates, random addition with 100 addition sequences each; Felsenstein 1985) were calculated in PAUP, and Bremer indices (Bremer 1988) were calculated using AutoDecay (Eriksson 1998).

Floral morphology and pollen–ovule (P/O) ratio

Fresh floral material from both morphs was collected in the field and measured under a stereomicroscope. Fig. 1 illustrates the parameters measured. Morphological differences between the two floral morphs were compared by one-way analysis of variance. We determined the pollen–ovule (P/O) ratio according to Cruden (1977) and Kearns and Inouye (1993). We opened both locules under a stereomicroscope and counted the ovules. The pollen of the buds already used for ovule counting was prepared by opening anthers in an isotonic solution (0.9% NaCl), and pollen grain number and size were calculated with a Cell Counter and Analyzer System (CASY, Schärfe System). The total number of pollen grains per flower was estimated by counting the number of one closed anther and multiplying by the number of anthers per flower (four). We first calculated P/O ratios for individual buds by dividing the number of pollen grains by the number of ovules. Then we calculated P/O–ss/ls, i.e. the ratio of averaged ss morph pollen grains to averaged ovule numbers of the ls morph, as well as the inverse ratio (P/O–ls/ss).

Table 1. Voucher information

Accession	Morph	Country, province, locality	Collection	EMBL no.
A. aristatum Standl.	ls	Ecuador, Carchi, El Àngel	Wolff 25 (MO, UBT)	AM182061
A. capitatum (Benth) K. Schum.	ls	Ecuador, Loja, Cajanuma	Wolff 4 (MO, UBT)	AM182062
A. capitatum (Benth) K. Schum.	ss	Ecuador, Loja, Cajanuma	Wolff 4 (MO, UBT)	AM182063
A. ciliolatum Standl.	?	Ecuador, Zamora-Chinchipe, Valladolid	Wolff 36 (MO, UBT)	AM182064
A. filiforme (Ruiz & Pav.) Standl.	ss	Ecuador, Azuay, Cajas	Wolff 1 (MO, UBT)	AM182065
A. filiforme (Ruiz & Pav.) Standl.	ls	Peru, Amazonas, Chachapoyas, Leimebamba	Wolff 47 (MO, UBT)	AM182066
A. lavarum K. Schum.	?	Costa Rica, Cartago, Cerro de la Muerte	Wolff 59 (MO, UBT)	AM182067
A. macbridei Standl.	?	Peru, Amazonas, Chachapoyas, Leimebamba	Wolff 34 (MO, UBT)	AM182068
A. macbridei Standl.	ls	Ecuador, Loja, Cajanuma	Wolff 5 (MO, UBT)	AM182069
A. muticum (Wedd.) Standl.	?	Costa Rica, Cartago, Cerro de la Muerte	Wolff 60 (MO, UBT)	AM182070
A. rivetii Danguy & Cherm.	ss	Peru, Amazonas, Chachapoyas, Leimebamba	Wolff 35 (MO, UBT)	AM182071
A. setosum (Ruiz & Pav.) Standl.	ss	Ecuador, Azuay, Cajas	Wolff 2 (MO, UBT)	AM182072
A. setosum (Ruiz & Pav.) Standl.	ls	Ecuador, Azuay, Cajas	Wolff 2 (MO, UBT)	AM182073
A. thymifolium (Ruiz & Pav.) Standl.	?	Peru, Lima, Yauyos-Laraos	Beltrán s.n. (UBT)	AM182074
A. thymifolium (Ruiz & Pav.) Standl.	?	Ecuador, Imbabura, Cuicocha	Homeier s.n. (UBT)	AM182075
A. vernicosum Standl.	ls	Ecuador, Loja, Cajanuma	Wolff 3 (MO, UBT)	AM182076
Hedyotis nigricans (Lam.) Fosb.	?	USA, Eastern USA	Church 2003	AF381461 (ITS1) AF381494 (ITS2)
Houstonia longifolia Gaertn.	?	USA, South and Central USA Mexico, Northern Mexico	Church 2003	AF381477 (ITS1) AF381510 (ITS2)

Abbreviations: ls = long-styled, ss = short-styled. Unless otherwise indicated, the material sequenced was sterile.

Nectar sugar composition

Nectar of several individuals was sampled from unbagged flowers with microcapillaries (0.5, 1 and 2 µl). The nectar amount obtained reflects both secretion and depletion by visitors. Nectar of each morph was pooled in 70% alcohol and frozen for nectar sugar analysis and determination of nectar concentration. To this end, samples were dried in a vacuum centrifuge, diluted with 200 µl water and filtered on a Waters high-performance carbohydrate column to avoid contamination. The injection volume was 10 µl; elution took place with an acetonitrile–water mixture (71:28), at a flow rate of 1.4 ml/min and a temperature of 35 °C. Glucose, fructose and sucrose were detected with a refraction index detector of 410, and quantified with the Millenium Software from Waters. Since we collected nectar under natural conditions, we could not avoid nectar dilution caused by heavy rain, nor high nectar viscosity caused by evaporation. These sampling artifacts may influence nectar volume and nectar concentration. Nectar concentration was converted from µg/µl to sucrose-equivalent, % weight per total weight, using Table 63 in the 50th edition of the Handbook of Chemistry and Physics (Weast 1969).

Seed set, flower visitors

We collected fruits of several individuals and both morphs in the field and determined seed set as the percentage of seeds per fruit to the averaged number of ovules. Finally, we observed diurnal pollinator activity in the species studied, using fixed periods of 15 min. All visits on flowers in the observed patches were counted.

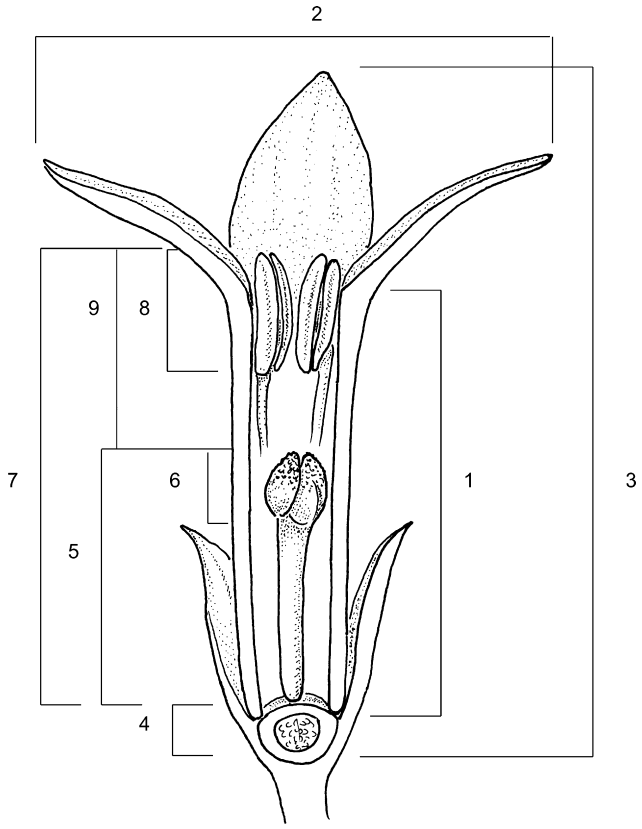

Fig. 1. Schematic flower of *Arcytophyllum* (short-styled morph), indicating the distances measured. Numbers correspond to the following measurements for both morphs: 1 = corolla tube length, 2 = corolla diameter, 3 = total flower length, 4 = ovary length, 5 = stigma level, 6 = stigma lobe length, 7 = anther level, 8 = anther length, 9 = anther–stigma separation.

Observations took place from late morning until early afternoon (from 10.00 a.m. to 4.30 p.m.); longer observation times were not possible because of the remote locations of the plants. The population of *A. capitatum* was observed for three (non-consecutive) days, the populations of *A. thymifolium*, *A. filiforme*, *A. macbridei* (Ec) and *A. vernicosum* were observed for two (non-consecutive) days and the remaining populations could be observed for only one day each.

Results

Phylogeny

The ITS alignment, available on the website of SLS and TreeBase (Sanderson et al. 1994), comprised 16 taxa and 587 characters (study accession number = S1447, matrix accession number = M2604). For the two outgroup taxa, 85 positions in the 5.8s region are missing, due to separate sequencing of ITS1 and ITS2 (Church 2003). The matrix contains 88 parsimony informative characters; branch-and-bound search (Hendy and Penny 1989) resulted in four trees of 231 steps (CI = 0.76, RI = 0.79, RC = 0.68). The strict consensus tree (Fig. 2) confirms the position of *A. thymifolium* as sister to all other *Arcytophyllum* species with high bootstrap support. All other species form an equally well-supported clade ('core *Arcytophyllum* clade'), which comprises four unresolved subclades. The sister-group position of *A. aristatum* and *A. muticum* found by Andersson et al. (2002) is retrieved, albeit unsupported, and this species pair is sister to *A. rivetii*, again unsupported. The two accessions of *A. filiforme* form a well-supported subclade with *A. capitatum*. The largest subclade comprises a well-supported triplet of the two accessions of *A. macbridei*, *A. vernicosum* and *A. setosum*. The sister-group position of the Central American *A. lavarum* to this triplet is moderately supported.

Combining both matrices, and analyzing only those nine species for which both ITS and cpDNA data are available, results in 123 parsimony informative characters and a single tree of 359 steps (CI = 0.79, RI = 0.8, RC = 0.72; Fig. 3). In this tree, both the monophyly of *Arcytophyllum* and the position of *A. thymifolium* as sister to all remaining species are strongly supported. Likewise, the sister-group relationship of *A. ciliolatum* and *A. rivetii* is strongly supported, whereas the relationships of the remaining taxa are only weakly to moderately supported.

Adding the remaining four species, for which one of the partial sequences is missing (cpDNA for *A. capitatum* and *A. filiforme*, ITS for *A. ericoides* and *A. nitidum*), the number of parsimony informative characters rises to 132, and two equally parsimonious trees of 378 steps each are retrieved (CI = 0.78, RI = 0.8, RC = 0.71). In the strict consensus tree (Fig. 4), *A. capitatum* and *A. filiforme* form a well-supported clade that is sister to the remaining clades within the core *Arcytophyllum* clade. *Arcytophyllum ericoides* is added to the *A. rivetii*/*A. ciliolatum* clade, and this monophyletic group of three species is also well supported. *Arcytophyllum nitidum* is sister to *A. setosum* with very good support, and this species pair is sister to the unsupported pair *A. macbridei*/*A. vernicosum*. The position of *A. lavarum* as sister to these latter two species pairs corresponds to the position of this species in the other analyses. Support for the pair *A. aristatum*/*A. muticum* increases slightly compared to the other analyses.

Floral morphology

The flowers of *Arcytophyllum* are 5–13 mm long (Table 2), with the tube slightly longer than the

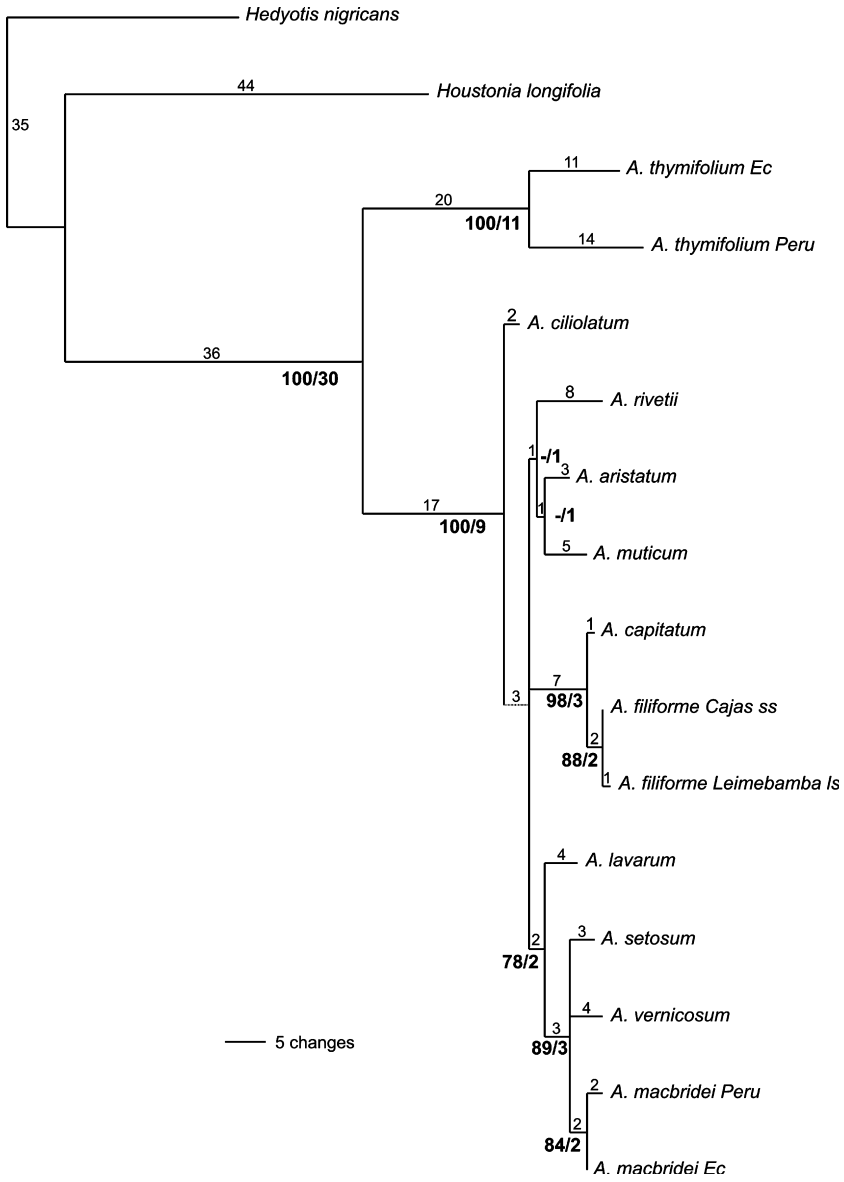

Fig. 2. One of the four most parsimonious trees retrieved from branch-and-bound analysis of the ITS dataset (l = 231 steps, CI = 0.76, RI = 0.79, RC = 0.68). Numbers indicate branch lengths, bold numbers indicate bootstrap percentages/decay indices (Bremer values).

four lobes (Fig. 1). The flowers of all investigated *Arcytophyllum* species/populations are distylous, with significant differences in morphology between the morphs (Tables 2, 3; Fig. 5). In the ss morphs (Fig. 1), the anthers are positioned at the mouth of the corolla tube overtopping the style and stigma, whereas in the ls morphs, the style and stigma overtop the anthers, which are sunken in the corolla tube. Fig. 5 shows a clear separation of morphs in all *Arcytophyllum* species when stigma level is plotted against anther level. However, an overlap in anther levels between the morphs was observed in *A. macbridei* (Ec), *A. rivetii*, *A. thymifolium* and *A. vernicosum.* There was an overlap of anther and stigma levels between the two morphs in *A. filiforme*. In the populations of *A. filiforme*, *A. thymifolium* and *A. vernicosum* studied, individuals with anthers and stigmas on the same levels occur (Fig. 5). Regarding averaged values of stigma–anther separation, however, significant differences occur between the morphs in all investigated species. The flowers of all A*rcytophyllum* species/populations studied show further significant differences between the morphs in averaged stigma levels. The stigma level depends on the length of the style and the size of the ovary (Richards and Barrett 1992). The ovary is significantly longer in ls flowers than in ss ones, with the exception of *A. macbridei* (Peru), *A. aristatum, A. setosum*, *A. ciliolatum*, *A. filiforme* and *A. rivetii.* The averaged anther levels differ significantly between ls and

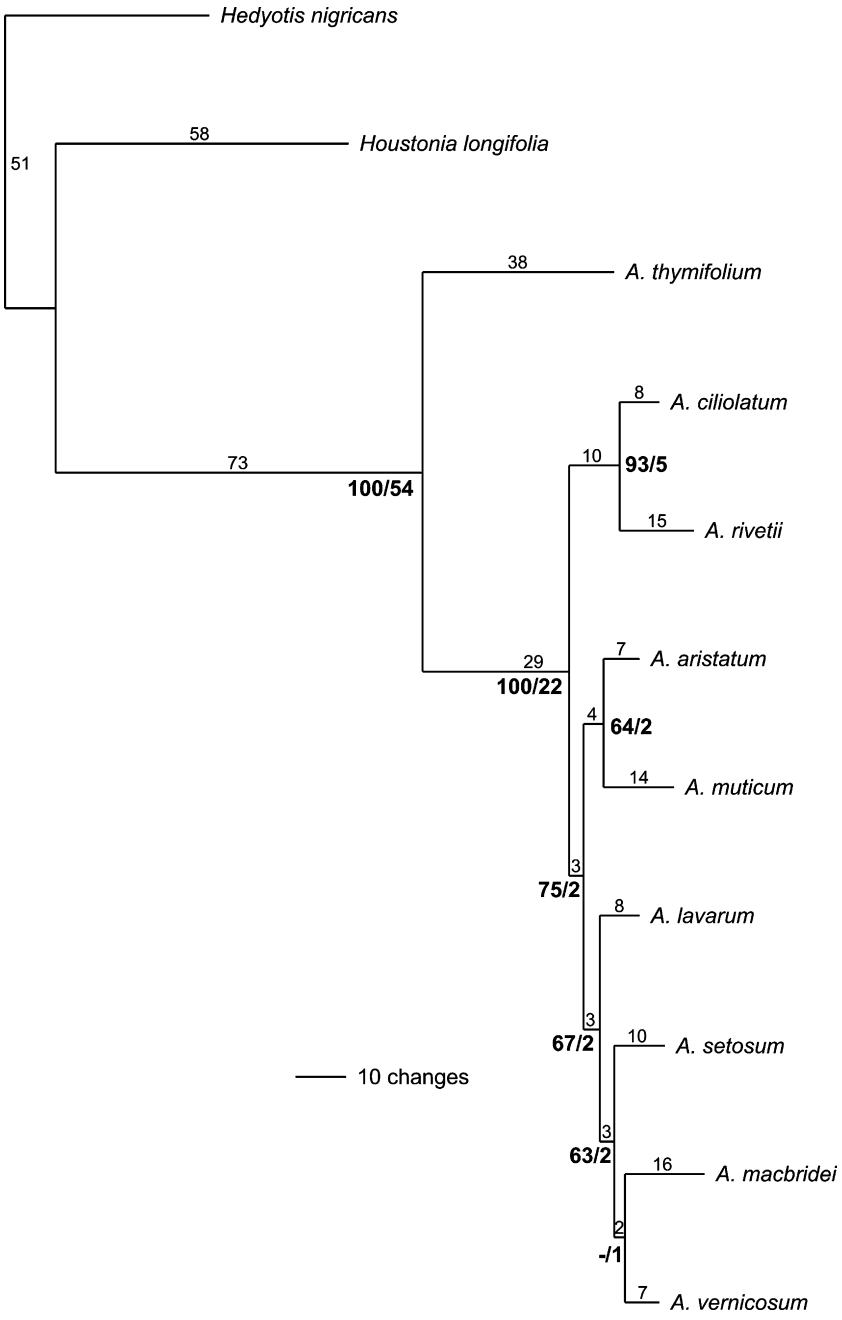

Fig. 3. Single most parsimonious tree derived from from branch-and-bound analysis of the nine *Arcytophyllum* species, for which both ITS and cpDNA (*rps*16 intron and the *trn*L-F region) data were available (l = 359 steps, CI = 0.79, RI = 0.8, RC = 0.72). Numbers indicate branch lengths, bold numbers indicate bootstrap percentages/decay indices (Bremer values).

ss morphs in all species studied except *A. filiforme* (Table 2). Ss flowers have significantly longer corolla tubes in *A. ciliolatum*, *A. thymifolium*, *A. capitatum*, *A. rivetii*, *A. lavarum* and *A. macbridei* (Peru). Stigma lengths are significantly shorter in ls morphs, with the exception of *A. ciliolatum*, *A. lavarum*, *A. macbridei* (Peru) and *A. setosum*. Ss flowers have longer anthers in *A. capitatum*, *A. rivetti*, *A. macbridei* (Ec), *A. macbridei* (Peru) and *A. vernicosum*.

Pollen dimorphism, P/O ratio and seed set

Ovule number varied from about six in *A. rivetii* to 28 in *A. vernicosum* (ss morph). In six species both floral morphs had similar numbers of ovules (Table 5). In *A. thymifolium*, *A. macbridei* (Ec) and *A. macbridei* (Peru), ovule number was significantly higher in the ls morph, whereas in *A. vernicosum* and *A. lavarum* ovule number was significantly higher in the ss morph.

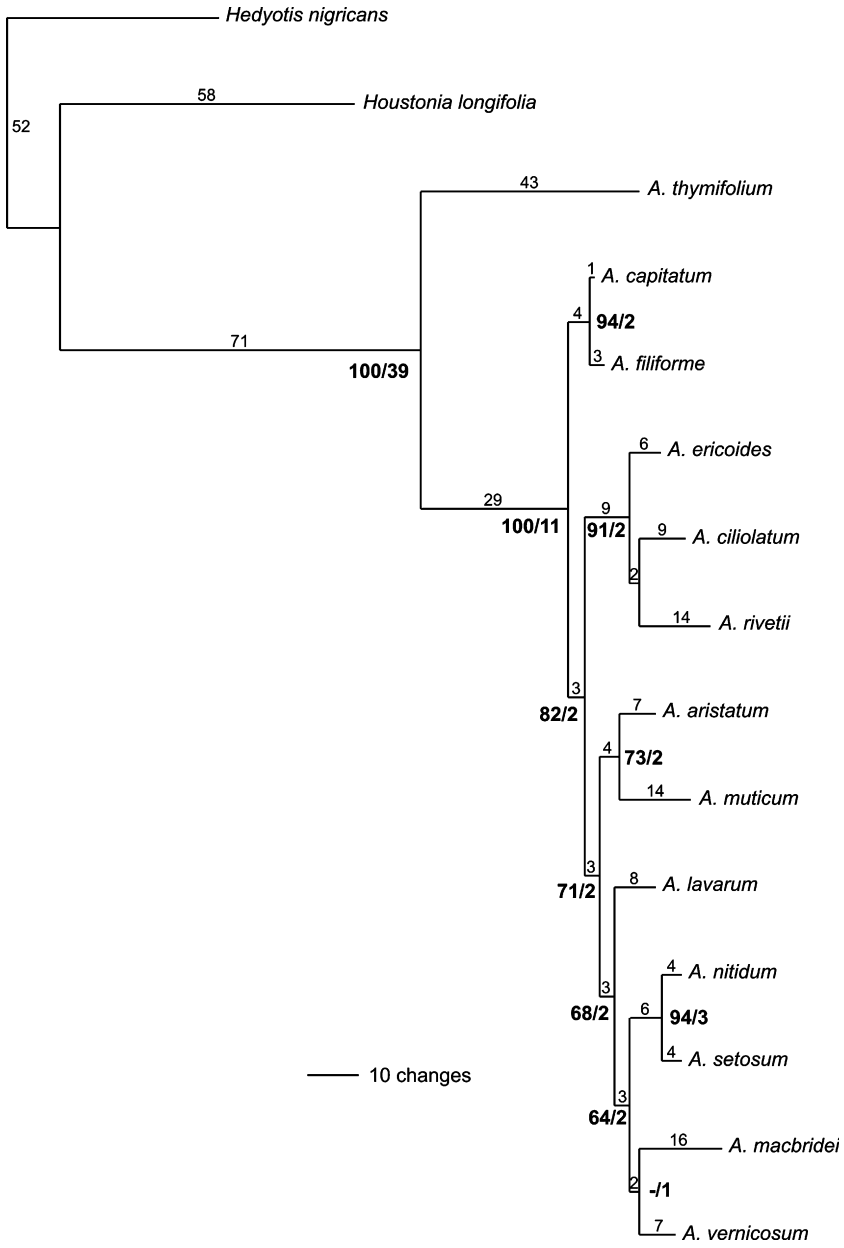

Fig. 4. One of the two most parsimonious trees resulting from analysis of ITS and cpDNA (*rps*16 intron and the *trn*L-F region) for all taxa, with missing partial sequences coded as "*n*" (l = 378 steps, CI = 0.78, RI = 0.8, RC = 0.71). Numbers indicate branch lengths, bold numbers indicate bootstrap percentages/decay indices (Bremer values).

Interspecific variation concerning the number of pollen grains was observed, with lowest records in *A. filiforme* (approx. 2800 grains per flower) and highest values in *A. macbridei* (Ec) (approx. 13,000 grains per flower). Furthermore, a high intraspecific variation could be observed, with ls flowers presenting a significantly higher number of pollen grains than ss ones in all species investigated, except in *A. filiforme* (Table 5). The ratio of ls to ss pollen grain number ranges from 0.95 in *A. filiforme* to 1.7 in *A. thymifolium*. Pollen grain size in ls morphs was significantly smaller than in ss morphs, with the exception of *A. filiforme*. The lowest P/O ratios of ls pollen to ovules of the ss flowers, and vice versa, were found in *A. vernicosum*, followed by *A. filiforme* and *A. aristatum*, whereas the highest ratios were found in *A. rivetii*. The P/O ratios of ls pollen to ovules of the ss flowers exceeded those of the reverse type, except in *A. lavarum* and *A. setosum*.

Seed production (Table 6) in fruits of *A. aristatum*, *A. lavarum* and *A. vernicosum* was significantly higher in ss patches than in ls patches. In the other species, there is only a tendency for higher seed set in ss flowers compared to ls flowers.

Table 2. Floral measurements (mm) in 11 *Arcytophyllum* species/populations

	Morph	*n*	Corolla tube length $x \pm$ s.d.	Corolla diameter $x \pm$ s.d.	Total flower length $x \pm$ s.d.	Ovary length $x \pm$ s.d.	Stigma level $x \pm$ s.d.	Stigma lobe length $x \pm$ s.d.	Anther level $x \pm$ s.d.	Anther length $x \pm$ s.d.	Anther–stigma separation $x \pm$ s.d.
A. thymifolium	ls	11	3.6±0.4	7.8±0.4	7.9±0.6	1.6±0.3	6.7±0.6	0.8±0.2	3.1±0.1	1.0±0.1	−3.5±0.5
A. thymifolium	ss	10	4.3±0.7	7.3±1.4	7.7±1.3	1.1±0.2	3.4±0.8	1.2±0.4	6.4±0.7	1.0±0.1	3.0±0.8
A. capitatum	ls	17	4.3±0.4	8.7±0.9	9.1±0.7	1.4±0.5	7.5±1.0	1.0±0.1	3.4±0.5	1.1±0.1	−4.1±1.2
A. capitatum	ss	16	4.7±0.7	6.8±0.7	7.6±1.4	1.1±0.1	3.6±0.6	1.2±0.3	6.3±0.7	1.4±0.4	2.7±0.9
A. filiforme	ls	10	3.9±0.7	4.8±0.9	6.3±1.2	0.9±0.1	5.3±1 1	0.3±0.2	4.0±0.8	0.7±0.3	−1.3±0.6
A. filiforme	ss	10	4.2±0.9	5.1±0.6	6.0±0.7	0.9±0.1	3.4±0.6	0.5±0.2	4.7±0.9	0.8±0.2	1.3±0.8
A. ciliolatum	ls	12	2.4±0.3	8.4±1.2	5.9±0.6	1.4±0.4	4.6±0.5	0.6±0.2	2.6±0.3	0.9±0.1	−2.0±0.4
A. ciliolatum	ss	12	3.0±0.4	7.7±1.2	6.4±0.9	1.3±0.4	2.3±0.4	0.6±0.2	4.8±0.7	0.9±0.2	2.5±0.6
A. rivetii	ls	11	3.5±0.4	8.0±0.6	7.1±0.2	1.4±0.3	5.6±0.8	0.7±0.2	3.5±0.6	0.9±0.1	−2.0±0.6
A. rivetii	ss	10	4.1±0.4	6.3±0.7	6.5±0.7	1.4±0.4	3.0±0.4	1.0±0.1	5.3±0.6	1.0±0.1	2.2±0.6
A. aristatum	ls	12	4.2±0.2	7.0±0.3	6.4±0.4	1.1±0.2	5.4±0.5	0.6±0.1	3.8±0.2	0.9±0.2	−1.7±0.5
A. aristatum	ss	12	4.1±0.2	7.0±0.7	6.3±0.3	1.0±0.0	4.2±0.2	1.0±0.0	5.5±0.5	0.8±0.2	1.3±0.4
A. lavarum	ls	10	2.5±0.4	7.6±0.5	5.8±0.5	1.4±0.3	4.7±0.4	0.7±0.3	2.7±0.3	1.0±0.0	−2.0±0.5
A. lavarum	ss	11	3.4±0.8	7.7±1.0	6.5±0.7	1.1±0.1	2.3±0.6	0.6±0.2	5.0±0.7	1.0±0.0	2.7±0.6
A. setosum	ls	10	5.3±0.5	9.0±1.3	10.4±0.7	2.0±0.1	8.1±0.8	0.9±0.2	5.4±0.6	1.3±0.3	−2.8±0.8
A. setosum	ss	10	5.6±0.7	8.7±1.5	10.0±1.2	1.8±0.3	4.2±0.8	1.2±0.5	7.4±0.8	1.3±0.2	3.2±0.7
A. macbridei (Peru)	ls	15	4.3±0.8	11.0±1.9	8.9±1.3	2.3±0.6	6.9±0.5	1.0±0.0	4.2±0.6	1.7±0.5	−2.7±0.5
A. macbridei (Peru)	ss	15	6.1±0.5	9.4±1.2	11.0±2.0	2.3±0.3	4.5±0.4	1.0±0.3	8.1±1.0	2.1±0.5	3.6±1.0
A. macbridei (Ec.)	ls	30	7.6±0.9	12.0±1.3	12.5±1.6	1.5±0.5	9.4±1.0	1.1±0.2	5.7±0.6	1.9±0.3	−3.7±1.0
A. macbridei (Ec.)	ss	23	7.8±0.6	10.2±1.4	12.8±1.1	1.0±0.1	5.2±0.4	1.3±0.2	8.2±0.7	2.2±0.2	3.0±0.7
A. vernicosum	ls	14	4.5±1.6	6.5±1.1	7.2±0.3	2.0±0.0	5.1±0.5	1.0±0.1	2.8±0.4	1.0±0.1	−2.3±0.5
A. vernicosum	ss	14	4.9±1.7	6.5±0.9	6.4±0.4	1.7±0.4	3.2±0.4	1.1±0.1	5.0±0.9	1.4±0.5	1.8±1.1

ls = long-styled, ss = short-styled, n = number of flowers measured, x = mean, s.d. = standard deviation.

Table 3. Results of one-way ANOVA tests for floral dimorphism in 11 *Arcytophyllum* species/populations ($p \geqslant 0.05 = -$; $p<0.05 = *$, $p<0.01 = **$, $p<0.001 = ***$).

	Corolla tube length		Corolla diameter		Total flower length		Ovary length		Stigma level		Stigma lobe length		Anther level		Anther length		Anther–stigma separation	
	F	*p*	*F*	*P*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
A. thymifolium	8.73	**	1.17	—	0.04	—	6.98	*	124.15	***	9.65	**	205.40	***	0.52	—	496.51	***
A. capitatum	4.97	*	46.48	***	5.84	*	13.23	**	177.14	***	4.63	*	215.07	***	6.44	*	335.60	***
A. filiforme	0.75	—	0.54	—	0.20	—	0.68	—	25.27	***	7.24	*	2.97	—	0.07	—	66.58	***
A. ciliolatum	20.66	***	2.64	—	0.68	—	2.17	—	152.53	***	0.32	—	95.94	***	0.10	—	486.04	***
A. rivetii	8.69	**	33.44	***	24.57	***	0.30	—	86.98	***	13.87	**	44.14	***	5.88	*	237.06	***
A. aristatum	0.85	—	0.00	—	4.31	*	1.69	—	56.88	***	166.38	***	135.56	***	0.36	—	232.01	***
A. lavarum	9.51	**	0.14	—	8.35	**	6.74	*	114.86	***	0.46	—	91.38	***	0.96	—	417.46	***
A. setosum	1.07	—	0.39	—	5.07	*	1.01	—	119.15	***	2.19	—	37.33	***	0.07	—	330.99	***
A. macbridei (Peru)	50.15	***	8.33	**	25.53	***	0.76	—	263.32	***	0.05	—	174.37	***	5.13	*	499.01	***
A. macbridei (Ec.)	1.05	—	22.04	***	0.05	***	11.55	**	396.62	***	13.39	***	183.35	***	9.69	**	715.76	***
A. vernicosum	0.47	—	0.00	—	8.28	—	26.86	***	108.61	***	15.54	***	72.55	***	13.38	**	169.97	***

Scent, flower color and nectar

The flowers of *A. macbridei* (Ec) emitted a very intensive, sweet, perfume-like fragrance, whereas those of *A. macbridei* (Peru) were odorless to human noses. The flowers of *A. lavarum* were also odorous but less intensely so. All other species did not produce a scent recognizable to humans. The corolla color was pink in *A. macbridei* (Ec), whereas *A. macbridei* (Peru) and all other species had a white corolla.

Arcytophyllum species showed a large variability in nectar sugar composition (Table 7). Most species contained hexose-rich nectar according to the classification of Baker and Baker (1983), which might be the ancestral condition for the genus. Nectar sugar composition is sucrose-rich in *A. macbridei* and sucrose-dominant in *A. vernicosum.* Corolla tube length and sugar ratio were significantly positively correlated ($r = 0.52$, $t = 2.61$, $p = 0.0175$). There was also a significant positive correlation between corolla tube length and the percentage of sucrose ($r = 0.51$, $t = 2.52$, $p = 0.0214$). Within the morphs, nectar sugar composition is more or less similar (Table 7). Nectar volume of unbagged flowers was similar within the morphs; significant differences were found only in *A. lavarum* and *A. ciliolatum.* Nectar concentration averaged $28.5 \pm 10.2\%$ [wt/total wt], calculated from 20 nectar samples of both morphs in the 10 investigated species/populations.

Pollinator activity

The main floral visitors on the species studied were Hymenoptera, Diptera and Coleoptera. Thrips were often present in the corolla tubes of all investigated species. Low floral visitation frequency (less than one record per hour) was observed in *A. aristatum*, *A. rivetii* and *A. filiforme*, whereas the flowers of *A. setosum* received, on average, six visits per hour (Table 8). Insect activity was very irregular, and dependent on good weather conditions. Highest visitor frequency was observed in dry weather in the late morning.

Discussion

Phylogeny

The recent interest in *Arcytophyllum* is a striking example for the wealth of information emerging on a group of organisms as soon as its basic taxonomic structure has been worked out and it becomes possible to identify its members by means of a morphological key (in this case Mena 1990).

In the present paper a range extension is recorded for *A. macbridei*, which was known only from the Department of Amazonas in Peru (Mena 1990). The collections from Cajanuma and Fiero Urco were first considered a new species, because they differ from the known collections of *A. macbridei* in shorter lamina length (4–6 mm) and shorter stipular teeth (0.2–1.5 mm). In addition, the flowers emit a sweet scent, whereas the Peruvian individuals are odorless. However, analysis of the other characters shows that the population indeed belongs to *A. macbridei*, a result also supported by the low sequence difference of 2 bp in ITS.

The large ITS sequence divergence between the two populations of *A. thymifolium* may be due to the wide geographical and altitudinal range of the species (Colombia to Peru, 400–4000 m; Mena 1990). Monachino (1954) described a variety of *A. thymifolium*, var. *scolnikii* Monach., from Cajamarca, Peru. However, the

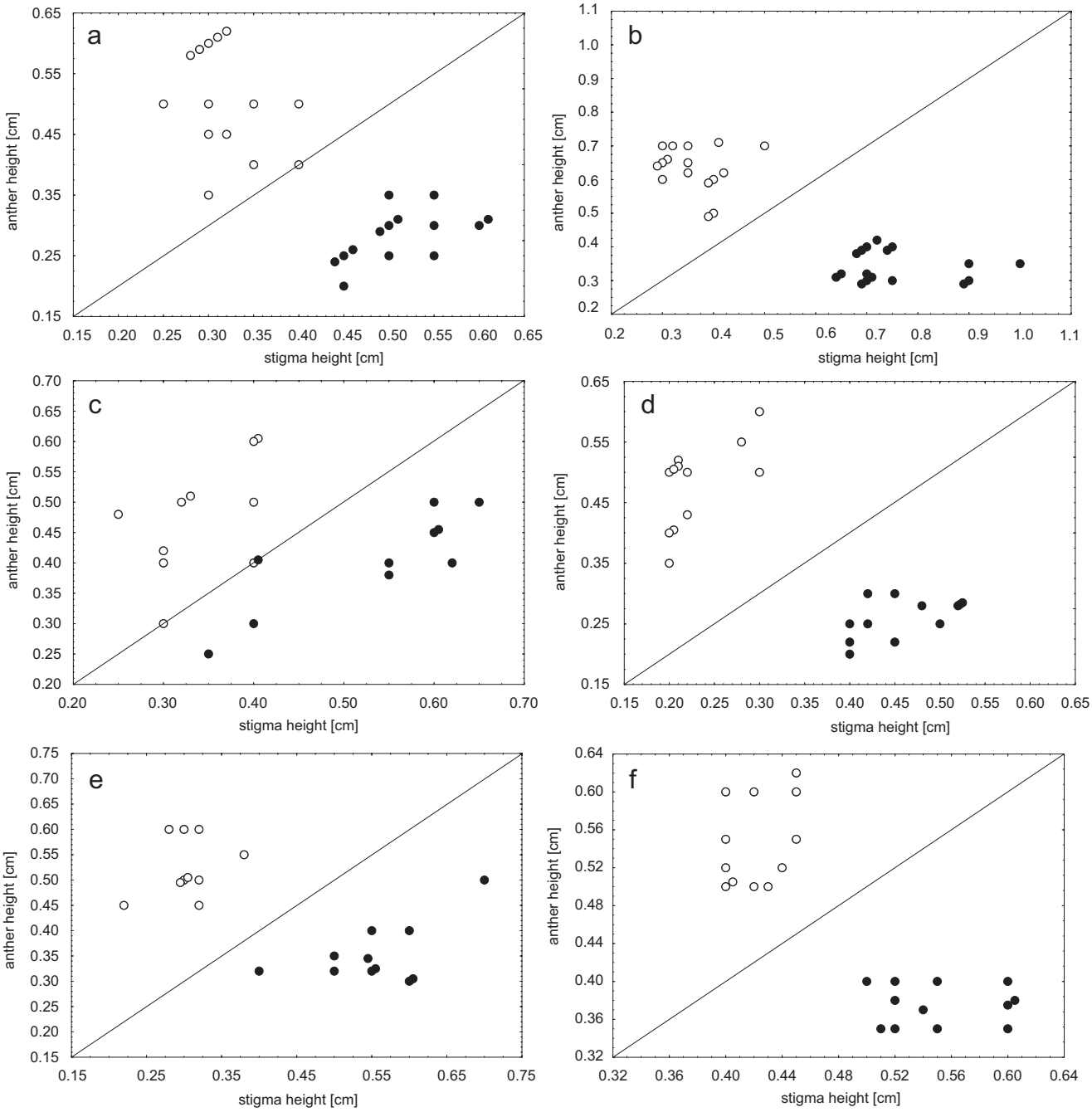

Fig. 5. Anther–stigma separation. Arrangement of species according to the phylogeny in Fig. 4; open circles = short-styled morph, solid circles = long-styled morph. (a) *Arcytophyllum thymifolium*. (b) *A. captitatum*. (c) *A. filiforme*. (d) *A. ciliolatum*. (e) *A. rivetii*. (f) *A. aristatum*. (g) *A. lavarum*. (h) *A. setosum*. (i) *A. macbridei* (Peru). (k) *A. macbridei* (EC). (l) *A. vernicosum*.

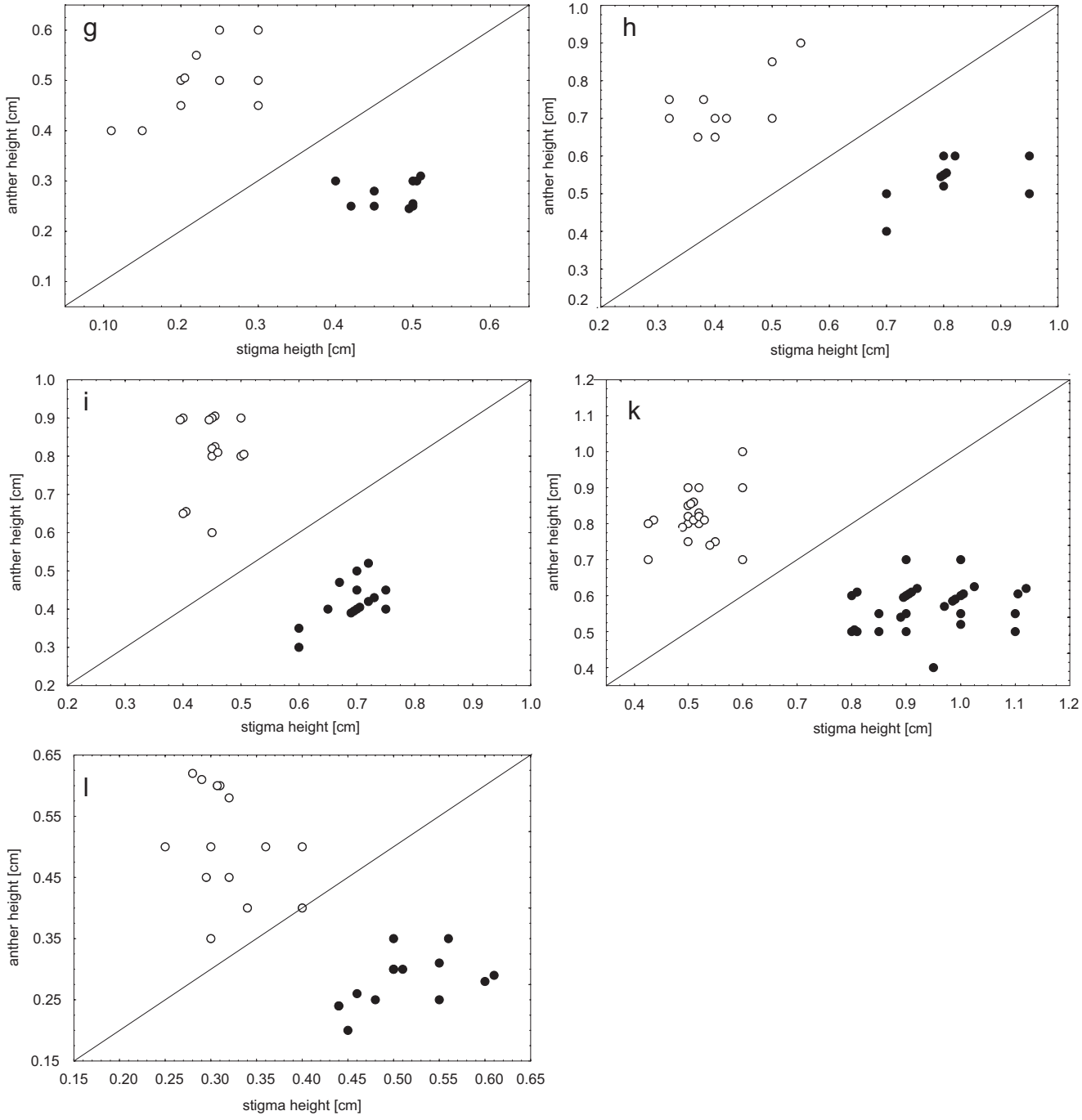

Fig. 5. (*Continued*)

main distinguishing character of var. *scolnikii* is its prostrate growth form, which does not correspond with our Peruvian collection. Mena (1990) subsumed var. *scolnikii* under *A. thymifolium* and described this species as certainly the most variable of all *Arcytophyllum* species. However, in order to suggest a subdivision of *A. thymifolium*, a larger sample of populations would be necessary. *Arcytophyllum ciliolatum*, *A. ericoides* and *A. rivetii* are geographically restricted to southern Ecuador and northern Peru, and form a well-supported clade in our combined analysis (Fig. 4), confirming the results of Andersson et al. (2002). The *A. capitatum*/*A. filiforme* and *A. nitidum*/*A. setosum* clades are formed by one northern and one southern species each. *A. capitatum* occurs in Colombia and Ecuador, whereas *A. filiforme* ranges from Ecuador to Bolivia; *A. nitidum* exists in Venezuela and Colombia, whereas *A. setosum* is distributed from Colombia to Bolivia (Mena 1990). It might be hypothesized that both the *A. capitatum*/*A. filiforme* and the *A. nitidum*/*A. setosum* pairs of sister

Table 4. Pearson correlation on corolla tube length versus anther height and stigma height, respectively, for each morph of 11 *Arcytophyllum* species/populations ($p \geqslant 0.05 = -$; $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$).

	Morph	Corolla tube length							
		Versus anther height				Versus stigma height			
		$r(X \cdot Y)$	r^2	t	p	$r(X \cdot Y)$	r^2	t	p
A. thymifolium	ls	−0.18	0.03	−0.54	—	0.07	0.01	0.22	—
A. thymifolium	ss	0.80	0.64	3.79	**	0.24	0.06	0.70	—
A. capitatum	ls	0.04	0.00	0.15	—	−0.17	0.03	−0.66	—
A. capitatum	ss	0.71	0.51	3.79	**	−0.45	0.21	−1.91	—
A. filiforme	ls	0.69	0.47	2.67	*	0.61	0.37	2.18	—
A. filiforme	ss	0.31	0.10	0.92	—	0.44	0.19	1.38	—
A. ciliolatum	ls	0.34	0.12	1.16	—	0.13	0.02	0.42	—
A. ciliolatum	ss	0.60	0.36	2.38	*	0.48	0.23	1.72	—
A. rivetii	ls	0.43	0.18	1.41	—	0.10	0.01	0.29	—
A. rivetii	ss	0.69	0.47	2.69	*	0.31	0.09	0.91	—
A. aristatum	ls	−0.23	0.05	−0.74	—	−0.72	0.52	−3.27	**
A. aristatum	ss	−0.45	0.20	−1.59	—	−0.42	0.17	−1.45	—
A. lavarum	ls	0.18	0.03	0.52	—	0.36	0.13	1.09	—
A. lavarum	ss	0.63	0.39	2.42	*	0.58	0.34	2.15	—
A. setosum	ls	0.62	0.39	2.24	—	0.69	0.47	2.67	*
A. setosum	ss	0.91	0.83	6.33	***	0.43	0.19	1.36	—
A. macbridei (Peru)	ls	0.49	0.24	2.04	—	0.28	0.08	1.05	—
A. macbridei (Peru)	ss	0.37	0.13	1.42	—	0.76	0.58	4.26	***
A. macbridei (Ec.)	ls	0.29	0.09	1.61	—	0.54	0.29	3.42	**
A. macbridei (Ec.)	ss	0.47	0.22	2.43	*	0.37	0.14	1.83	—
A. vernicosum	ls	0.49	0.24	1.92	—	0.63	0.39	2.78	*
A. vernicosum	ss	0.87	0.76	6.11	***	−0.38	0.14	−1.40	—

species are descendents of a respective common ancestor from which speciation occurred due to geographical separation.

Floral morphology

As in most other heterostylous species (Ganders 1979), *Arcytophyllum* flowers are sympetalous, with the stamens inserted into the corolla tube and the filaments partially adnate to it. Unfortunately, we did not measure filament insertion position, which determines anther level in *Hedyotis caerulea* (L.) Hook. (Ornduff 1980). However, there is a significant positive correlation between corolla length and anther position in ss flowers in eight of the 11 investigated species/populations, whereas in ls flowers a correlation of corolla length and stigma level is found in four species/populations (Tables 2, 4). Irregularities were found in *A. macbridei* (Peru) in which corolla length of the ss morphs was significantly correlated with stigma position, and in *A. filiforme* in which corolla length was significantly correlated with anther position in ls flowers. Anther level mainly depends on corolla tube length in other Rubiaceae, e.g. in *Gaertnera vaginata* Lam. (Pailler and Thompson 1997), *Bouvardia ternifolia* (Cav.) Schltdl. and *Psychotria chiapensis* Standl. (Faivre 2000). The facts that stigma lengths are shorter in ls morphs and that ss flowers have longer anthers are similar to many other distylous species, e.g. *Palicourea padifolia* (Willd. ex Roem. & Schult.) C.M. Taylor & Lorence (Rubiaceae; Contreras and Ornelas 1999). Longer corolla tubes on ss flowers, observed in six of 11 investigated *Arcytophyllum* species/populations, are an ancillary feature of the heterostylous syndrome (Ganders 1979; Dulberger 1992) and have been observed in several distylous species of Rubiaceae (Baker 1956; Ornduff 1980; Sobrevila et al. 1983; Feinsinger and Busby 1987; Murray 1990; Richards and Koptur 1993; Passos and Sazima 1995; Riveros et al. 1995; Stone 1995; Pailler and Thompson 1997; Ree 1997; Contreras and Ornelas 1999; Faivre and McDade 2001). Longer corolla diameters and therefore larger flower displays may enhance flower attractiveness; however, significant differences in corolla lengths between the morphs may favor the attractiveness of one morph and lead to reproductive conflict within the species. Therefore, it is interesting that the corolla diameter is significantly larger in ls flowers of *A. capitatum*, *A. rivetii* and *A. macbridei* (both populations). The Rubiaceae contain

Table 5. Pollen grain number per flower, results of one-way ANOVA for differences in number of pollen grains between long-styled (ls) and short-styled (ss) morphs, ratio of pollen grain numbers ls/ss, pollen grain size, results of one-way ANOVA for differences in pollen grain size between morphs, ratio of pollen grain sizes ss/ls, ovule number, results of one-way ANOVA for differences in ovule number between morphs, ratio of ovule numbers ls/ss and pollen-ovule-ratios ls/ss (pollen of ls to ovule of ss), ss/ls (pollen of ss to ovule of ls), ls/ls (pollen of ls to ovule of ls) and ss/ss (pollen of ss to ovule of ss) for 11 *Arcytophyllum* species/populations ($p \geqslant 0.05 = -$; $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$).

	Morph	*n*	Pollen grain number per flower				Pollen grain size (μm)				Ovule number			Pollen–ovule ratios		
			$x \pm$s.d.	*F*	*p*	ls/ss	$x \pm$s.d.	*F*	*P*	ss/ls	$x \pm$s.d.	*F*	*p*	ls/ss	ss/ls	ls/ls ss/ss
A. thymifolium	ls	10	5446±545	66.55	***	1.70	27.1±0.6	193.04	***	1.17	11.3±1.3	8.81	**	633.3	283.0	481.9
A. thymifolium	ss	10	3198±680				31.8±0.9				8.6±2.6					371.9
A. capitatum	ls	20	4938±952	4.71	*	1.13	27.6±1.2	87.63	***	1.15	8.9±1.3	0.06	—	563.6	491.6	558.0
A. capitatum	ss	21	4351±765				31.7±1.6				8.8±0.9					496.6
A. filiforme	ls	10	2712±685	0.25	—	0.95	22.2±0.5	1.65	—	1.02	9.3±2.7	2.90	—	361.6	306.5	291.6
A. filiforme	ss	10	2850±540				22.6±0.7				7.5±2.0					380.0
A. ciliolatum	ls	10	7442±2017	12.46	**	1.52	26.2±0.9	19.75	***	1.07	14.3±2.5	0.04	—	509.7	341.8	520.4
A. ciliolatum	ss	10	4888±1081				28.1±1.0				14.6±3.7					334.8
A. rivetii	ls	10	6672±926	14.00	**	1.28	24.2±0.7	26.97	***	1.12	6.3±0.8	3.53	—	1191.4	825.7	1059.0
A. rivetii	ss	10	5202±828				27.1±1.6				5.6±0.8					928.9
A. aristatum	ls	10	5266±894	5.50	*	1.17	23.6±1.5	56.47	***	1.13	13.0±2.7	0.01	—	407.3	344.8	405.1
A. aristatum	ss	14	4483±741				26.7±0.4				12.9±1.0					346.7
A. lavarum	ls	11	6098±864	4.87	*	1.17	27.5±1.1	43.87	***	1.15	10.8±1.6	9.20	**	432.5	483.4	563.7
A. lavarum	ss	10	5230±940				31.5±1.7				14.1±3.2					370.9
A. setosum	ls	15	7532±2141	4.87	*	1.20	33.0±1.7	17.43	***	1.07	15.1±3.7	0.15	—	484.6	558.0	497.7
A. setosum	ss	24	6293±1377				35.4±1.8				15.5±2.8					404.9
A. macbridei (Peru)	ls	10	12372±2700	9.71	**	1.29	29.3±0.6	39.66	***	1.08	22.9±3.0	4.95	*	609.5	419.5	540.3
A. macbridei (Peru)	ss	10	9608±760				31.6±1.0				20.3±2.2					473.3
A. macbridei (Ec.)	ls	10	13866±1180	4.39	*	1.10	27.1±0.6	100.82	***	1.14	24.9±2.6	14.89	**	684.0	506.7	556.9
A. macbridei (Ec.)	ss	11	12616±1513				31.0±1.1				20.3±2.9					622.3
A. vernicosum	ls	12	8062±882	8.47	**	1.28	25.3±1.3	33.44	***	1.10	24.3±3.7	6.29	*	286.9	259.5	332.5
A. vernicosum	ss	10	6292±1741				27.9±0.5				28.1±3.4					223.9

Table 6. Number of seeds per fruit, results of one-way ANOVA for differences in number of seeds per fruit between long-styled (ls) and short-styled (ss) morphs, and percent seed set for 11 *Arcytophyllum* species/populations ($p \geq 0.05 = -$; $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$).

	Morph	Seeds per fruit			Seed set (%)
		$x \pm$ s.d.	F	p	
A. thymifolium	ls	9.3 ± 2.4 (26)	1.71	—	82.3
A. thymifolium	ss	8.4 ± 2.5 (25)			97.7
A. capitatum	ls	3.9 ± 2.1 (21)	0.54	—	43.8
A. capitatum	ss	4.4 ± 2.1 (21)			50.0
A. filiforme	ls	4.7 ± 2.4 (21)	0.57	—	50.5
A. filiforme	ss	5.2 ± 2.1 (21)			69.3
A. ciliolatum	ls	8.3 ± 4.1 (23)	1.11	—	58.0
A. ciliolatum	ss	9.5 ± 3.5 (21)			65.1
A. rivetii	ls	5.4 ± 1.4 (19)	0.36	—	85.7
A. rivetii	ss	5.0 ± 1.6 (21)			89.3
A. aristatum	ls	7.0 ± 3.2 (17)	8.60	**	53.8
A. aristatum	ss	10.2 ± 3.2 (17)			79.1
A. lavarum	ls	9.5 ± 2.3 (26)	12.16	***	88.0
A. lavarum	ss	12.7 ± 4.2 (29)			90.1
A. setosum	ls	12.5 ± 5.2 (22)	0.21	—	82.8
A. setosum	ss	13.1 ± 4.6 (23)			84.5
A. macbridei (Peru)	ls	12.4 ± 5.1 (20)	0.78	—	54.1
A. macbridei (Peru)	ss	11.0 ± 5.1 (22)			54.2
A. macbridei (Ec.)	ls	12.5 ± 6.3 (26)	0.15	—	50.2
A. macbridei (Ec.)	ss	13.2 ± 5.8 (27)			65.0
A. vernicosum	ls	15.2 ± 5.2 (20)	32.56	***	62.6
A. vernicosum	Ss	24.1 ± 4.8 (21)			85.8

more heterostylous genera than all other Angiosperm families combined (Ganders 1979). Many heterostylous Rubiaceae show similar patterns of differences between ss and ls flowers as discussed above. These species, however, do not form a monophyletic group. The distribution of heterostyly in the family indicates that it is unlikely that heterostyly has evolved only once in all rubiaceous taxa that share these characteristics (Anderson 1973; Faivre and McDade 2001). Graham and Barrett (2004) came to the same conclusion for heterostyly in *Narcissus* (Amaryllidaceae). Within the genus *Arcytophyllum* the morphological expression of heterostyly cannot be deduced from the phylogenetic position of a species. For example, in the well-supported species pair *A. capitatum* and *A. filiforme*, the floral morphs of *A. capitatum* differ significantly in all nine morphological features investigated, whereas the floral morphs of *A. filiforme* show significant differences only in stigma level, stigma lobe length and anther-stigma separation. Likewise, in the well-supported species triplet *A. ciliolatum*, *A. rivetii* and *A. ericoides* (not studied), the morphs of *A. rivetii* show significant differences in all investigated features (except ovary length), whereas the morphs of *A. ciliolatum* differ only in corolla tube length, stigma level, anther level and anther-stigma separation. In the two populations of *A. macbridei*, corolla tube length and total length were not significantly different in ls and ss morphs of the Ecuadorian population, whereas they were significantly different in the Peruvian population. On the other hand, ovary and stigma lobe lengths did not differ significantly between ls and ss morphs in the Peruvian population, but did in the Ecuadorian one. Features in the expression of heterostyly may thus vary among closely related species and among populations, and show a great variability of floral morphology. The fact that the majority of floral morphological differences between the two morphs, which were observed in other species, are missing in the probably self-pollinated species *A. filiforme* indicates that there might be considerable selective pressure on the distylous features of floral morphology by the pollinators. Homostyly as a derived condition in *Arcytophyllum* is not a singular phenomenon, but one also found in a study of *Turnera* (Truyens et al. 2005).

Pollen dimorphism, P/O ratio and seed set

Pollen dimorphisms in number (ls > ss) and/or size (ss > ls) have been observed in several Rubiaceae: *Hedyotis* (Ornduff 1980; Riveros et al. 1995) and *Palicourea* Aubl. (Sobrevila et al. 1983; Contreras and Ornelas 1999). Ls flowers usually produce more pollen

Table 7. Nectar sugar composition, sugar ratio, nectar sugar concentration, nectar volume and results of one-way ANOVA for differences in nectar volumes between long-styled (ls) and short-styled (ss) flowers of 11 *Arcytophyllum* species/populations ($p \geqslant 0.05 = —$; $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$).

	Morph	Fructose (%)	Glucose (%)	Saccarose (%)	Sugar ratio	Nectar conc. (%wt/wt)	Nectar volume		
							$x \pm$s.d. (µl) (n)	F	p
A. thymifolium	ls	44.8	42.8	12.4	0.14	37	0.36±0.18 (5)	2.30	—
A. thymifolium	ss	45.2	43.8	11.1	0.12	14	0.17±0.15 (3)		
A. capitatum	ls	46.1	44.7	9.2	0.10	42.5	0.19±0.16 (25)	0.67	—
A. capitatum	ss	38.9	43.9	17.2	0.21	24.0	0.15±0.13 (17)		
A. filiforme	ls	30.8	36.3	33.0	0.49	32.0	0.18±0.14 (10)	0.18	—
A. filiforme	ss	35.4	38.0	26.6	0.36	49.0	0.16±0.1 (10)		
A. ciliolatum	ls	37.5	38.9	23.5	0.31	30	1.26±1.01 (20)	15.99	***
A. ciliolatum	ss	37.9	37.2	24.9	0.33	20	0.41±0.26 (24)		
A. rivetii	ls	32.7	35.3	32.0	0.47	22	0.40±0.18 (6)	0.23	—
A. rivetii	ss	33.6	35.3	31.2	0.45	33.5	0.47±0.3 (6)		
A. aristatum	ls	—	—	—	—	—	0.15±0.14 (10)	1.22	—
A. aristatum	ss	—	—	—	—	—	0.14±0.1 (8)		
A. lavarum	ls	37.8	39.6	22.6	0.29	32	0.24±0.11 (12)	18.94	***
A. lavarum	ss	34.4	35.0	30.6	0.44	36	0.52±0.2 (12)		
A. setosum	ls	34.4	40.6	25.0	0.33	13.5	0.58±0.37 (10)	1.22	—
A. setosum	ss	32.5	35.3	32.3	0.48	13	0.4±0.33 (10)		
A. macbridei (Peru)	ls	30.2	27.8	42.1	0.73	22	1.17±0.51 (10)	4.21	—
A. macbridei (Peru)	ss	29.0	27.5	43.5	0.77	38	0.8±0.29 (11)		
A. macbridei (Ec.)	ls	28.6	23.3	48.1	0.93	28	0.58±0.63 (14)	1.13	—
A. macbridei (Ec.)	ss	29.7	25.0	45.3	0.83	40	0.89±0.97 (22)		
A. vernicosum	ls	22.5	24.0	53.5	1.15	24	0.6±0.28 (10)	0.07	—
A. vernicosum	ss	20.9	24.0	55.1	1.23	20	0.55±0.47 (15)		

Table 8. Observed flower visits and composition of visitor fauna for 11 *Arcytophyllum* species/populations

	Observ. time (h)	Visits/15 min $x \pm$ s.d. (n)	Visits/h	Coleoptera (%)	Hymenoptera (%)	Diptera (%)
A. thymifolium	6	0.38 ± 0.65 (24)	1.50	33.3	66.7	0.0
A. capitatum	10.5	0.74 ± 1.33 (42)	2.95	19.4	80.6	0.0
A. filiforme	4	0.19 ± 0.54 (16)	0.75	0.0	0.0	100.0
A. ciliolatum	5	0.64 ± 0.99 (20)	2.60	15.4	84.6	0.0
A. rivetii	3	0.17 ± 0.39 (12)	0.67	0.0	50.0	50.0
A. aristatum	3	0.0 ± 0.0 (12)	0.00	0.0	0.0	0.0
A. lavarum	4.25	0.59 ± 0.94 (17)	2.35	20.0	50.0	30.0
A. setosum	4	1.5 ± 1.59 (16)	6.00	8.3	91.7	0.0
A. macbridei (Peru)	3	0.33 ± 0.65 (12)	1.33	0.0	100.0	0.0
A. macbridei (Ec.)	6	0.67 ± 0.92 (24)	2.67	0.0	100.0	0.0
A. vernicosum	5.25	0.81 ± 1.08 (21)	3.24	29.4	58.8	11.8

than ss flowers, the ls/ss ratio (from 0.95 in *A. filiforme* to 1.7 in *A. thymifolium*) ranges at the lower end of distylous species reported by Ganders (1979; ls/ss ratio 1.13–3.12). The ratio of ss to ls morph pollen size in *Arcytophyllum* ranges from 1.02 to 1.17, which is similar to *H. caerulea* (1.2, Ornduff 1977) and *H. salzmannii* (DC.) Steud. (1.31; Riveros et al. 1995), and fits well into the range reviewed by Dulberger (1992), with values from 1.06 to 1.80. Pollen number and size differed markedly between closely related species, e.g. *A. filiforme* and *A. capitatum*, while large variation in ovule numbers was observed between *A. ciliolatum* and *A. rivetii*. There is no evidence that the numbers of pollen or ovules are under phylogenetic constraints, as also found by Wyatt et al. (2000) for Apocynaceae–Asclepiadoideae. Environmental factors have great impact on pollen number and/or size, which is negatively affected by low nutrient content of the soil, as shown by experimental results (Lau and Stephenson 1993, 1994) or by the loss of leaves (Frazee and Marquis 1994; Quesada et al. 1995; Aizen and Raffaele 1998; Lehtilä and Strauss 1999).

It is noteworthy that individuals with stigmas and anthers at the same level (homostylous) occurred in populations of *A. filiforme* and *A. vernicosum* (lowest P/O ratios), and of *A. thymifolia*. However, these three species occur in separate clades (Figs. 2–4), so that a phylogenetic tendency toward homostyly cannot be assumed. According to Cruden (1977), the breeding system ranges from facultatively autogamous (*A. vernicosum*) to facultatively xenogamous (*A. rivetii*). The small size of *A. filiforme* and *A. aristatum* (both are mat forming) and their solitary flowers may result in low pollinator activity. Interestingly, *A. thymifolium*, which is sister to all other species studied, is at the same time the most widespread one and the one with the highest seed set (98%) in the ss morph. The tendency in all species/populations (except for *A. macbridei* Peru and *A. rivetii*) *A. rivetii* that the ss morph had a higher percentage of seeds per ovules confirms the results of Garcia-Robledo and Mora-Kepfer (2004) on *A. lavarum* from Costa Rica, that the ss morphs display higher female reproductive success.

Nectar

The relationship between longer corolla tubes and higher sucrose proportions in the nectar reported here for *Arcytophyllum* corresponds to the results of Torres and Galetto (2002) in Asteraceae flowers. There is a tendency toward a higher percentage of sucrose in the species of the terminal clades, with the species pair *A. macbridei* and *A. vernicosum* showing high sucrose proportions. In contrast, *A. thymifolium*, the sister to all remaining species, has a very low sucrose/hexose ratio. Our classification of flower visitors is quite rough, and we did not measure the proboscis lengths of the flower visitors. Therefore, we can neither judge whether the observed visitors act as pollinators nor whether the nectar is composed according to the pollinators' preferences. Apart from the possible phylogenetic implications of nectar sugar content, the morphological features associated with heterostyly do not show any phylogenetically interpretable pattern, nor does the P/O ratio (Fig. 4; Tables 3, 5).

While the basic features of distyly are investigated here, many unresolved questions for further research remain. Detailed comparisons between populations, concerning morph ratios within populations, pollen carryover, stigmatic pollen loads and controlled pollination experiments for some of the species studied are presently being carried out.

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