Pollination Biology of Gentianales in a Southern Ecuadorian Montane Forest

Dissertation

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1 General Introduction

It has been recognized that the flora of the Andes is far richer in species than the flora of the much larger Amazon basin (Henderson *et al.* 1991). Neotropical montane forests generally are characterized by their high biodiversity, caused by the presence of large numbers of endemic taxa (Churchill *et al.* 1995, Luna-Vega *et al.* 2001). Unfortunately, these "hotspots of biodiversity" are greatly threatened. Disturbances caused by human activities restrict these communities to nearly inaccessible slopes. According to Hamilton *et al.* (1995), the Ecuadorian Andes have lost 90% of their original forest cover. Nevertheless, Balslev (1988) estimates that half of the Ecuadorian plant species grow at elevations between 900 m and 3000 m. The southern Ecuadorian Andes (especially the region of the Podocarpus National Park) is an outstanding hotspot of biodiversity (Barthlott *et al.* 1996, Myers *et al.* 2000, Brummitt and Lughadha 2003) and, according to Pitman *et al.* (2000), the Podocarpus National Park is the protected area with the largest number of endemic plants in the country. An understanding of these threatened forests is essential to their conservation. This dissertation forms part of an interdisciplinary research project established in 1997 in southern Ecuador (Beck and Müller-Hohenstein 2001), investigating the diversity and functioning of a montane rainforest system.

An outstanding feature of the Angiosperms is the amazing diversity in form and color of the flowers. Flowers inspired great art for centuries, fuel a major industry, and give pleasure to us at windows, in gardens and parks, and even serve as a solace for suffering mankind. Yet, the flower is merely a sex organ, having no other function than promoting reproduction by seed. The beautiful, weird, sinister, astounding forms into which flowers have developed are strictly pragmatic, and have encouraged the ecological diversification and dominance of the flowering plants (Richards 1997).

While the primitive flower types are visited only for pollen, and the most primitive flower visitors cannot utilize nectar, specialized flower visitors (e.g., bees, wasps, butterflies, moths, birds, and bats) crucially depend on the production of nectar by many flower types (Richards 1997). The first nectar feeders probably utilized stigmatic secretions. Sophisticated flowers, such as Asclepiadoideae, which receive pollinia within stigmatic cavities, may also

produce large quantities of nectar in these cavities to be utilized by visitors (Kevan *et al.* 1989). Nectar is essentially a phloem secretion, in which sucrose usually predominates, with fructose and glucose also present (Richards 1997, Pacini *et al.* 2003, De la Barrera and Nobel 2004). The amount and activity of invertases determines the relative nectar concentration of sucrose versus its hexose components, fructose and glucose (Elias *et al.* 1975, Nicolson 2002, De la Barrera and Nobel 2004). Floral nectar drives pollination efforts as the primary floral reward for most pollinators in angiosperms (Simpson and Neff 1983). The behavior of pollinators strongly influences pollen flow, and the ways that plants have adapted to these behavioral characteristics play an overriding role in gene dispersal, and the genetic structure of plant populations (Richards 1997). Reproductive features are very important in the explanation of the general patterns of diversity and community structure encountered in tropical montane forests (Baker 1959, Ashton 1969, Bawa 1974, Kaur *et al.* 1978, Bawa 1990, Oliveira and Gibbs 2000), with the underlying idea that plant diversity and spatial distribution is dependent on reproductive processes (Heithaus 1974). In order to understand the coadaptation of zoophilous plants to pollinators with respect to gene flow, field observations on phylogenetically related taxa are necessary.

The order Gentianales comprises the families Apocynaceae (including Asclepiadoideae), Gelsemiaceae, Gentianaceae, Loganiaceae, and Rubiaceae (Backlund *et al.* 2000). Members of the Gentianales share several vegetative, floral, and phytochemical traits. The majority of members of the order are woody with internal phloem, opposite, entire leaves, often with stipules and colleters, generally with regular pentamerous flowers, nucleate endosperm formation, and frequent occurrences of indole alkaloids (Schumann 1891, Schumann 1895, Gilg 1895, Hakki 1980, Kisakürek and Hesse 1980). The Gentianales comprise almost all size classes and growth forms from small alpine herbs to large rain forest trees, including many ornamentals and economically important plants (e.g., *Catharanthus*, *Cinchona*, *Coffea*, *Gardenia* and *Strynchnos*).

Phylogenetic studies indicate that there are two major evolutionary lineages within the monophyletic order, one comprising the families Gentianaceae, Apocynaceae, Gelsemiaceae, and Loganiaceae, and the other consisting solely of the Rubiaceae (Backlund *et al.* 2000). This dichotomy is also supported by the occurrence of superior ovaries and internal phloem in the Gentianaceae-Apocynaceae-Gelsemianaceae-Loganiaceae lineage, as well as by differences in biosynthesis of iridoid and indole alkaloid compounds (Jensen 1991, 1992). The Rubiaceae are world-wide distributed with a distinct tropical preference. The family compromises approximately 10200 species (Mabberley 1997) and is considered a

monophyletic group, consisting of three well-defined subfamilies. The cosmopolitan family Gentianceae consists mostly of herbs, rarely shrubs or trees, and comprises approximately 1200 species (Mabberley 1997). It occurs predominantly at high elevations in mountain systems around the world. The generic composition of the smallest Gentianales family, Loganiaceae, is still a matter of debate (Struwe *et al.* 1994, Leeuwenberg 1997); however, the family is absent from the study area. Most members in the Apocynaceae are tropical or subtropical, and the family consists of approximately 4800 species in five subfamilies (Endress and Bruyns 2000). Many systematists have argued that the pollinia-forming Asclepiadoideae should be recognized as family Asclepiadaceae (Cronquist 1981, Takhtajan 1987, Rosatti 1989a, 1989b, Nicholas and Baijnath 1994), but it has been shown that this group of taxa represents the most derived clade within the monophyletic Apocynaceae (Sennblad and Bremer 1996, Sennblad 1997, Backlund *et al.* 2000, Potgieter and Albert 2001).

Rubiaceae range among the most predominant Andean families in floristic studies [e.g., Gentry 1988 (high Andes), Madsen and Øllgaard 1994 (southern Ecuador non-ridge forest 2900 m), Jørgensen and León-Yánez 1999 (Ecuador 1500–2500 m), Webster and Rhode 2001 (northern Ecuador 1100–2800 m), Dorr *et al.* 2000 (Venezuela 1500–2800 m), Homeier 2004 (southern Ecuador, 1800–2400 m)]. Nearly 100 of the approximately 500 species of Rubiaceae occuring in Ecuador are endemic to the country. Every other Asclepiadoideae species (42 of 85 species), and over 40% of Gentianaceae (28 of 65 species) in Ecuador are endemic (Pitmann *et al.* 2000). According to Grant and Struwe (2003) the Podocarpus National Park represents one of the areas of highest species diversity for *Macrocarpaea* (Gentianaceae), and the study site is the only locality in Ecuador where four species occur sympatrically. Besides the large number of species in the Gentianales, the order was chosen because it includes all major life forms, such as trees, shrubs, herbs, and vines, and the flowers show morphological adaptations to ornithophily, melittophily, myiophily, chiropterophily, and sphingophily.

The reproductive system in Gentianales is quite diverse and includes a large proportion of heterostylous species. In these species, two distinct hermaphroditic floral morphs coexist in populations at roughly equal frequencies, with flowers having reciprocally placed anthers and stigmas (reciprocal herkogamy). In the long-styled morph, the anthers are often sunken in the corolla tube, in the short-styled one the anthers are positioned at the mouth of the corolla tube. Typically, these morphs are cross-compatible, but lack intramorph compatibility. This dialectic incompatibility system, which prevents self- and intramorph

pollination (Ganders 1979, Barrett 1992), has been reported in more than 90 genera of Rubiaceae (Bir Bahadur 1968, Ganders 1979). Because about half the individuals in a dimorphic species are unavailable for mating, heteromorphy should be a rather inefficient outcrossing mechanism (Richards 1997). However, Darwin (1877) proposed that the heterostyly should encourage legitimate pollen flow between the morphs, thus increasing the efficiency of pollen usage.

In the gynostegium-forming Apocynaceae-Asclepiadoideae, both dichogamy and herkogamy prevail. Flowers are pollinated by a complex pollination mechanism, in which one of the two pollinia of a pollinarium is inserted into one of the five guide rails, each formed by two adjacent anther wings (e.g., Kunze 1991, 1995). Legs or proboscis of visitors are trapped in these guide rails and the struggle for release leads to pollinium attachment. Whereas this general mechanism of pollination is quite well understood, information on specific pollination processes and pollinators is rare and focuses on just a few of the ca. 3000 species accepted in Asclepiadoideae (cf. Ollerton and Liede 1997, Meve 2002). In general, most tropical plant reproductive biology investigations focus on conspicuous flowers and specialized animal-plant interactions, while flowers with small and/or rather unspecialized morphologies receive less attention. This study investigates the reproductive biology of more than 50 mostly small-flowered species and various degrees of specialization.

This dissertation comprises four manuscripts:

1. Nocturnal versus diurnal pollination success in Isertia laevis (Rubiaceae)

Pollination syndromes are suits of floral traits proposed to reflect adaptations to one or another pollinator type (Waser *et al*.1996). Flowers that are adapted primarily for pollination and feeding by hummingbirds tend to have mostly thick-walled, tubular, vivid red or yellow colored corollas, with large quantities of nectar accumulating at the base of the corolla tube (Faegri and van der Pijl 1979). Flowers adapted primarily to pollination and feeding of sphingids have a longer, more slender floral tube than "hummingbird-flowers" (Grant and Temeles 1992). Furthermore, the flowers possess white to pale colored corollas, emitting an intense perfume-like scent and are usually open in the late evening or at night (Silberbauer-Gottsberger and Gottsberger 1975, Faegri and van der Pijl 1979). Floral characteristics and their relevance for the interaction of flower and pollinators were investigated in *Isertia laevis* (Rubiaceae). Comparisons of diurnal and nocturnal patterns of nectar availability in covered and uncovered flowers were conducted in the natural habitat. In order to examine pollination

efficiency by hummingbirds and sphingids, two types of enclosure experiments were performed to exclude either diurnal or nocturnal visitors. A comparison of visitor effectiveness is interesting because it is assumed that the most effective pollinator may have an overriding selective influence on floral morphology.

2. Pollination in the small flowering Asclepiadoideae (Apocynaceae)

Pollination of Asclepiadoideae is remarkably little studied (Ollerton and Liede 1997), except for some species of American *Asclepias*. However, *Asclepias* is untypical for Asclepiadoideae in several respects. The plants are erect, possess many-flowered inflorescences of rather large (ca. 1 cm diam.), often brightly colored flowers, and occur in rather numerous individuals in relatively small areas of American grasslands. The average member of the family, in contrast, is a twiner with few-flowered inflorescences of small, whitish or dull colored flowers, and occurs in very few individuals in large areas in tropical and subtropical forest margins and scrubs. To date, only more or less casual pollination observations are available for the latter type of Asclepiadoids (e.g., Liede 1994) and the present study is the first to attempt a comparison of strategies between several members of the subfamily growing in the same area. These strategies have frequently been hypothesized as motor of the extreme homology in floral morphology between phylogenetically only distantly related taxa of the subfamily and are therefore fundamental to the understanding of the evolution of the subfamily.

To resolve how small flowered species of Asclepiadeae are pollinated, pollinarium removal and insertion rates were calculated for nine species, and floral visitors were observed. Reproductive phenology was monitored for two years. Additional data on nectar sugar composition of the Asclepiadoideae, excluded from the analysis mentioned below (manuscript 3) because of their special pollination system, were included.

3. Nectar sugar composition and volumes of Gentianales

Nectar is the most important reward from flowers pollinated by animals (Simpson and Neff 1983). Although nectars contain a wide variety of chemical constituents such as proteins, lipids and amino acids (e.g., Baker and Baker 1975, 1982; Gottsberger *et al*. 1984), three sugars (sucrose, glucose and fructose) dominate the total dissolved materials in floral nectar and represent the major energy source for visitors. Of special interest is whether nectar features, such as nectar sugar composition, nectar concentration, and nectar volume, are related to the type of pollinator, or whether they are relatively constant within taxonomically

related species groups. Nectar volumes were sampled from covered (24 h production) and uncovered (standing crop) flowers of 47 taxonomically related plant species (Gentianaceae and Rubiaceae) in the natural habitat. Sucrose, fructose, and glucose were quantified in the nectar using high performance liquid chromatography. Nectar concentration and composition, volume of covered and uncovered flowers, and flower visitors of 47 plant species from such a hitherto data-scarce region are presented here. Nectar features were linked to floral visitors and pollination syndromes observed.

4. Phylogeny and Reproductive Biology of the distylous Arcytophyllum (Rubiaceae)

A phylogenetic study of *Arcytophyllum* based on an additional marker Internal Transcribed Spacer (ITS) was conducted and compared with an earlier study based on cpDNA (Andersson *et al.* 2002) in order to improve the previously obtained phylogenetic results. Floral visitors and floral morphology of ten heterostylous species in eleven populations were investigated. Charlesworth and Charleswoth (1979) hypothesized that heterostyly would evolve in populations with high levels of inbreeding depression. Therefore, the breeding system was classified by using pollen-ovule ratio (Cruden 1977). If floral morphological variation and nectar sugar composition have a strong phylogenetic component, one would expect closely related species to be similar in the expression of heterostyly and in their nectar sugar ratio. Different expressions of heterostyly, pollen-ovule ratio, and nectar sugar composition are interpreted in the light of phylogeny.

The aforementioned ecological aspects of animal-plant-interactions, as well as the evolutionary questions concerning floral morphology and nectar features have rarely been addressed explicitly for such a large number of taxa, that is both phylogenetically and spatially defined. This study reports new data on floral biology which are important for the understanding of the ecosystem of the highly threatened Andean montane forests.

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2 Nocturnal versus diurnal pollination success in *Isertia laevis* (Rubiaceae): a sphingophilous plant visited by hummingbirds

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2.1 Abstract

Isertia laevis (Rubiaceae) possesses flowers with traits typical for the pollination syndrome of sphingophily. Diurnal flower observation showed that nine different hummingbirds (*Trochilidae)* and one flower piercer (*Coerebidae)* were frequent visitors. Their activity on the flowers peaked in the morning hours. Very low nectar volumes were found in the morning (8.00 h) in unbagged flowers. Nectar volumes, however, reached their peaks (27 µl) at night (2.00 h) in bagged as well as in unbagged flowers. At night few individuals of sphingids were observed. Pollination experiments showed that flowers presented to nocturnal pollinators from 18.00 h to 6.00 h had low fruit set (14%) but high seed set (59%). Flowers accessible from 6.00 h to 18.00 h for diurnal flower visitors showed high fruit set of 63% but low seed set of 14%. This suggests that pollination of individual flowers is less effective during daytime. Regarding relative reproductive success, i.e. efficiency of pollination defined as fruit set x seed set, both diurnal and nocturnal pollinators, however, are equally successful. We conclude that frequently occurring, but not very effective pollinators contribute substantially to seed production, when the expected pollinators are scarce.

2.2 Key words

Rubiaceae, Sphingidae, Trochilidae, nectar production, pollination effectiveness, pollination syndromes, reproductive system, fruit set, seed set.

2.3 Introduction

Floral characters are often related to the interaction of flowers and pollinators (Campbell 1989, Campbell *et al.* 1996, Galen 1996; Galetto 1998; Gentry 1990). Ornithophilous flowers tend to have characteristics that facilitate bird pollination (Smith *et al.* 1996), such as long, narrow, tubular corollas, and, in many cases, vivid coloration (Faegri and van der Pijl 1979, Willmott and Búrquez 1996). Sphingophilous flowers frequently have narrow tubes as well; however, they show a different set of characters compared to ornithophilous ones, typically including anthesis at dusk, white to yellowish flowers and intense sweet floral scent (Faegri and van der Pijl 1979).

Individual plant species are often associated with a specific "pollination syndrome", i.e. classified as having "moth-flowers", "bird-flowers", "bumble-bee flowers", etc., which may suggest that there are no other visitors (Ollerton and Watts 2000). Pollination syndromes, however, cannot serve to automatically characterize the pollinator spectrum of a given plant, because any visitor at any time may act as a pollen carrier as long as flowers are open (Baker 1961, Herrera 1988). Pollinators commonly show a plastic behaviour, choosing flowers based on rewards, and, as a consequence, visiting a variety of plant species without as much regard for their floral traits as might be expected (Waser and Price 1990, Waser *et al.* 1996). Plant species restricted to a single pollinator are more the exception than the rule. But for the plant not every pollinator grants the same reproductive success. Numerous studies have investigated the different levels of pollinator effectiveness in plants with diverse pollinator assemblages (e.g., Baker 1961; Motten *et al.* 1981, Haber and Frankie 1982; Murcia 1990, Wilson and Thomson 1991, Harder and Barrett 1993, Sazima *et al.* 1994).

In the present paper we investigate *Isertia laevis* (Triana) B. M. Boom (Rubiaceae), a neotropical tree with flowers exhibiting the characteristics of sphingophily. During daytime, however, we frequently observed nine species of hummingbirds visiting flowers of *Isertia laevis.* According to Stone (1996) pollinator effectiveness depends on a combination of variables including visitation frequency, time distribution of visitation in relation to floral

anthesis, pollen transfer capability and the balance of visitation frequency among different plant individuals. In order to examine efficiency of pollination by diurnal and nocturnal pollinators we conducted an exclosure experiment. We used relative reproductive success, defined as fruit set multiplied by seed set, to measure pollination efficiency of different visitors. In this paper we try to assess the contribution of hummingbirds to the reproductive success in the "sphingophilous" *Isertia laevis.*

2.4 Material and methods

2.4.1 Plant material and study site

Isertia is a neotropical genus of 14 species, distributed mainly in the Amazon basin and the Guianas (Andersson and Ståhl 1999). Flowers are normally white, fragrant, and nocturnal, but in some cases red, odorless, and diurnal. *Isertia laevis* (Triana) B. Boom is a tree up to 15 m high with nocturnally open, sweetly fragrant, homostylous flowers. The corolla tube is usually white to cream-colored, sometimes reddish. The lobes are always white. The species is distributed from southern Nicaragua through South America as far as northern Bolivia and occurs in humid forests, mainly in disturbed sites (Andersson and Ståhl 1999). The study site is located between Loja and Zamora in southern Ecuador, Province of Zamora-Chinchipe, bordering the Podocarpus National Park (03° 58′ S, 79° 04′ W). Population density at the study site is 14.4 individuals per hectare (Merkel, unpubl. data). Investigations were carried out at elevations between 1800 and 2100 m above sea level and field work took place mainly from February 2000 to June 2000 and in February 2001.

2.4.2 Phenology, anthesis, and flower morphology

Every second day the number of buds, flowers and fruits on 35 marked individuals was estimated. On 25 inflorescences marked in bud stage, the number of open flowers was counted every evening. To describe flower anthesis we marked 150 individual buds and documented the anthesis in different weather conditions. We described flower morphology by measuring the following parameters: maximum corolla diameter, maximum corolla tube length, style length, stigma length, filament length, and anther length. Eleven individual flowers were measured 1 hour after flower opening. Six flowers were measured 6 hours and another eleven 12 hours after flower opening.

2.4.3 Nectar dynamics and nectar sugar composition

On four individual trees, we sampled nectar every two hours from unbagged flowers and every four hours from bagged flowers. Each individual flower was sampled only once. Due to a limited number of accessible flowers, samples were taken over several days. Sampling sizes ranged from eight to 29 for unbagged flowers and from 16 to 29 for bagged flowers. Nectar was taken up with microcapillaries, conserved in 70% alcohol and frozen until analysis. For sugar concentration and composition analysis we randomly chose ten samples (in two cases less than 10) for each sampling time and pooled them. We pooled the total volume of nectar obtained from ten uncovered flowers to determine the energy which would have been offered to a potential visitor. In order to obtain the average nectar concentration for bagged flowers, 1 µl of nectar from each of the ten flowers was pooled. 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 and the eluation took place with an acetonitrile-water-mixture (71:28), with 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. The nectar samples of the unbagged flowers taken at 8:00 h contained too little nectar for nectar sugar analysis.

2.4.4 Flower visitation

We observed flower visitors over a period of 16 days in observation blocks from 6.00 h to 18.00 h for a total of 69 hours of observation. Visits of hummingbirds were recorded as soon as one flower was visited legitimately. If all flowers were visited from outside only at the base of corolla the visit was not recorded. The species observed were identified according to Hilty and Brown (1986) and Del Hoyo *et al.* (1999). We recorded nocturnal flower visitors three times between 18.00 h and 21.00 h, by observing inflorescences with an infrared night vision device. Only two or three inflorescences were visible at once through this device. Therefore, some nocturnal visits to a plant may have been missed.

2.4.5 Reproductive system

To investigate the ability of plants to self spontaneously, we bagged seven inflorescences of four individuals with nylon mesh and determined fruit and seed production. We also conducted part-time exclosure experiments to investigate potential pollinators. During seven days, we bagged a total of seven inflorescences on three plant individuals to

deny access to diurnal flower visitors between 6.00 and 18.00 h. We bagged seven other inflorescences on the same three individual trees from 18.00 to 6.00 h to deny access to nocturnal flower visitors. We marked open flowers within these part-time covered inflorescences and measured fruit and seed production. Additionally, we marked and hand-pollinated 26 flowers at 10.00 h; and 28 additional flowers at 22.00 h. In both cases pollen was taken from bagged flowers of other plant individuals. The flowers were bagged in bud stage and remained bagged after hand pollination until fruit- and seed production could be determined two months later. We also determined fruit and seed production on twelve untreated inflorescences (five plant individuals) for comparison.

We calculated fruit set by dividing the number of mature fruits by the total number of presented flowers. The absolute seed production is the average number of seeds in a fruit, and we defined the relative seed production as the ratio of the total number of seeds produced in all fruits to all presented flowers. Based on a total of 14 buds from five different trees, we calculated seed set as the average number of seeds per mature fruit (= absolute seed production) as a percentage of the average number of ovules per flower. Finally, we defined relative reproductive success as fruit set multiplied by seed set.

We determined the pollen-ovule ratio according to Cruden (1977) and Kearns and Inouye (1993). The pollen of nine 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 (six). We first calculated pollen-ovule ratios for individual buds by dividing the number of pollen grains by the number of ovules.

2.5 Results

2.5.1 Phenology, anthesis, and flower morphology

Isertia laevis is widespread in second growth woodland. Flowering occurs from February to July, usually peaking from March to April. Numbers of inflorescences and open flowers vary widely among plants; on the majority of flowering inflorescences, three to five flowers open daily in mid-season, but some inflorescences open as many as twelve flowers simultaneously. A single inflorescence blooms between three to five weeks and contains

between 40 and 80 flowers. The flowers open between 16.00 h and 19.00 h and fade the next day between 14.00 h and 18.00 h, depending on weather conditions. The anthers are open as early as in bud-stage. Flower tube length is about 40 mm and its diameter 6.5 mm on average. Measurements of floral parts (Table 2.1) showed no siginficant differences (Mann-Whitney-U-test) during anthesis. Nectar is accumulated at the bottom of the corolla tube.

Table 2.1 Flower morphology of *Isertia laevis*. *x* = mean, *sd* = standard deviation, *n* = 28 investigated flowers.

	Corolla tube, length [mm]	Corolla, diameter [mm]	Corolla tube, diameter [mm]	Style, length [mm]	Stigma, length [mm]	Filament, length [mm]	Anther, length [mm]
$x \pm sd$	41.0 ± 0.3	32.4 ± 0.34	6.5 ± 0.07	42.0 ± 0.2	5.0 ± 1.0	33.0 ± 0.4	8.0 ± 1.0

2.5.2 Nectar dynamics and nectar sugar composition

Nectar volume and sugar concentration of bagged flowers are given in Figures 2.1 and 2.2. During the first part of the night, the flowers contained little nectar, whereas the highest nectar volume was reached at 2.00 h with a median of 27 µl. During the day, nectar volume was about 10 µl except for the sample at 10.00 h, for which the median was slightly higher (16 µl). Freshly opened flowers contained a high nectar sugar concentration (100 µg/µl). The lowest nectar sugar concentration of 46 µg/µl was reached at 2.00 h. At the same time the flowers contained the highest nectar volume. Sugar concentrations in bagged flowers (Figure 2.2) were generally high when volumes were low.

Nectar volumes in unbagged flowers (Figure 2.3) increased during the first half of the night and reached their maximum (27 µl) at 2.00 h, as in bagged flowers. In the second half of the night, the median of nectar volumes declined, but single flowers contained up to 70 µl. Unbagged flowers sampled at 8.00 h contained nearly no nectar and at 14.00 h nectar volumes were also low in general. Nectar volumes increased slightly in the later morning hours until noon. Nectar sugar concentrations were higher in unbagged than in bagged flowers (Figure 2.4). During the night, the average nectar concentration was about 100 µg/µl, ranging from 88 µg/µl at 18.00 h to 124 µg/µl at midnight. During the following day, nectar sugar concentrations were generally higher than during the night, but they were minimal (80 µg/µl)

at 14.00 h while maximal concentrations were reached at noon (197 µg/µl). The nectar sugars glucose, fructose, and sucrose were present in almost equal portions in both bagged and unbagged flowers.

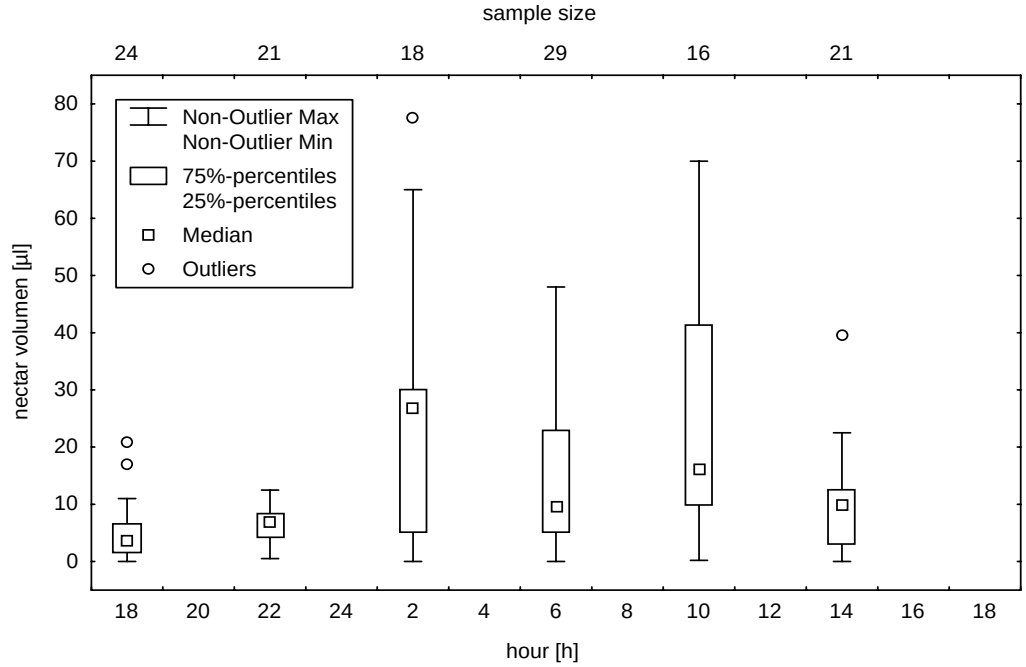

Figure 2.1 Nectar volumes in covered flowers. Outliers are those values which fell beyond ± 1.5 from the length range of the 25 – 75% percentiles box.

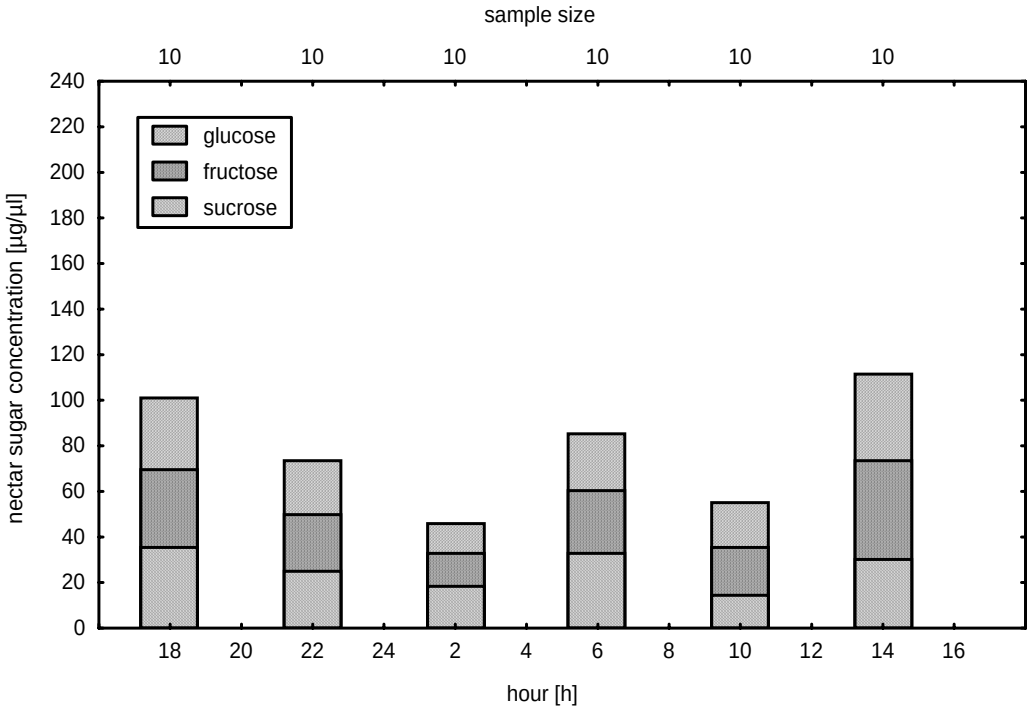

Figure 2.2 Nectar sugar concentration and composition of covered flowers.

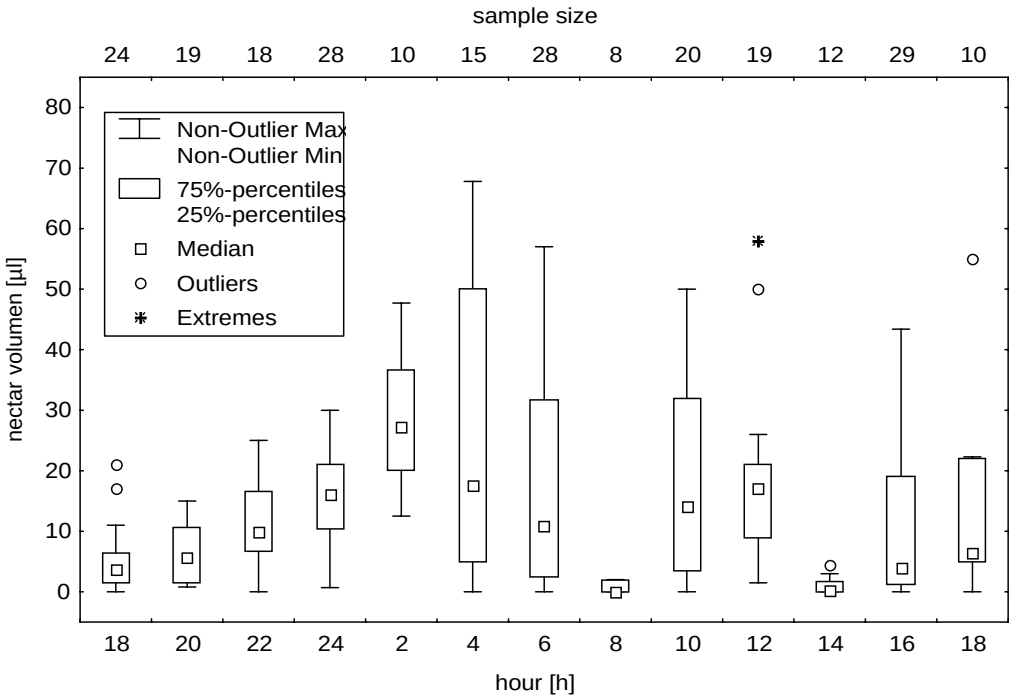

Figure 2.3 Nectar volumes in uncovered flowers (standing crop). Outliers are those values which fell beyond ± 1.5 from the length range of the 25%-75% percentiles box. Extremes are those values which fell beyond ± 3 from the length range of the 25%-75% percentiles box.

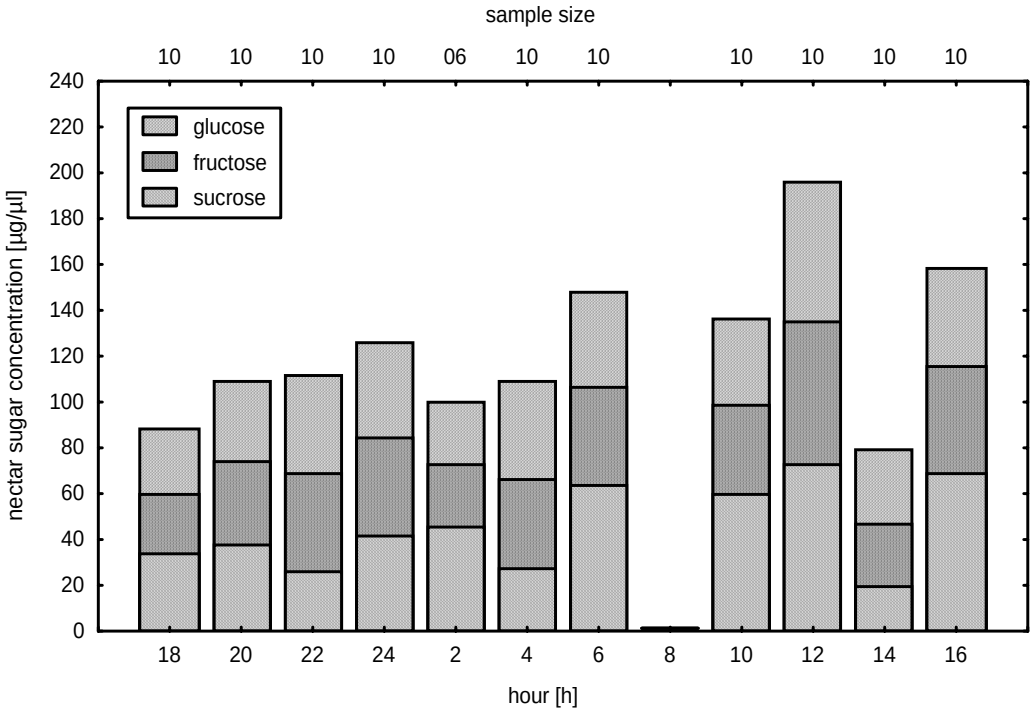

Figure 2.4 Nectar sugar concentration and composition of uncovered flowers (standing crop).

2.5.3 Flower visitation

During daytime we observed nine hummingbird species and one honeycreeper at flowers of *I. laevis* (Table 2.2). Flowers were most frequently visited in the morning between 7.00 to 9.00 h, averaging between 5.5 and 10.5 hummingbird visits per hour and tree (Figure 2.5). During the remainder of the day, we observed between 2 and 4.5 visits per hour and tree. The most frequent visitor was *Aglaiocercus kingi* (34% of all observed visits); the second most frequent visitor was *Chaetocercus mulsant* (27% of observed visits); followed by *Ocreatus underwoodii* (12%) and *Diglossa albilatera* (11%) (Table 2.2). *Diglossa albilatera* (Coerebidae) visited the plant frequently, but robbed nectar by piercing the flowers or taking nectar at the base of broken flowers and therefore never contacted the reproductive flower parts. *Aglaiocercus kingi* and *Chaetocercus mulsant* often acted as "nectar thieves", but also visited flowers legitimately**.** In addition, bumble-bees visited the flowers occasionally, taking nectar on the base of broken flowers or by piercing them. Some bees, wasps and stingless bees were observed eating pollen. During nine hours of observation from 18.00 to 21.00 h over three days we observed only a total of three night-active sphingids. Thrips were found in buds and flowers during the night as well as day.

Table 2.2 Diurnal flower visits of hummingbirds and sugar birds on flowers of *Isertia laevis*. Bill length after Feinsinger (1990): 1 < 28 mm, 2 > 28mm.

Taxa/Species	Bill length	Visits (69 h observed)	Visits per hour	Percentage of total visits
Trochilidae (Trochilinae)				
Aglaiocercus kingi (Lesson)	2	76	1.10	34%
Chaetocercus mulsant (Bourcier)	2	61	0.88	27%
Ocreatus underwoodii peruanus (Gould)	2	27	0.39	12%
Colibri coruscans (Gould)	2	18	0.26	8%
Heliodoxa rubinoides (Bourcier and Mulsant)	2	7	0.10	3%
Heliodoxa leadbeateri (Bourcier)	2	7	0.10	3%
Adelomyia melanogenys (Fraser)	2	1	0.01	0.4%
Colibri thalassinus (Swainson)	2	1	0.01	0.4%
Coeligena coeligena (Lesson)	1	1	0.01	0.4%
Coerebidae				
Diglossa albilatera (Lafresnaye)	-	24	0.35	11%

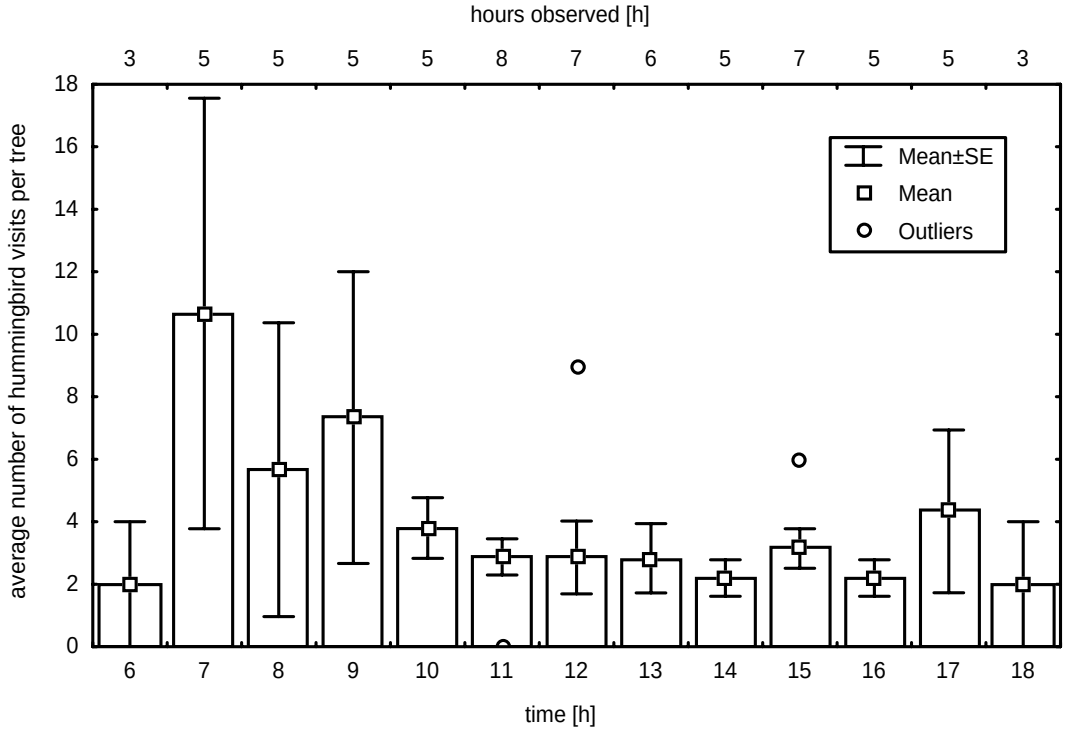

Figure 2.5 Mean numbers of hummingbird visits at *I. laevis* from 06.00 to 18.00h. Outliers are those values which fell ± 1.5 SE from the mean.

2.5.4 Reproductive system

Fruit- and seed set varied substantially between day and night as the experiment of part-time exclosure for pollinators showed (Table 2.3). Significantly more fruits were developed on flowers presented during the day (63.3%) vs. during the night (14.3%). Seed set was much higher on flowers accessible to nocturnal visitors (59%) than on flowers accessible to diurnal visitors (14%). Relative seed production was slightly higher during the day. In flowers of *I. laevis* the average number of ovules was 543 ± 108 (n = 14). Hand-pollinated flowers showed an almost identical percentage of fruit set (80%), independent of the time of pollination (10.00 h or 22.00 h). Seed set, however, was significantly higher in flowers hand-pollinated at night than in those hand-pollinated during the day (Table 2.4), although the difference was not as marked as in the exclosure experiment. The flowers showed low selfing ability (Table 2.4). We estimated a mean number of 122000 ± 21000 pollen grains per flower (n = 9), and the pollen-ovule ratio was 236 ± 44 (n = 9).

Table 2.3 Absolute and relative seed production. x = mean, sd = standard deviation, n = number of investigated fruits$_1$ and flowers$_2$. [a, b] indicate pairs with $p \leq 0.001$ (Mann-Whitney U-test).

	Inflorescences open during night	Inflorescences open during day
Absolute number of seeds		
$x \pm sd$ (n_1)	320[a] ± 94 (9)	78[a] ± 79 (43)
Seed set [%]	59	14
Fruit set [%]	14.3	63.3
Relative number of seeds		
$x \pm sd$ (n_2)	46[b] ± 118 (63)	49[b] ± 73 (68)
Relative seed production [%]	8.5	9.0

Table 2.4 Seed production. x = mean, sd = standard deviation, n = number of investigated fruits. [a, b] indicate pairs with $p \leq 0.001$ (Mann-Whitney U-test).

	Inflorescences covered during flowering	Control Inflorescences untreated	Flowers pollinated by hand at 22 h	Flowers pollinated by hand at 10 h
number of seeds				
$x \pm sd$ (n)	21[a] ± 34 (29)	116[a] ± 112 (61)	291[b] ± 51 (24)	208[b] ± 69 (22)
seed set [%]	3.9	21.4	53.6	38.3

2.6 Discussion

2.6.1 Nectar sugar composition

Isertia laevis displays characters typical of sphingophilous flowers. Flower morphology, sweet scent and the daily phenology of flowers, opening in the late afternoon and starting nectar secretion at night, indicate an adaptation to nocturnal pollination. This interpretation is supported by hand pollination being more successful at night than during the

day. However, the flowers were frequently visited by hummingbirds. The flowers of *I. laevis* are highly attractive to hummingbirds because of their rich nectar supply accumulated during the night due to the low visitation rate of the sphingids. The hummingbirds may use the plant as nectar source also because nectar production continues during the day. Nectar sugar composition was claimed to be a useful predictor of pollinators with sucrose-dominant nectar being indicative for lepidoptera, hummingbirds and large bees (Baker and Baker 1990, Stiles and Freeman 1993). Galetto *et al.* (1998) on the other hand found no correlation between sugar ratio and pollinator adaptation and assumed that nectar sugar composition is a conservative character and thus not directly indicative for flower visitors. In *I. laevis*, almost equal concentrations of glucose, fructose and sucrose were found. In contrast to Bernadello *et al.* (1994) who found either glucose or fructose dominant nectar in two species of the closely related but ornithophilous genus *Palicourea* Aubl., we found sucrose dominant nectar in all ten *Palicourea* species growing at the study side (Wolff, unpubl. data). According to Stiles and Freeman (1993), nectar of six out of seven hummingbird-visited Rubiaceae species is also sucrose dominant. Preference of the sucrose by hummingbirds traces back to their specialized digestive system (Rio 1990, Rio *et al.* 1992) which allows the metabolism of sucrose with the same efficiency as hexose sugars. In nature, the preferred food types are not always available in sufficient quantity. Thus hummingbirds, although liking sucrose dominant nectar, will visit hexose-rich flowers if they are short of succrose-rich ones (Baker and Baker 1990).

2.6.2 Flower visitors

According to Feinsinger and Colwell (1978), the Trochilidae observed to visit *I. laevis* follow the low reward trap-line strategy; however, in the study area, they behaved more like opportunistic visitors. At the study site, between 23 and 29 ornithophilous species flowered during the period from March to July (Dziedzioch 2001), in contrast, the number of sphingophilous flowers competing for visitors is low (Wolff, unpubl. data), so that pollen loss and contamination of stigma with pollen of other species is lower in flowers accessible only during the night. During the day, the number of visited plant species and visitation patterns can influence pollen carryover on a given species (Herrera 1988). Various studies on pollen carryover by hummingbirds visiting plant populations mainly in the heterostylous genus *Palicourea* showed that every intervening plant species further reduced pollen transfer from donor to recipient (Feinsinger and Busby 1987; Feinsinger *et al.* 1988; Murcia and Feinsinger 1996). The indiscriminate foraging behavior of the most frequent hummingbirds *Aglaiocercus kingi, Chaetocercus mulsant,* and *Ocreatus underwoodii* may reduce intraspecific pollen

transfer and lead to stigma contamination with loads of foreign pollen (Waser 1978, Brown and Kodric-Brown 1979; Motten *et al.* 1981). Furthermore, *Aglaiocercus kingi* and *Chaetocercus mulsant* often visited the flowers illegitimately by piercing the corolla base or by licking nectar at the base of broken flowers.

Apart from pollinator behavior, time of anthesis and anther dehiscence are important components of pollination effectiveness. While bumble-bees remove a large proportion of available pollen during the first few flower visits, hummingbirds only export a tiny fraction of the available pollen at every visit (Mitchell and Waser 1992). The high percentage of seed set in night-accessible flowers of *I. laevis* indicates that sphingids, like bumble-bees, remove a large proportion of the available pollen during the first flower visits. The number of pollen grains is also reduced by numerous thrips feeding on them. While this effect could not be quantified in the present study, there was a tendency to a reduced number of pollen grains found in anthers of buds with a large thrips population. Even flowers that have not been visited by nocturnal visitors may therefore offer a reduced number of pollen grains to diurnal flower visitors.

Nectar volumes increased during the night and, at night, unbagged flowers frequently contained as much nectar as bagged flowers. This, as well as the poor fruit set of flowers accessible to pollinators only during the night, suggests that sphingids are rare flower visitors at our study site. This view is supported by the scarcity of sphingid observations on *I. laevis*. At the study site six species of sphingids may act as nocturnal pollinators (Brehm and Suessenbach, personal communication), but these are far fewer than the numbers of species at even slightly lower elevations. For example, the Monteverde Cloudforest Reserve, Costa Rica (1550 m above sea level) supports 52 species of sphingids (INBio, 2002). While fruit set was poor in flowers accessible only during the night, seed set was high. This high seed set may have two explanations: either nocturnal visitors are excellent pollen vectors compared to diurnal ones, or stigma receptivity, and/or pollen viability are higher during the night than during the day. Flowers pollinated by hand at 22.00 h showed significantly higher seed set than those pollinated at 10.00 h, but this difference only explains part of the extraordinary discrepancy between flowers visited exclusively during the night and those accessible only during the day. We therefore suggest that nocturnal visitors are more effective pollinators. In addition, Sphingids probably contribute significantly to the outcrossing rate of *I. laevis*, because they cover long distances rapidly, move readily between plants and are not known to forage within a home range, while hummingbirds tend to forage within a given territory, reducing cross-pollination (Haber and Frankie 1989). *I. laevis* provides hexose-rich nectar.

Haber and Frankie (1989) found mainly sucrose-dominant nectars in flowers visited by hawkmoths in Costa Rica, and could show experimentally that hawkmoths prefer sucrose dominant nectar to hexose dominant nectar if given a choice, but feed on hexose dominant nectar as well. Probably the low percentage of competing species with sphingophilous flowers at the study site (Wolff, unpubl. data), the low species numbers of sphingids together with an acceptable, but not particularly favored nectar composition accounts for the relatively low, but constant visitation rate of *I. laevis* by sphingids at the study site.

2.6.3 Pollination effectiveness

Although *I. laevis* conforms closely to the pollination syndrome of sphingophily, it can be misleading to infer from floral characters alone which flower visitor contributes most to reproductive success at a given place or time, or to call hummingbirds only opportunistic nectar thieves. The contribution of different visitors to a plant's relative reproductive success is determined both by pollen transfer of compatible pollen to a flower per visit (quality) and by visitation rate (quantity). This principle is confirmed by the results of Beattie (1972), Motten *et al.* (1981), Waser and Price (1983), Schemske and Horvitz (1984), Herrera (1987, 1989), Olsen (1997), Mayfield *et al.* (2001). Waser and Price (1990) investigated *Delphinium nelsonii* Greene which conforms to a classical bee pollination syndrome. In experiments they showed that a bee deposits about ten times as much pollen while visiting a flower as a hummingbird does, and causes about ten times as many seeds to be developed. At the level of entire pollinator populations, however, hummingbird visitation rates may be more than ten times as high as those of bees. Birds and bees have similar contributions to relative seed set showing that individual pollination efficiency must be distinguished from population-level effectiveness, and that the pollination syndrome of a flower may not indicate present-day effectiveness of its visitors. Even with low seed set by hummingbirds we can assume that they have the same contribution to relative reproductive success than sphingids caused by the fact that hummingbirds are frequent flower visitors (fruit set > 60%). On the other hand, sphingids are rare flowers visitors but they result high seed set. The pollination syndrome concept can give an orientation as to which pollinator can be expected, but it is common for plants to have multiple visitors pollinating to some degree, as shown by our investigations and other examples [e.g. Bertin and Willson 1980 for *Asclepias* (Apocynaceae); Locatelli and Machado 1999 for *Cereus fernambucencis* Lem. (Cactaceae); Mayfield *et al.* 2001 for *Ipomopsis aggregata* (Pursh) V. Grant (Polemoniaceae); Young 2002 for *Silene alba* Burnat (Caryophyllaceae)].

The flowers of *I. laevis* are better adapted to sphingids than to hummingbirds as pollinators. However, relative seed production shows that less efficient pollinators visiting frequently are equally important as pollinators on the whole. Further investigations at lower elevations would be needed to test whether the scarcity of sphingid visits to *I. laevis* is an altitudinal effect. Sphingids are more common at lower elevations and therefore they might be more frequent visitors there, thus reducing nectar available to hummingbirds in the morning, resulting in a loss of interest in *Isertia* by hummingbirds. The sphingophilous characters of *I. laevis* might have evolved in different habitats, e.g. at a lower elevation. At our study site in a tropical mountain forest, however, hummingbirds and sphingids are equally effective as pollinators of *I. laevis*. The only other observations available for *Isertia* point to an involvement of hummingbirds in pollination in the genus as both *I. hypoleuca* Benth. and *I. rosea* Spruce ex K. Schum. have been observed to be pollinated by hummingbirds in Colombia at 200—300 m altitude (Rosero, unpubl. data). A highly speculative, but testable hypothesis is that the variable *I. laevis* might shift between favoring hummingbirds and sphingids over time, depending on altitude, e.g. by a shift in nectar production towards daytime and/or by starting with flower anthesis in the early afternoon. Our permanent site in Ecuador should allow *Isertia* to be studied again in future years, to see whether such a shift occurs.

2.7 Acknowledgements

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3 Observations on pollination in small flowering Asclepiadoideae (Apocynaceae) of Southern Ecuador

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3.1 Abstract

Analysis of plant-pollinator interactions is fundamental for the understanding of terrestrial ecosystems, but most investigations on the reproductive biology of tropical plants focus on specialized animal-plant interactions. Inconspicuous flowers have received little attention. This paper studies phenology and pollination ecology of nine small-flowered neotropical species of *"Cynanchum"*, *Ditassa*, *Jobinia*, *Oxypetalum* and *Scyphostelma* (tribe Asclepiadeae). The relatively large numbers of small flowers, blooming simultaneously in many species, are important in attracting insects. Numerous kinds of floral visitors were observed, however, pollinaria were carried only by four insect species. The flowers show a comparatively low pollinaria removal rate with an average of 0.32 ± 0.13, and an even lower average was recorded for the pollinia insertion rate 0.13 ± 0.07. The percentage of inserted pollinia to removed pollinaria is comparatively high with an average of 42.7% ± 22.3%. This shows that if an insect did achieve pollinia transfer, they did it very effectively. The complex floral morphology of the Asclepiadoideae has often been interpreted as a general trend toward specialization, but observations of pollination indicate at least some degree of generalization. Floral longevity varies between three to five days and floral longevity is shortened by successful pollinia insertion. The nectar of seven out of eight investigated species is sucrose-dominated, nectar of the one remaining species is classified as sucrose-rich. There is no association between nectar sugar composition and sugar preference of pollinators. This suggests that nectar sugar composition in the subfamily is under phylogenetic constraint.

3.2 Key words

Asclepiadoideae; "*Cynanchum*"; *Ditassa*; fruit set; nectar; *Oxypetalum*; pollination biology; phenology; *Scyphostelma*

3.3 Introduction

Most tropical plant reproductive biology investigations focus on specialized animal-plant interactions, and, mostly, conspicuous flowers. However, inconspicuous flowers with no apparent specialization have received little attention. The derived floral structure and pollinating system in the Apocynaceae-Asclepiadoideae has often been understood as very close flower-pollinator interaction (Ollerton and Liede 1997). The flowers are pollinated through a complex mechanism in which one of the two pollinia of a pollinarium – which are held together via a translator centered by a corpusculum with clamp function for the insect's hairs, tarsi or mouth parts – is inserted into one of the five guide rails, each formed by two adjacent anther wings (e.g., Kunze 1991, 1995; see also Figures 3.1, 3.2). After insertion, the pollinium is pulled up until the translator breaks (Figure 3.2C) and the insect escapes with the second pollinium still attached. The pollinium is then partially or completely placed within the guide rail (Figure 3.2C), behind which, in the stigmatic chamber, the pollen tubes germinate and penetrate the style-head for fertilization (Figure 3.2D). Whereas this general mechanism of pollination is quite well understood, information on specific pollination processes and pollinators is rare and focuses on just a few of the ca. 3000 species accepted in Asclepiadoideae (cf. Meve 2002). A compilation of published and unpublished pollination data from Asclepiadoideae and Periplocoideae is online with the ASCLEPOL database at *http://www.pflanzensystematik.uni-bayreuth.de/*, and an analysis of these data is presented by Ollerton and Liede (1997). There are three main pollination strategies: 1. Generalists such as *Asclepias* or *Cynanchum* are pollinated by some to many different Hymenoptera and Lepidoptera (e.g., Bertin and Willson 1980, Morse 1985, Kunze and Liede 1991, Ollerton and Liede 1997). 2. Another large group of taxa is pollinated by just a specific group of pollinators like Diptera in Stapeliinae (Meve and Liede 1994), with 1-10 different pollinating species observed (Ollerton and Liede 1997). 3. Rarely, a high degree of specialization is reached where single pollinating species interact with singe asclepiad species as in *Calotropis gigantea* with the bee *Xylocopa tenuiscapa* (Wanntorp 1974), *Marsdenia fraseri* with

Metriorrhynchus lateralis (Coleoptera, Forster 1989), or *Microloma* with nectarinid birds (Pauw 1998). Only few field observations on Latin American species are published (Skutch 1988, Kunze and Liede 1991, Liede 1994, Krings 1999, Vieira and Shepherd 1999a). The most comprehensive study was contributed by Vieira and Shepherd (1999b), giving a detailed survey on the pollinators of seven *Oxypetalum* species in Brazil. All these works typically deal with comparatively large flowered, rather easily accessible plants. Small flowered neotropical species of the tribe Asclepiadeae as presented here, however, never have been subject of a pollination study before.

The present investigation was conducted on the Estación Científica San Fransisco, which lies on the eastern Andean mountain range (Cordillera Real) of southern Ecuador, bordering the Podocarpus National Park. The Andes are a region with a high rate of endemism; every second Asclepiadoideae species in Ecuador is endemic (Valencia *et al.*, 2000). Unfortunately the Ecuadorian Andes have lost about 90% of their original forest cover (Hamilton *et al.* 1995), and with 1.1% the annual rate of loss in tropical montane forests exceeds the rate (0.8%) of tropical lowland forests (Doumenge *et al.* 1995). We examine the pollination biology of nine species of Apocynaceae-Asclepiadoideae, tribe Asclepiadeae, in order to provide basic information urgently needed for the protection of these highly threatened forests.

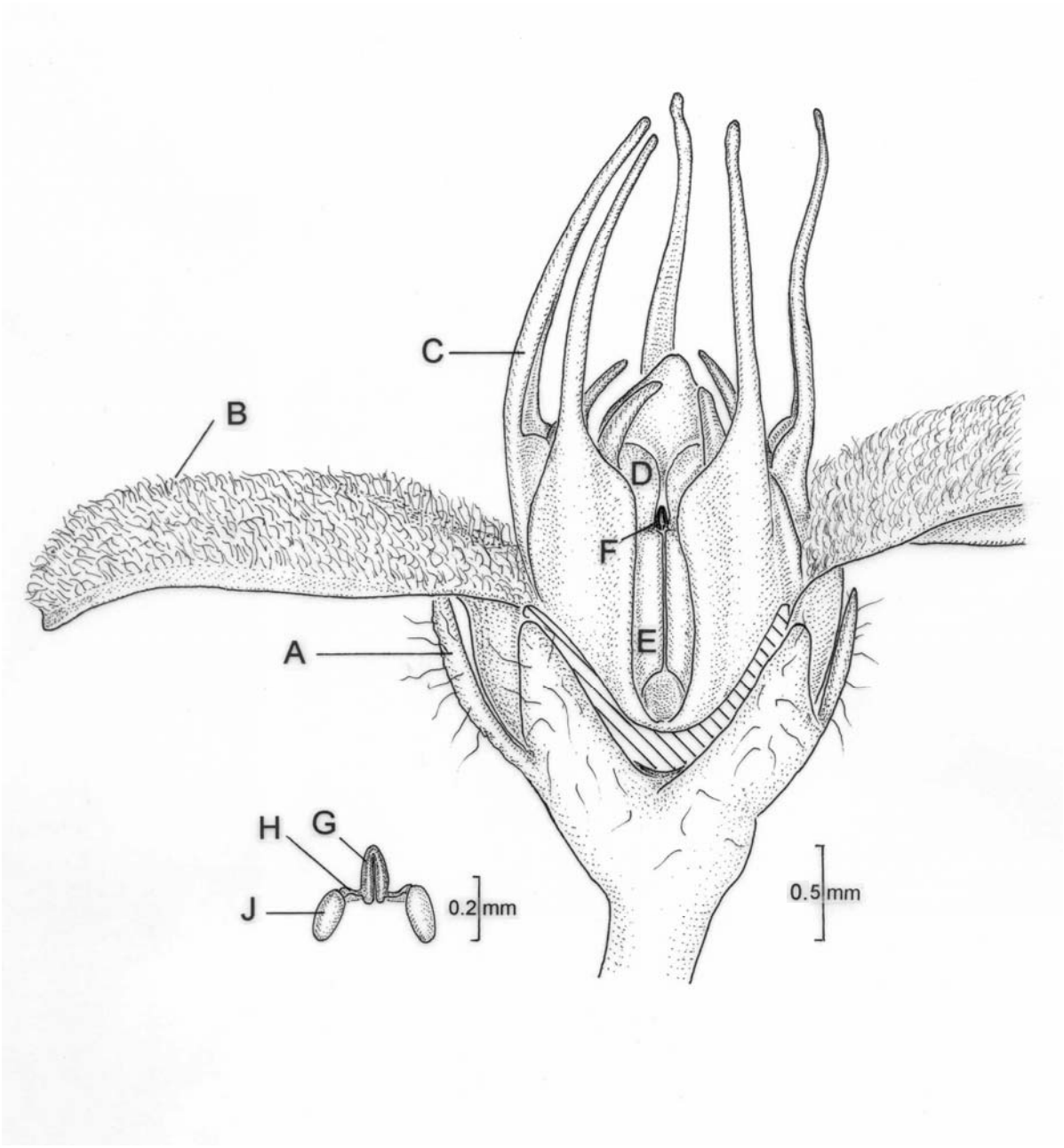


Figure 3.1 Floral structures in Asclepiadoideae-Asclepiadeae I: Flower of *Ditassa endoleuca,* with parts of the corolla removed, and pollinarium (A, sepal; B, corolla lobe; C, corona; D, anther appendage; E, guide rail; F, corpusculum terminating guide rail; G, corpusculum of isolated pollinarium; H, caudicle; J, pollinium (drawn by U. Meve from Matezki 419)).

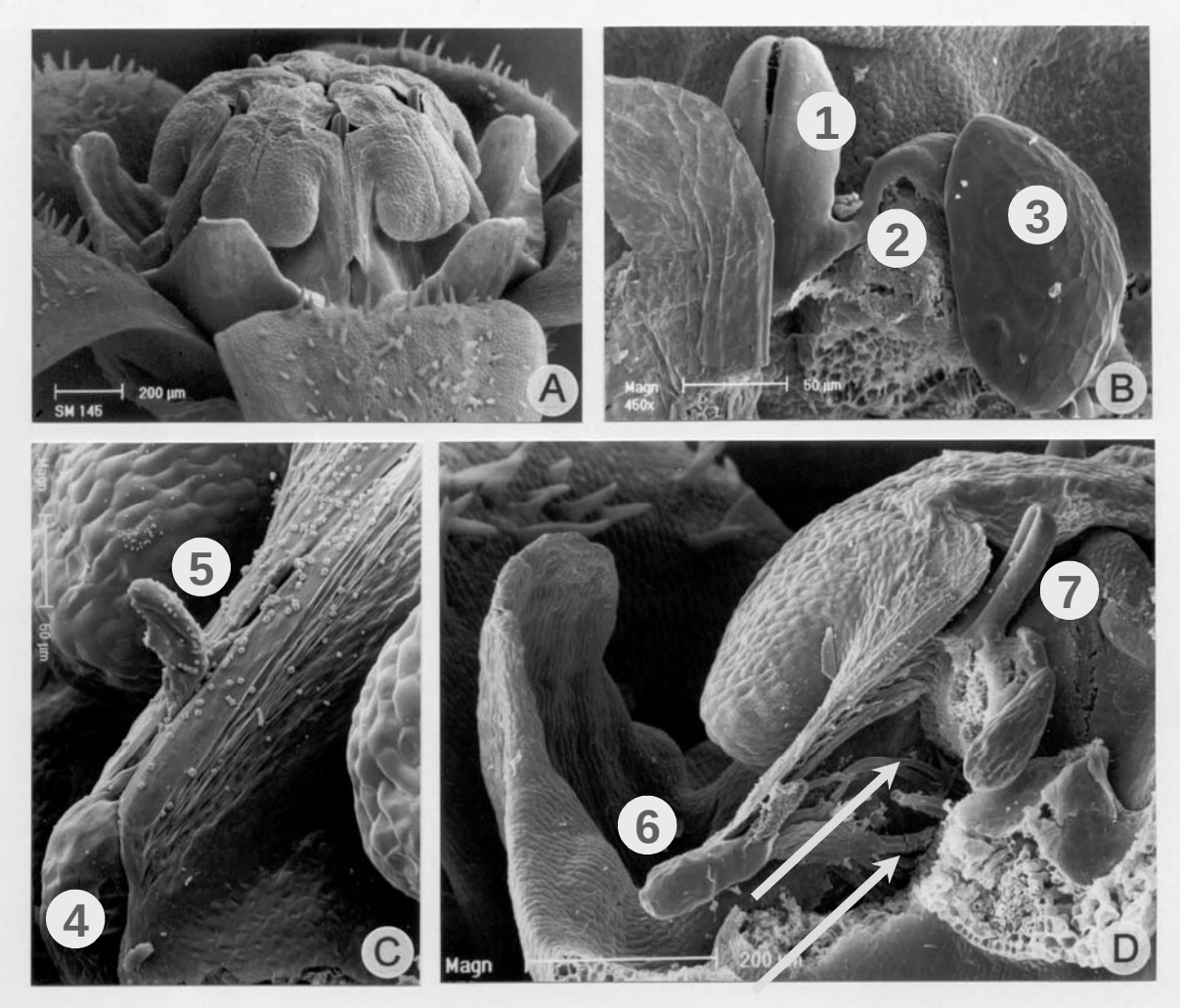


Figure 3.2 Floral structures in Asclepiadoideae-Asclepiadeae II: SEM of gynostegium and pollinarium. A, Corona and gynostegium in lateral view; B, Pollinarium at stylar head corpusculum (1), caudicle (2), pollinium (3) (one anther removed); C, Pollinium (4) with caudicle (5) inserted into guide rail mouth; D, Germinated pollinium (6) with pollen tubes grown into the style-head (arrows) and pollinarium at stylar head (7) (one complete anther removed). A, C & D from *Scyphostelma* sp. A (Matezki 145), B from *Jobinia* sp. (Matezki 169).

3.4 Material and methods

3.4.1 Study site

Pollination ecology of eight species of the subfamily Asclepiadoidae (Apocynaceae) were studied at the area of "Estación Científica San Fransisco" (03° 58´ S, 79° 04´ W) which is located at the eastern slopes of the Cordillera Real of the southern Ecuadorian Andes. The study site is bordering the Podocarpus National Park and elevation ranges from 1800 to 3200 m a.s.l. The annual mean temperature is 17.3°C and annual rainfall is 2283 mm, with a wet season from March to August (up to 280 mm rainfall in July) and a drier period from September to February (Maldonado 1985: 105 mm precipitation in November). The ninth species investigated, *Scyphostelma* sp. C, is found at the Cajanuma Reserve (Prov. Loja), 30 km W of the main study site at the western slopes of the Eastern Cordillera. Here, it is part of subpáramo vegetation which marks the transition zone to the treeless alpine vegetation at an altitude of 3000 m (Table 3.1).

3.4.2 Phenology

Field observations were made from March to June 2000, September to February 2001 and August to December 2001. The phenological study includes only individuals for which complete data were available. Some individuals of several species were lost during the two years of observation due to drought or landslides. Tagged individuals of *Ditassa anderssonii* (2), *Ditassa endoleuca* (9), *Jobinia* sp. (2), *Orthosia ellemannii* (7), *Scyphostelma* sp. A (3) and *Scyphostelma* sp. B (7), were monitored for open flowers. The counting was done in the following "logarithmic" classes: 0, 1-10, 11-25, 26-50, 51-100, 101-250, 251-500, 501-1000, 1001-2500. The above ranges of the classes were chosen due to the fact that blooming flowers could be counted exactly for vines with a low number of blooming flowers whereas it had to be estimated or roughly counted for vines with high numbers of blooming flowers.

For an evaluation in time intervals of every two weeks, the empiric mass distribution function has been calculated. From these, the median ($Q_{0,5}$), the lower- ($Q_{0,25}$) and the upper quartile ($Q_{0,75}$) have been calculated in order to get an evaluation for a standard interval $[Q_{0,25}; Q_{0,75}]$ with median-value $Q_{0,5}$ for the number of blooming flowers within a vine over the two years starting in January 2001 and ending in December 2002. Young and mature pods were counted exactly. Reproductive phenology of "*Cynanchum*" *harlingii* (7 individuals) was observed at every second census. As Liede-Schumann *et al.* (2005) have shown, all so-called

"*Cynanchum*" species of the Americas, except for subgen. *Mellichampia* (Sundell 1981) are not even distantly related to "*Cynanchum*". Therefore, for species which have not yet been formally transferred to other genera, "*Cynanchum*" is put into quotation marks.

3.4.3 Flower visitors

Flower visitors and insect frequency on the flowers of each species were observed from 6.00 a.m. to 6.00 p.m. in blocks of 2 hours. "*Cynanchum*" *harlingii* was observed from 10.00 a.m. to 5.00 p.m. because of its remote growing site. The single species of subpáramo vegetation in Cajanuma, *Scyphostelma* sp. C, was observed on 22.-24.04., 09.-11.06. and 29.10- 2.11. 2000. Number of visiting insects and number of visited flowers were noted. After observation DW tried to catch visiting insects (which was difficult because of the topology of the study site with up to 60° inclination and the scrambling to twining life form of the plants) and checked for pollinaria under a dissecting microscope. Flowers were preserved in 70% ethanol and the numbers of removed pollinaria and inserted pollinia were determined later under the dissecting microscope.

3.4.4 Nectar sugar composition

For the determination of nectar production flowers were covered with nylon mesh. Nectar was sampled by inserting 0.5 µl microcapillaries and nectar was rinsed into tubes of 100 µl ethanol (70%). Repeated rinsing guaranteed the complete transfer of the nectar. The samples were frozen and dried in a vacuum centrifuge for sugar composition and concentration analysis (Savant Speed Vac SC 100). The dried samples were diluted with 200 µl water and filtered in order to avoid pollen contamination on the HPLC column. The analyses were made with a Waters High Performance Carbohydrate Column. Injection volume was 10 µl, and elution took place with an acetonitrile-water-mixture (71:28). The flow rate was 1.4 ml/min and the temperature was 35°C. Sugars (glucose, fructose, sucrose) were detected with a refraction index detector 410 and quantified with the Millenium Software from Waters. Statistical analyses were performed using STATISTICA™, Version 7.0 from StatSoft, Inc. (2004).

Field work with these mostly very small-flowered, inconspicuous plants, which occur only very locally and scattered in their often nearly inaccessible habitats such as steep gorges, makes high demands on the field worker´s (DW) physical fitness and endurance. And even if this is given, a successful study is not guaranteed because of absence of pollinators on the flowers observed, heavy rain diluting the nectar to be analyzed, or inflorescences being too

difficult to access for pollinator study or catches. Most important, however, the general scarcity of flowers in some of the species diminishes the amount of data which can be collected within a reasonable time.

3.5 Results

3.5.1 Flower morphology and flower scents

A demonstration of the flowers and their sometimes peculiar structures is given in Figures 3.1 to 3.3; flower colors are reported in Table 3.1. The size of the flowers ranges from three to eight millimeters in diameter, only the flowers of "*Cynanchum*" *harlingii* and *Oxypetalum* sp. are slightly larger, measuring 10-12 mm in diameter. The gynostegium and the nectar bearing corona is usually openly presented with the guide rails easily accessible, only in *Ditassa endoleuca* (Figure 3.1) and *Oxypetalum* sp. these structures are more hidden in the campanulate and very hairy corolla. In most species the flowers are presented subsequently in less dense and few-flowered inflorescences, only in *Jobinia* sp. and *Oxypetalum* sp. flush flowering of dense and many-flowered inflorescences is the rule. Inflorescence organisation was classified as laxly cymose in *Jobinia* and "*Cynanchum*" *harlingii* and sciadioidal (umbel-shaped inflorescence derived from a cyme by reduction of the rhachis, cf. Weberling 1989) in the others. The flowers are odorous, emitting very sweet and perfumed fragrances (*Ditassa anderssonii, D. endoleuca, Jobinia* sp., *Oxypetalum* sp.), or more or less odorless for human noses ("*Cynanchum*" *harlingii, Orthosia ellemannii, Scyphostelma* spp.).

Table 3.1 Origin, flower colors, habit, habitats, and pollinators of the material studied at the investigation sites in southern Ecuador (ECSF = Estación Científica San Francisco, Prov. Zamora-Chinchipe). *Endemic to the region of Podocarpus National Park acc. to Valencia *et al.* (2000).

Taxon	Voucher	Altitude	Color of Corolla	Color of Corona/ Gyno-stegium	Habit and Habitat	Flower visitors (V) and Pollinators (P)
"*Cynanchum" harlingii* Morillo*	ECSF: D. Wolff 167 (B, UBT); S. Liede & U. Meve 3460 (UBT)	2400 m	rose	bright yellow	tiny climber along streams, in gorges	Drosophilidae (V) Lauxaniidae (V, P) Small moths (V)
Ditassa anderssonii Morillo* (var. nov. ined.)	ECSF: S. Matezki 132 (UBT); Wolff s.n. (UBT)	2100 m	yellow	creme	climbing and tangling, widespread in forests and forest openings	Different flies (V) Small bees (V)
Ditassa endoleuca Schltr.	ECSF: S. Matezki 419 (UBT)	2250 m	white	white	climbers on trees and on shrubs in "elfin forest"	Different Flies (V) Small bees (V)
Jobinia sp.	ECSF: S. Matezki 169 (UBT)	1950 m	white	white	large climber, in forests	Ichneumonoidea (P) Halictidae (V) other indet. Bees (V) Brachycera (V) Moskitos (V) Empididae (V) Small moths (V, P)
Orthosia ellemannii (Morillo) ined.= "*Cynanchum*" *ellemannii* Morillo*	ECSF: D. Wolff 48 (B, UBT); Matezki 161 (UBT)	1850 m	creme	creme	climbing and tangling in forest and thickets	Empididae (V) Drosophilidae (V) medium large Brachycera (V)
Oxypetalum sp.	ECSF: S. Matezki 80 (UBT)	1850 m	pale yellow	pale yellow	large and floriferous climber in forest openings, edges and thickets	Small Lepidoptera (V) Small bees (V) Small moths (V)
Scyphostelma sp. A	ECSF: D. Wolff 58 (B, UBT); Matezki 145 (UBT)	1900 m 2700 m	vine red	yellow	climbing and tangling in forest openings, thickets	Drosophilidae (V, P)
Scyphostelma sp. B	ECSF: Wolff 117 (UBT)	2200 m	whitish/ rose	rose	tiny climber in trees and on shrubs in the "elfin forest"	Small flies (V)
Scyphostelma sp. C	Cajanuma (Prov. Loja): S. Liede & U. Meve 3462 (UBT)	3000 m	yellow	white	small climber/ subshrub in subpáramo	

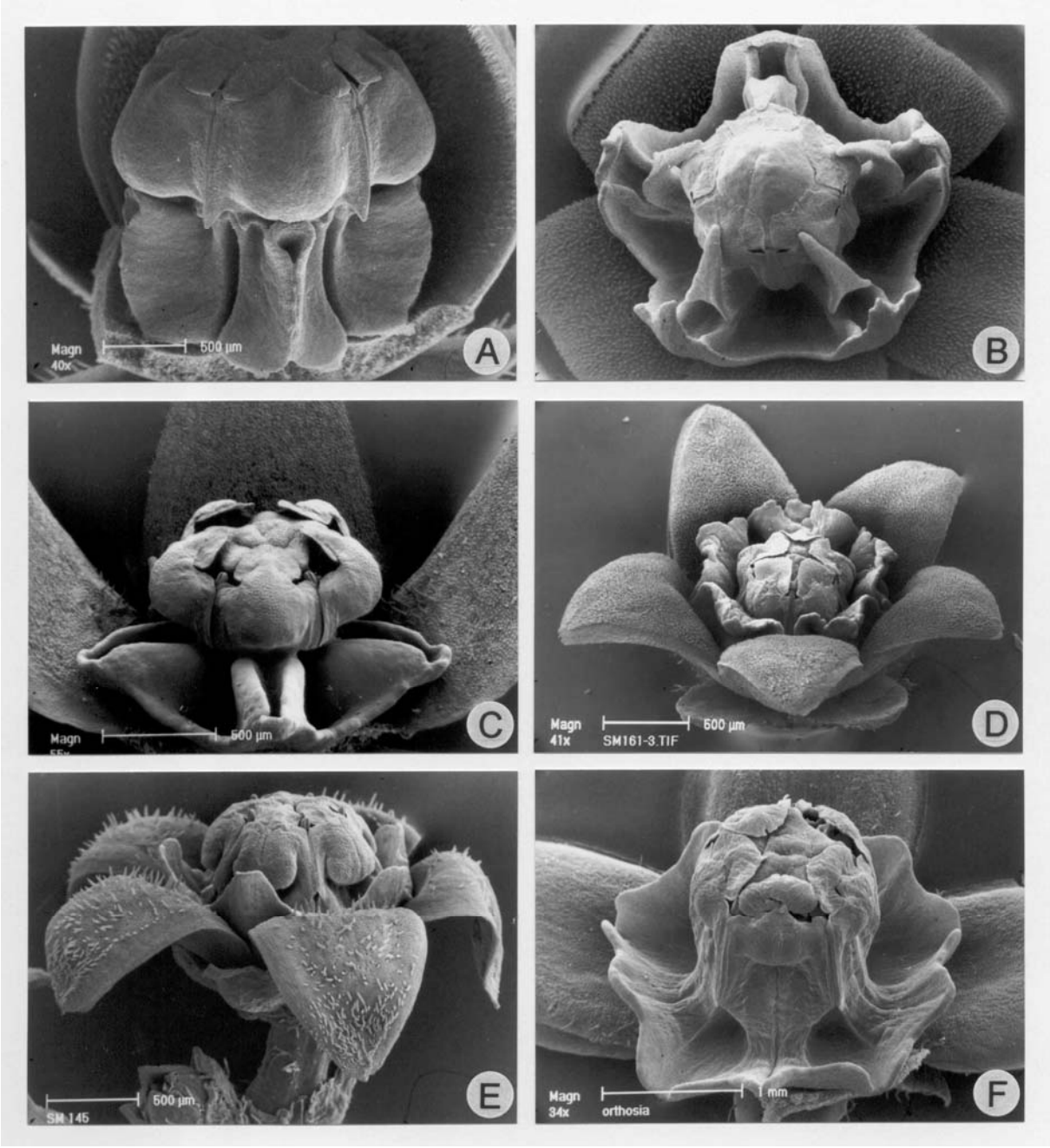
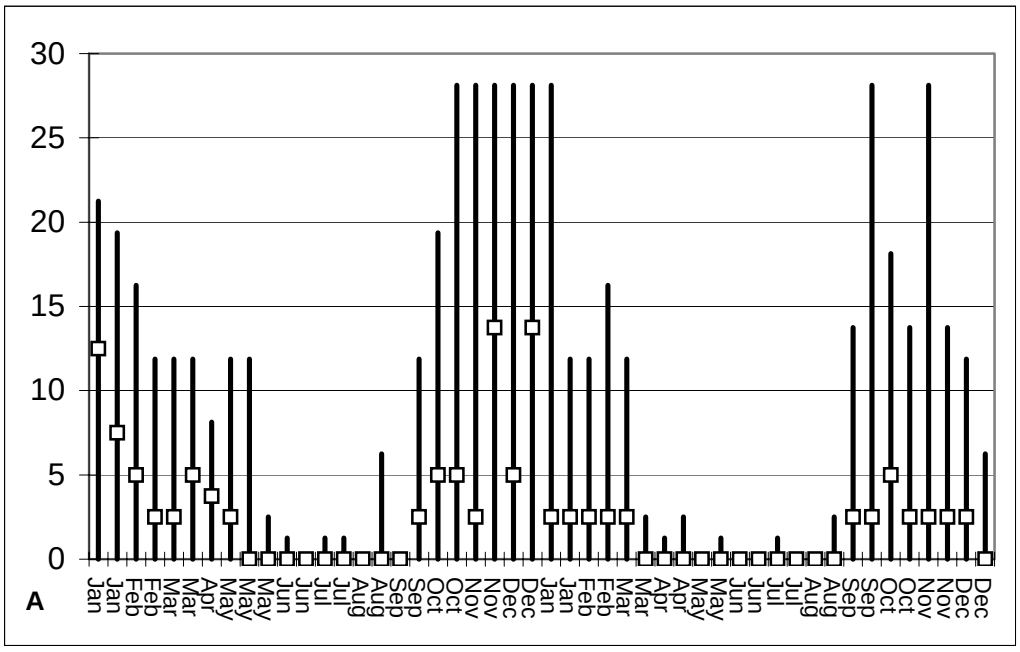


Figure 3.3 Flowers of a selection of the investigated species (SEM). A, "*Cynanchum*" *harlingii* (Liede & Meve 3460); B, *Ditassa anderssonii* (Wolff s.n.); C, *Jobinia* sp. (Matezki 169); D, *Orthosia ellemannii* (Matezki 161); E, *Scyphostelma* sp. A (Matezki 145); F, *Scyphostelma* sp. B (Wolff 117).

3.5.2 Phenology

Orthosia presents a continuous flowering pattern at population level, however, analysis at an individual level indicates that only two plants showed such a continuos pattern. Blooming peaks between September and March (Figures 3.4A and 3.5). An irregular flowering pattern is found in *Scyphostelma* sp. B, where single individuals are flowering for two to five months (Figure 3.4B). A clear flowering seasonality is shown by *Ditassa endoleuca* which flowers during the dry period from October to March (Figure 3.4C). As far as any conclusions are possible from the two individuals of *Ditassa anderssonii* observed phenologically, there is a trend that the species bloom sequentially from January to May after the flowering period of *Ditassa endoleuca* (Figure 3.5). "*Cynanchum*" *harlingii* presents a continuous flowering pattern with always more than 70% flowering and fruiting individuals. The intensity of flowering, however, seems to show some seasonality, peaking from February to May, as shown in Figure 3.4D. The three observed individuals of *Scyphostelma* sp. A and the two of *Jobinia* sp. flowered irregularly, leading to the assumption that flowering is unpredictable within these small samples. *Ditassa anderssonii, D. endoleuca, Oxypetalum* sp. and *Scyphostelma* sp. A show a typical flush-flowering with many flowers open at the same time, whereas the other species bloom more subsequently. We observed individual differences in the flowering of "*Cynanchum*" *harlingii* and *Orthosia ellemannii* between the years with more flowers in 2001 than 2002 (Figure 3.4). *Scyphostelma* sp. B produced more flowers in 2002 than in 2001, whereas no change between the years was found in *Ditassa endoleuca*. Life span of the flowers varied between the species on average between three to five days (Table 3.5). There is a significant negative correlation between life span and pollinia insertion rate (Table 3.6) as well as between life span and pollinium removal-pollinaria insertion ratios (Table 3.6).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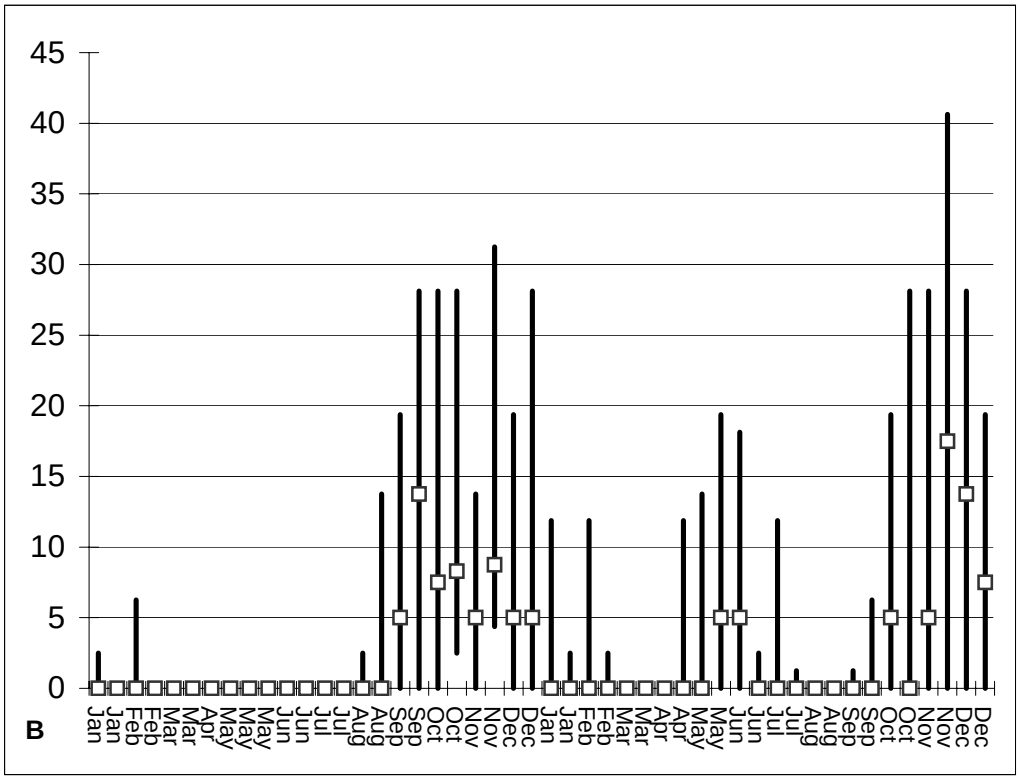
45
40
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Jan Jan Feb Feb Mar Mar Apr Apr May May Jun Jun Jul Jul Aug Aug Sep Sep Oct Oct Nov Nov Dec Dec Jan Jan Feb Feb Mar Mar Apr Apr May May Jun Jun Jul Jul Aug Aug Sep Sep Oct Oct Nov Nov Dec Dec

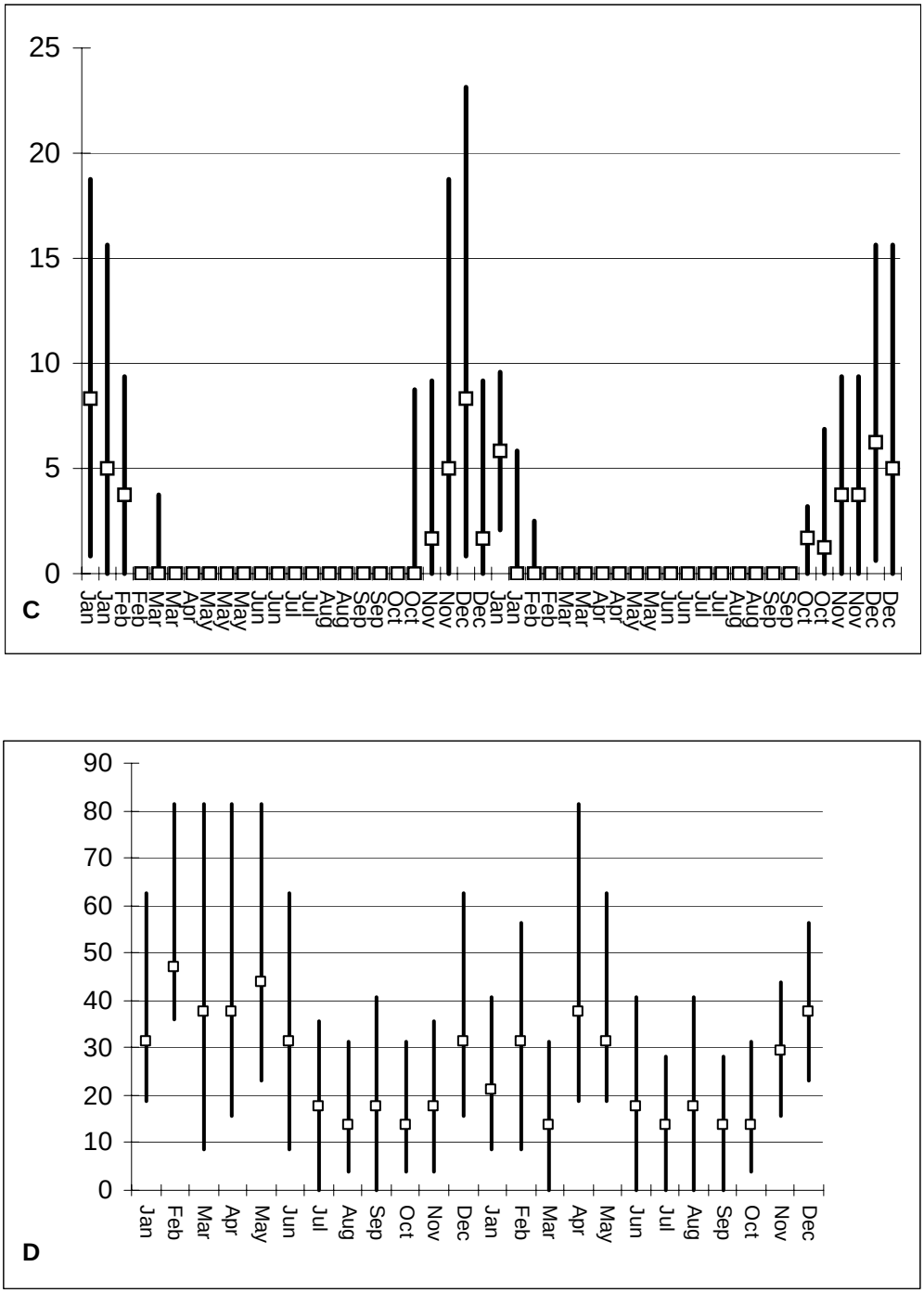

Figure 3.4 Flowers per individual over the two years time scale from Jan. 2001 to Dec. 2002 (Median quartile): A, *Orthosia ellemannii*; B, *Scyphostelma* sp. B; C, *Ditassa endoleuca*; D, "*Cynanchum*" *harlingii*.

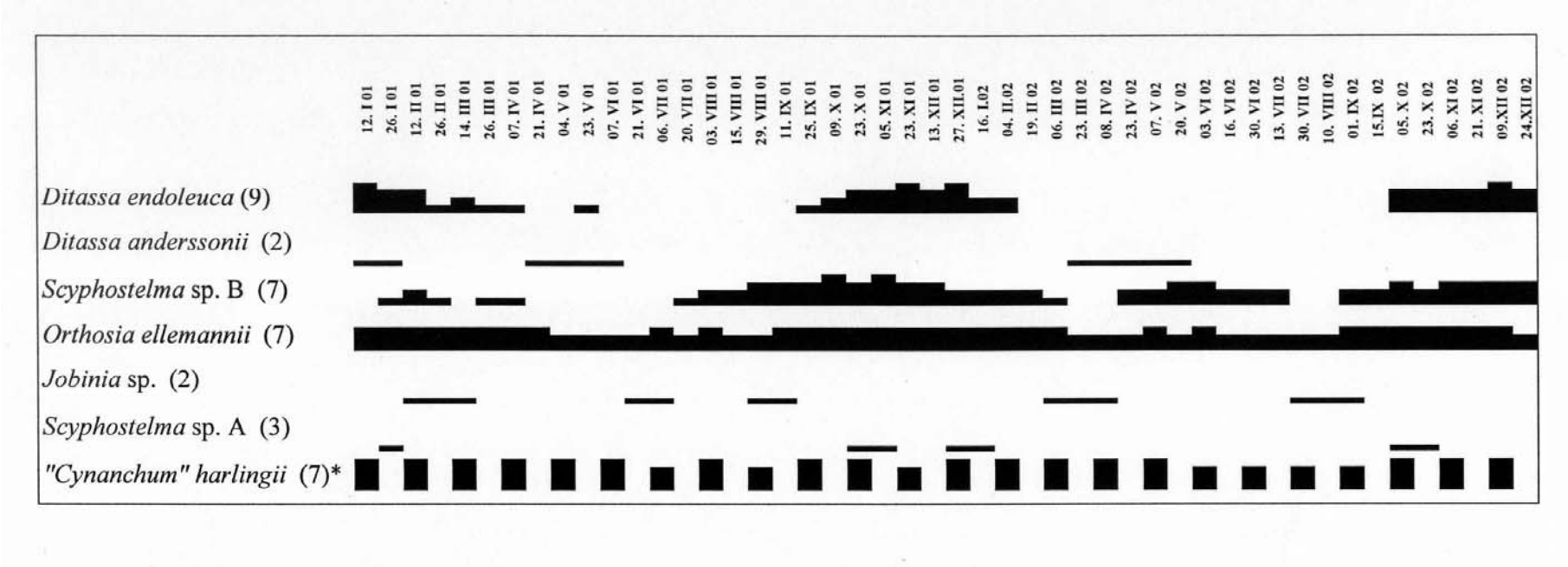

Figure 3.5 Flowering phenology of seven species in 2001 and 2002 (One quarter represents 1-25%, two quarters 26-50%, three quarters 51-75% and four quarters >75% of flowering individuals, dashed lines represent one, two or three flowering individuals; * *"Cynanchum" harlingii* was controlled only every second census).

3.5.3 Pollinaria removal and pollinia insertion

Pollinarium removal rate allows an estimation of insect activity in Asclepiadoideae (Willson and Rathke 1974). In the nine species investigated the average pollinarium removal rate was very low with an average of 0.32 ± 0.13 pollinaria removed per flower (6.4% of all possible pollinaria, Table 3.2). The variability between species was low, with a maximum of 0.62 pollinaria per flower (12.3% of all possible pollinaria) removed in the *Jobinia* flowers (Table 3.2). In "*Cynanchum*" *harlingii* and *Oxypetalum* sp. pollinaria were removed in every fifth flower (0.19 pollinaria per flower), which is less than 4% of all possible pollinaria. In 77.1% of the 633 flowers checked not a single pollinarium was removed. One pollinarium was removed in 110 flowers (17.4%) and two in 28 flowers (4.4%, Table 3.2). Single or double removal accounted for 88% of all 189 removed pollinaria, and in only 12% of the cases three to all five pollinaria were removed (Table 3.2).

Table 3.2 Pollinaria removal and pollinia insertion in nine species of Asclepiadoidae (n = number of flowers, 100% removed pollinaria = 5*n, 100% inserted pollinia = 5*n; insertion rate = percentage of removed pollinaria to inserted pollinia).

		Pollinaria removed number/flower (% pollinaria removed)						Removed pollinaria (% removed pollinaria)	Removed pollinaria/ flower	Inserted pollinia (%inserted pollinia)	Inserted pollinia/ flower	Percentage of inserted to removed pollinia
	n	0	1	2	3	4	5					
"Cynanchum" harlingii	102	88 (86%)	11 (11%)	2 (2%)	0 (0%)	1 (1%)	0 (0%)	19 (3.7%)	0.19	14 (2.8%)	0.14	73.7%
Ditassa andersonii	24	18 (75%)	5 (21%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)	7 (5.8%)	0.29	2 (1.7%)	0.08	28.6%
Ditassa endoleuca	65	51 (78%)	11 (17%)	2 (3%)	0 (0%)	1 (2%)	0 (0%)	19 (5.8%)	0.29	4 (2%)	0.06	21.1%
Jobinia sp.	34	22 (64%)	5 (15%)	5 (15%)	2 (6%)	0 (0%)	0 (0%)	21 (12.3%)	0.62	6 (3.1%)	0.18	28.6%
Orthosia ellemannii	93	68 (73%)	21 (23%)	3 (3%)	1 (1%)	0 (0%)	0 (0%)	30 (6.5%)	0.32	4 (0.9%)	0.04	13.3%
Oxypetalum sp.	83	72 (87%)	7 (8%)	3 (4%)	1 (1%)	0 (0%)	0 (0%)	16 (3.9%)	0.19	8 (1.9%)	0.10	48.7
Scyphostelma sp. *A*	180	130 (72%)	40 (22%)	9 (5%)	1 (1%)	0 (0%)	0 (0%)	61 (6.8%)	0.34	46 (5.1%)	0.26	75.4%
Scyphostelma sp. *B*	38	29 (76%)	7 (19%)	2 (5%)	0 (0%)	0 (0%)	0 (0%)	11 (5.8%)	0.29	6 (3.1%)	0.16	54.5%
Scyphostelma sp. *C*	14	10 (71%)	3 (22%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)	5 (7.1%)	0.36	2 (2.8%)	0.14	38.9%

To assess the relationship between inflorescence size and number of removed pollinaria we analysed 295 umbels with a total of 523 flowers in 5 species (Tables 3.3a-3.3e). In *Ditassa endoleuca* and *Oxypetalum* sp. a significant tendency for increasing pollinaria removal with inflorescence size was found (r = 0.9, t = 3.6, p = 0.037 and r = 0.99, t = 11,35, p = 0.008 respectively). There is a negative, significant tendency for the percentage of flowers with no removed pollinaria and increasing sciadioid size in "*Cynanchum*" *harlingii* and *Oxypetalum* sp. (r = –0.96, t = –6.2, p = 0.009 and r = –0.95, t = –4.51, p = 0.046 respectively), suggesting that larger sciadioids are more attractive to insects than smaller ones. In "*Cynanchum*" *harlingii*, again, a significant tendency for pollinaria removal increasing with inflorescence size was found (r = 0.87, t = 3.5, p = 0.025). The number of flowers without pollinarium removal, however, was not correlated with sciadioid size (r = -0.49, t = -1.13, p = 0.32). However, on single flowers no pollinarium was removed. In *Orthosia ellemannii* and *Scyphostelma* sp. A, there is no significant correlation between the number of removed pollinaria and pseudumbel size(r = -0.79, t = -1.3, p = 0.42 and r = –0.91, t = -3.3, p = 0.087, respectively). Further, the percentage of flowers with no pollinaria removed did not change significantly with sciadioid size in these two species (r = 0.83, t = 1.49, p = 0.38 and r = 0.53, t = 0.87, p = 0.475). Unfortunately, in *Jobinia* sp., *Ditassa anderssonii*, *D. endoleuca,* and also in *Scyphostelma* sp. C of the Cajanuma Reserve (Loja), flowering was too weak to further analyse correlations between sciadioid size and pollinarium removal and pollinia insertion.

Table 3.3a Pollinaria (poll.) removal and inflorescence size in *Ditassa endoleuca.*

	Open flowers per inflorescence				
	1	2	3	4	5
No. flowers	10	16	12	12	15
No. inflorescences	10	8	4	3	3
Removed poll./flower					
0	9	14	9	9	10
1	1	1	3	2	4
2	0	1	0	0	1
3	0	0	0	0	0
4	0	0	0	1	0
5	0	0	0	0	0
Removed poll. in all	1	3	3	6	6
Poll./inflorescences	0.1	0.4	0.8	2.0	2.0
Poll./flower	0.1	0.2	0.3	0.5	0.4

Table 3.3b Pollinaria (poll.) removal and inflorescence size in *Scyphostelma* sp. A.

	Open flowers per inflorescence			
	1	2	3	4
No. flowers	75	72	21	12
No. Inflorescences	75	36	7	3
Removed poll./flower				
0	54	53	14	9
1	16	15	7	2
2	4	4	0	1
3	1	0	0	0
4	0	0	0	0
5	0	0	0	0
Removed poll. in all	27	23	7	4
Poll./inflorescences	0.36	0.64	1	1.33
Poll./flower	0.36	0.32	0.33	0.33

Table 3.3c Pollinaria (poll.) removal and inflorescence size in *Orthosia ellemannii.*

	Open flowers per inflorescence		
	1	2	3
No. flowers	47	28	18
No. inflorescences	47	14	6
Removed poll./flower			
0	32	22	14
1	14	3	4
2	1	2	0
3	0	1	0
4	0	0	0
5	0	0	0
Removed poll. in all	16	10	4
Poll./inflorescences	0.34	0.71	0.66
Poll./flower	0.34	0.36	0.22

Table 3.3d Pollinaria (poll.) removal and inflorescence size in *"Cynanchum" harlingii.*

	Open flowers per inflorescence					
	1	2	3	4	5	6
No. flowers	10	10	27	12	25	18
No. Inflorescences	10	5	9	3	5	3
Removed poll./flower						
0	10	9	25	10	17	17
1	0	1	3	1	4	2
2	0	0	0	1	0	1
3	0	0	0	0	0	0
4	0	0	0	0	1	0
5	0	0	0	0	0	0
Removed poll. in all	0	1	3	3	8	4
Poll./inflorescences	0	0.2	0.3	1	1.6	1.3
Poll./flower	0	0.1	0.12	0.25	0.32	0.22

Table 3.3e Pollinaria (poll.) removal and inflorescence size in *Oxypetalum* sp.

	Open flowers per inflorescence			
	1	2	3	4
No. flowers	17	36	18	12
No. Inflorescences	17	18	6	3
Removed poll./flower				
0	16	33	14	9
1	1	1	2	3
2	0	2	0	1
3	0	0	1	0
4	0	0	0	0
5	0	0	0	0
Removed poll. in all	1	5	5	5
Poll./inflorescences	0.1	0.3	0.8	1.7
Poll./flower	0.06	0.14	0.3	0.4

For the insertion rate even lower numbers were recorded (Table 3.2). A total number of 92 pollinia was found to be inserted in the 633 flowers examined. In the nine investigated species, the pollinia insertion rate was on average 0.13 ± 0.07 pollinia inserted per flower (2.6% of all possible pollinia, Table 3.2). The highest pollinia insertion was found in *Scyphostelma* sp. A in which every fourth flower was pollinated (0.26 pollinia inserted per flower or 5.1% of all possible pollinia), while the lowest values were found in *Orthosia ellemannii* in which pollinaria were inserted in just every 25th flower (0.04 pollinia inserted per flower or 0.9% of all possible pollinia). Double insertions (two pollina per gynostegium) were scarce with one in *Oxypetalum*, one in *Jobinia*, three in "*Cynanchum*" *harlingii*, and eight in *Scyphostelma* sp. A. Multiple insertions (>2 pollina per gynostegium) as well as multiple insertions into one guide rail (>1 pollinia in one guide rail) did not occur. The percentage of inserted pollinia to removed pollinaria was relatively high with an average of 42.7% ± 22.3%, varying from 13.3% in *Orthosia ellemannii* to 75.4% in *Scyphostelma* sp. A.

3.5.4 Fruit set

Against the understanding that every inserted pollinia leads to the formation of a follicle we calculated the *a priori* fruit set, which overestimated the number of mature fruits, except for *Orthosia ellemannii* (Table 3.4). Phenological observations available on six species, however, confirmed a higher number of young pods, which decreased during maturation.

Table 3.4 Fruit set of open flowers. Fruit set [*a priori* of total number of inserted pollinaria to number of observed flowers (cf. Table 3.2)], fruit set (young pods in % of initiated pods/flowers), and fruit set (mature pods of % initiated pods/flowers) (-- = no data obtained from phenological observations).

Species	Fruit set *a priori* [%]	Fruit set young pods [%]	Fruit set mature pods [%]
"Cynanchum" harlingii	13.7	--	7.8
Ditassa andersonii	8.3	6	2.1
Ditassa endoleuca	10	8	1.4
Jobinia sp.	17.6	20	21.8
Orthosia ellemannii	4.3	4	4.0
Oxypetalum sp.	--	--	8.9
Scyphostelma sp. *A*	25.6	15	12.3
Scyphostelma sp. B	15.8	5	3.2
Scyphostelma sp. C	14.3	--	--

3.5.5 Pollinators

Under favourable weather conditions insects were present on all species. On the average, the number of visits per hour is 1.7 ± 2.0. The number of visits per hour ranged from 0.5 in *Scyphostelma* sp. C to 6.8 in "*Cynanchum*" *harlingii* (Table 3.5). On average, 2.2 ± 1.0 flowers were frequented per visit (Table 3.5). There is a significant correlation between the number of open flowers and the number of floral visits received per hour ($r = 0.84$, $t = 4.15$, $p = 0.004$) as well as between the number of open flowers and the number of flowers visited by an insect ($r = 0.91$, $t = 5.9$ $p = 0.0006$). Moreover, there is a significant correlation between the number of visits per hour and the number of flowers visited (Table 3.6). However, even though the flowers of "*Cynanchum*" *harlingii* and *Oxypetalum* sp. had the highest number of flowers frequented per visit (Table 3.5) and received high numbers of visits per hour (Table 3.5), they had the lowest average of removed pollinaria (<4% of all possible pollinaria, Table 3.2). The number of flowers visited, as well as the number of visits per hour show no significant correlation to pollinarium removal or pollinium insertion rates (Table 3.6). Insect activity was very irregular, and dependent on good weather conditions. Highest visitor frequency was observed under dry weather in the late morning and again in the late afternoon.

A wide variety of different insects was found at the flowers (cf. Table 3.1); some could be caught, others just observed. Small bees were observed on flowers of *Ditassa anderssonii* and *D. endoleuca,* but these were outnumbered by small and medium-sized flies. *Oxypetalum* sp. received visits mainly by Hymenoptera (64%) and small Lepidoptera (25%), but small moths (11%) also visited their flowers. On flowers of *Orthosia ellemannii* we caught several individuals of Empididae but none were carrying pollinaria. Furthermore, we observed ants and many broken ant legs told of frequent visitation. Pollinaria carrying Drosophilidae were caught on flowers of *Scyphostelma* sp. A, and ants were found here, too. Drosophilidae and Lauxaniidae were frequently caught on flowers of "*Cynanchum*" *harlingii*, but only one of eight examined individuals of Lauxaniidae carried pollinaria at the legs, and no pollinarium was found on six individuals of Drosophilidae. Once, small moths were observed on "*Cynanchum*" *harlingii.* On *Jobinia* many different Diptera and Hymenoptera were observed as flower visitors (own observations and Matezki pers. comm., cf. Table 3.1). Only one small moth and two individuals of an Ichneumonoidea species carried a pollinarium at the leg or the mouth parts, respectively. At dusk two small moths were observed and the one caught was carrying a pollinarium at leg. On *Scyphostelma* sp. B small flies were observed, but could not be caught. Many floral visitors were observed on the flowers, however, only four insect species were observed extracting pollinaria (Table 3.1).

Table 3.5 Insect activity on flowers of nine species of Asclepiadoideae (n_1 = estimated number of flowers, x = mean, *s.d.* = standard deviation, n_2 = number of flowers observed for estimation of life span, -- = no data available. All species were observed for 12h, except for 24h in *Orthosia ellemannii.*

Species	n_1	Flowers visited $x \pm s.d.$	Visits/hour $x \pm s.d.$	Floral life span $x \pm s.d.$ (n_2)
"Cynanchum" harlingii	300	3.9 ± 3.4	6.8 ± 2.4	--
Ditassa andersonii	50	2.3 ± 1.5	0.8 ± 1.1	4.5 ± 0.9 (11)
Ditassa endoleuca	50	1.4 ± 0.5	1.1 ± 1.3	5.0 ± 1.1 (20)
Jobinia sp.	10	1.4 ± 0.6	1.3 ± 1.2	4.3 ± 1.5 (14)
Orthosia ellemannii	20	1.6 ± 0.7	0.8 ± 1.2	4.3 ± 0.8 (10)
Oxypetalum sp.	150	3.5 ± 2.3	1.3 ± 1.7	4.1 ± 1.0 (22)
Scyphostelma sp. *A*	200	2.7 ± 1.5	1.7 ± 2.0	3.1 ± 0.6 (8)
Scyphostelma sp. *B*	20	1.3 ± 0.5	0.7 ± 0.8	3.6 ± 1.2 (14)
Scyphostelma sp. *C*	10	1.3 ± 0.5	0.5 ± 0.7	--

Table 3.6 Pearson-Correlations between fruit set (FS), life span (LS), number of flowers visited per visit (F), number of visits per hour (V), pollinaria removal rate (removal), pollinia insertion rate (insert) and the percentage of inserted pollinia to removed pollinaria (ratio) (*p<0.05, ** p<0.01, ***p<0.001).

	FS			LS			F			V			ratio			insert		
	r	t	p	r	t	p	r	t	p	r	t	p	r	t	p	r	t	p
removal	**0.72**	2.56	0.04*	0.06	0.12	0.91	-0.6	-1.96	0.09	-0.35	-1	0.35	-0.35	-1.0	0.35	0.34	0.95	0.37
insert	0.63	1.99	0.09	**-0.8**	-2.99	0.03*	0.13	0.35	0.74	0.16	0.42	0.68	**0.73**	2.82	0.03*			
ratio	0.21	0.52	0.62	**-0.89**	-4.4	0.007**	0.65	2.25	0.06	0.59	1.93	0.10						
V	0.11	0.28	0.79	-0.42	-1.03	0.35	**0.72**	2.75	0.03*									
F	0.07	0.18	0.87	-0.36	-0.87	0.43												
LS	-0.3	-0.71	0.51															

3.5.6 Nectar composition

The nectar of *Scyphostelma* sp. A is sucrose-rich, whereas the nectar of the remaining seven species is classified as sucrose dominated [Table 3.7; classification according to Baker and Baker (1990)]. Nectar concentration correlated significantly with fruit set ($r = 0.81$, $t = 3.04$, $p = 0.029$) and with the percentage of inserted pollinia to removed pollinaria ($r = 0.79$, $t = 2.84$, $p = 0.036$).

Table 3.7 Nectar sugar composition of eight species of Asclepiadoideae (n_1 = number of investigated flowers, n_2 = number of nectar samples, x = mean, *s.d.* = standard deviation; n.d. = not defined). *Jobinia* flowers contained no nectar.

Species	n_1	n_2	Sugar [µg/µl] $x \pm s.d.$	Fructose [%] $x \pm s.d.$	Glucose [%] $x \pm s.d.$	Sucrose [%] $x \pm s.d.$	Sugar ratio S/(F+G)
Cynanchum harlingii	18	2	157.7 ± 10.2	7.4 ± 10.4	8.8 ± 12.4	83.9 ± 22.8	5.2
Ditassa andersonii	16	2	115.2 ± 10.7	0	0	100	n.d.
Ditassa endoleuca	21	3	122.2 ± 15.7	8.6 ± 2.6	6.1 ± 1.4	85.3 ± 3.1	5.8
Orthosia ellemannii	14	2	83.3 ± 37.0	0	0	100	n.d.
Oxypetalum sp.	12	1	220.9	3.2	2	94.7	18.2
Scyphostelma sp. *A*	15	1	216.2	26.7	28.4	44.9	0.8
Scyphostelma sp. *B*	6	1	161.5	0	0	100	n.d.
Scyphostelma sp. *C*	3	1	186.7	1.3	0.8	97.9	46.6

3.6 Discussion

3.6.1 Phenology

Patterns of continuous or irregular flowering so far have not been observed frequently. The majority of *Asclepias* species studied by Lynch (1977) showed a distinct seasonal variation in number of flowers per inflorescences with more flowers produced in the early season. However, these observations come from a temperate country, where the climatical seasonality is much more pronounced than at our study site. However, the suggestion of Lynch (1977) that the size of inflorescences may be correlated with the growth rate of the plant and explain variations within and between years certainly holds for our study site as well.

The life spans observed here are comparable to non-tropical *Asclepias* (Wyatt 1981, Chaplin and Walker 1982, Wyatt and Shannon 1986, Kephart 1987). Floral longevity of the milkweeks here investigated contrasts to the life span of the majority of Gentianales of the study site which seldom exceeds one day (Wolff, unpubl. data). This relatively long life span might be related to the low pollinator visitation rate or, as Wyatt (1981) speculated, to the low effective pollinator service. The significant negative correlation between life span and pollinia insertion rate as well as between life span and pollinium removal-pollinaria insertion ratios (Table 3.6) support the assumption that floral longevity is shortened by successful insertion of pollinia. Several studies on proterandrous species (e.g., Devlin and Stephenson 1985, Richardson and Stephenson 1989, Sargent and Roitberg 2000, Evanhoe and Galloway 2002) showed that morphological male-phase longevity was shortened by pollen removal and morphological female-phase longevity was shortened by pollen deposition.

3.6.2 Pollinaria removal and pollinia insertion

Compared to other New World species of the subfamily Asclepiadoideae the values found for pollinia removal here are the lowest recorded: 0.5 pollinaria/flower in *Matelea reticulata* in Texas, USA (Krings 1999), 0.9 pollinaria/flower in Mexican *Matelea reticulata* and *Cynanchum foetidum* (Liede 1994), 2.5 pollinaria/flower in Mexican *Funastrum clausum* and *F. pannosum* (Kunze and Liede 1991), 2.7 pollinaria/flower in Mexican *Funastrum arenarium* (Liede 1994), and 3.6 pollinaria/flower in US *Asclepias* spp. (Lynch 1977, Willson and Rathke 1974).

Inflorescence organization like sciadioid size is known to play an important role in pollinator attraction (Willson and Price 1977, Chaplin and Walker 1982, Harder *et al.* 2004). Our results for *"Cynanchum" harlingii* and *Oxypetalum* sp. also suggest that larger sciadioids are more attractive to insects than smaller ones. The fact that the percentage of flowers with no pollinaria removed did not change significantly with sciadioid size is similar to the findings of Liede (1994) for *Matelea reticulata* and *Cynanchum foetidum.*

Regarding pollinia insertion, Liede (1994) found corresponding low values for *Cynanchum foetidum* (0.02 pollinia per flower) and *Matelea reticulata* (0.19 pollinia per flower). While the high percentage of inserted pollinia to removed pollinaria suggest relatively effective pollinators, we cannot rule out that pollinia are inserted into flowers of the same plant they were excerpted. Our data indicate further, that the fly visiting *Orthosia ellemannii* (probably Empididae) is effective in extracting pollinaria but fails to insert pollinia. In *Scyphostelma* sp. A 46 pollinia were inserted into the guide rails. Only in 10 of

these cases the pollinarium terminating the occupied guide rail had been removed, in 36 cases the pollinarium remained at its original position (s. Figure 3.2D). However, the corpusculum of the terminating pollinarium does not serve as the point to which the translator arm of the inserted pollinium is pressed for final break-off from the corpusculum as found by Wyatt (1976) for *Asclepias tuberosa*, because the pollinium is ususally not completely roped into the guide rail structure at all (Figure 3.2C).

3.6.3 Fruit set

As suggested by Willson and Rathke (1974) and Willson and Price (1977) the availability of energy limits pod production. Availability of outcrossed pollinia versus pollinia from the same plant (geitonogamy) limits pod initiation (Fritz and Morse 1981). Meve (1997) and Meve *et al.* (2004) have demonstrated for stapeliads (Ceropegieae) that most of the (artificially crossed) species are self-sterile. Typically, the genetic barriers only break down in polyploid species, dipoids can be regarded as mostly self-sterile (Meve *et al.* 2004). For half of the species investigated here, diploidy has been proven (Meve, unpubl. data), supporting the idea of self-sterility. The low visitation rates of pollinators between genetically different individuals surely reduce the number of foreign pollinia exchange and with it pollination success. If nevertheless pod development sometimes is initiated by pollinia from the same clone, abortions observed might also be due to insufficient energy conditions or infestation. Fruits on *Ditassa endoleuca* were regularly infested by fungi and/or Tephritidae; these attacks also reduce pod maturation and seed development, respectively.

3.6.4 Pollinators

The here observed significant correlation between the number of open flowers and the number of floral visits received per hour as well as between the number of open flowers and the number of flowers visited by an insect demonstrates again the importance of inflorescence structure and phenology for insect attraction (cf. Harder *et al.* 2004). The high percentage of inserted pollinia to removed pollinaria shows that if an insect did achieve pollinia transfer, it did it very effectively. The fact that many insects were observed, but only a few were carrying pollinaria coincides with the observations of Liede and Whitehead (1991) on *Cynanchum (Sarcostemma) viminale* in South Africa. At the same study site in southern Ecuador, Wolff *et al.* (2003) showed for *Isertia laevis* (Rubiaceae) that the flower visitor achieving the highest seed set was also scarce.

3.6.5 Nectar composition

The sucrose dominated nectar of our study plants corresponds with the records for most species of *Asclepias* in which sucrose is the dominant sugar (Percival 1961, Southwick *et al*. 1981, Southwick 1983). The nectar of nine additional species of Asclepiadoideae is also sucrose-dominant (Wolff, unpubl. data). Short-tongued bees and flies, however, prefer hexose-dominant and hexose-rich nectars (Baker and Baker 1983). There seems to be no association between sugar ratios and pollinators. The data suggest that nectar sugar composition is relatively uniform within the subfamily. Wolff (submitted) showed that sucrose is predominant in nectar of 41 out of 47 species of Gentianales from the study site. Within many other families there are phylogenetic constraints on nectar sugar composition irrespective of pollinator guild, for example Antirrhineae (Scrophulariaceae; Elisens and Freeman 1988), *Lycium* (Solanaceae; Galetto *et al.* 1998), Sinningieae (Gesneriaceae; Perret *et al*. 2001).

The correlation of nectar concentration with fruit set and with the percentage of inserted pollinia to removed pollininaria agrees with the results of Wyatt and Shannon (1986) who found in *Asclepias exaltata* that plants producing more concentrated nectars showed higher levels of reproductive success. On the other hand, allocation of concentrated nectar can increase loss of energy that would have been required for, e.g., seed set as shown for *Blandfordia nobilis* (Liliaceae; Pyke 1991).

The reasons for the high diversity of Asclepiadoideae species in the Ecuadorian Andes are little understood. The nine species studied are mostly limited to a narrow geographical range, comprising southern Ecuador and northern Peru. It is not known whether the ranges of the pollinators correspond to those of the plants. As recent phylogenetic studies have shown (Liede-Schumann *et al.* 2005), all species under consideration here belong to a large, morphologically very diverse, exclusively New World clade. Rapini and Van den Berg (2005) attribute a relatively recent origin to this clade, and state that it is still in active speciation and radiation. Montane forests in many areas of the tropical Andes occur on very steep slopes, being subjected to frequent natural landslides. Such gaps are often preferred habitats for twining species such as the Asclepiadoideae investigated and are important factors for the dynamics of forest regeneration. Frequent formation of these habitats might therefore serve as an important supporting factor in the rapid radiation of small-flowered, ecologically undemanding and adaptable groups like *Scyphostelma*. This asclepiad genus is the most diverse of the Asclepiadoideae at our study site, in Ecuador as well as in other Andean countries, with a still high, uncounted number of recognized and so far undescribed species

(Liede-Schumann and Meve, unpubl.). The long flowering period of *Scyphostelma* together with its effective pollination and high fruit set are valuable prerequisites for the sucessful adaptative radiation observed.

In contrast to the other pollinaria-carrying family, the Orchidaceae, in which highly specialized plant-pollinator relationships are predominant (e.g., Dressler 1968, Manning and Linder 1992, Johnson *et al.* 1998), the issue of specialization versus generalization in Asclepiadoideae is less clear. Apart from highly specialized species (e.g., *Calotropis*, *Microloma*, s. Introduction), the best studied genus, *Asclepias*, is usually visited by a wide variety of insects (e.g., Morse 1985). In the tribe Ceropegieae, a specialization towards myiophily has taken place, but any fly of a certain size can act as pollinator (Meve and Liede 1994, Ollerton 2005). However, in the South African *Cynanchum viminale* (tribe Asclepiadeae, Liede and Whitehead 1991), a wide range of flower visitors was observed, but only very few carried pollinaria. Our present study suggests the same degree of limited specialization, with a rather large number of insects visiting, but only a few able to successfully transfer pollinaria, as indicated by the high percentage of removed to inserted pollinia. This observation of limited specialization coincides with the understanding of the American Asclepiadeae as a still actively radiating and rapidly evolving branch of the Apocynaceae.

Our study is one of the first analyzing the mostly twining, highly scattered inhabitants of forests, forest margins and thickets. We would expect to find similar patterns of plant-pollinator interactions in other geographical regions in which such Asclepiadoideae occur, be it in the Old or the New World. We would also expect that numerous seemingly generalistic species in the montane rain forest will exhibit some degree of specialization toward a particular pollinator guild upon closer examination.

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4 Nectar sugar composition and volumes of 47 species of Gentianales from a Southern Ecuadorian montane forest

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4.1 Abstract

This study investigates 47 taxonomically related species (Gentianales), all native to a tropical montane forest in southern Ecuador, in terms of nectar chemistry and nectar volumes in relation to pollination biology.

Nectar volumes of covered (24 h production) and uncovered (standing crop) flowers were measured in the natural habitat. Sucrose, fructose, and glucose were quantified in the nectar using high performance liquid chromatography. Flower visitors were observed.

Nectar sugar concentration did not differ significantly among the pollination syndromes. Regarding sugar composition, the only significant differences were found in chiropterophilous and myiophilous flowers, which had a significantly lower sugar ratio than sphingophilous flowers. A separation of chiropterophilous and myiophilous flowers from the other pollination syndromes is further substantiated by non-linear multidimensional scaling using CNESS index of dissimilarity based on nectar sugar compositions. Matrix test revealed no correlation of observed floral visitors to nectar concentrations, however a weak significant correlation was found between floral visitors and nectar sugar compositions. The nectar volumes of covered and uncovered flowers are related to, and differ significantly among, pollination syndromes. Matrix tests revealed correlation between floral visitors and nectar volume of covered flowers, and to a lesser extent, of uncovered flowers.

Sucrose is the predominant floral nectar sugar in the order Gentianales, suggesting that nectar sugar composition is a conservative characteristic. However, some degree of an adaptive convergence of floral nectar compositions to principal pollinator type within the constraints set by phylogenetic history is likely. The driving force to visitation appears to be the volume of nectar the visitor can expect to consume.

4.2 Key words

Nectar sugar composition, nectar volume, nectar standing crop, pollination syndrome, Rubiaceae, Gentianaceae, tropical montane forest, Ecuador

4.3 Introduction

Floral nectar is the most important reward offered to pollinators in angiosperms (Simpson and Neff 1983). The major sugars in nectar are the disaccharide sucrose and the hexose monosaccharides glucose and fructose (Baker and Baker 1983). Floral nectar characteristics such as sugar composition, sucrose-hexose proportions, concentration, volume, time of nectar secretion, and nectar dynamics are often related to the interaction of flowers and pollinators (Baker and Baker 1983, Freeman *et al.* 1984, Baker and Baker 1990, Stiles and Freeman 1993, Galetto *et al.* 1998, Perret *et al.* 2001, Pacini *et al.* 2003, Wolff *et al.* 2003, Wolff *et al.* in press). There are similarities in nectar features between taxonomically unrelated species in connection with the pollinator type. These convergences are often seen as a result of plant adaptation to preferences, digestive abilities, or sugar intake efficiencies of specific pollinators (Stiles 1976, Haber and Frankie 1989, Martínez del Rio *et al.* 1992, Baker *et al.* 1998). Other studies show homogeneity of nectar sugar composition among phylogenetically related taxa over various pollination syndromes (Galetto *et al.* 1998, Perret *et al.* 2001, Galetto and Bernardello 2003). Whether nectar features are related to the type of pollinator, or whether nectar sugar composition is a conservative feature relatively constant within taxonomically related species, or both, still remains uncertain.

Many field-studies of the nectar characteristics of flowering species sharing a single pollination syndrome carried out in natural plant communities reveal adaptation to this specific syndrome, such as hummingbird flowers (Stiles and Freeman 1993, Sazima *et al.* 1996, Dziedzioch 2001, McDade and Weeks 2004a, 2000b), moth flowers (Haber and Frankie 1989), or bat flowers (Sazima *et al.* 1999). Previous studies focusing on nectar sugar composition in phylogenetically related taxa comprising a large variety of pollination syndromes in, for example, Asteraceae (Baker and Baker 1982), Scrophulariaceae (Elisens and Freeman 1988), Fabaceae (Van Wyk 1993), Solanaceae (Galetto *et al.* 1998), Caryophyllaceae (Witt *et al.* 1999), and Gesneriaceae (Perret *et al.* 2001) were based primarily upon plant material from greenhouses or botanical gardens, such that the flower visitor impacts on nectar standing crop were unobserved. In an ecological context, however, decisions made by foragers are based upon rewards actually encountered (i.e., standing crop),

and those are quite different from nectar volumes protected from flower visitors (McDade and Weeks 2004a, 2000b). Field observations are necessary to determine the role of nectar features in the interactions between plants and flower visitors. This study investigates taxonomically related species (Gentianales) all native to a tropical montane forest in southern Ecuador, under natural conditions. The monophyletic order Gentianales includes Apocynaceae, Gelsemiaceae, Gentianaceae, Loganiaceae, and Rubiaceae (Backlund *et al.* 2000). Rubiaceae range among the most predominant Andean families in floristic studies (e.g., Gentry 1988, Madsen and Øllgaard 1994, Jørgensen and León-Yánez 1999, Dorr *et al.* 2000, Webster and Rhode 2001). According to Grant and Struwe (2003), the Podocarpus National Park presents one of the greatest species diversity in *Macrocarpaea* (Gentianaceae). Besides the large number of species existing at the study site, Gentianales exhibit flowers visited by bees, flies, butterflies, hummingbirds, and bats, so this order is ideal for testing nectar features. Nectar composition, volume of covered and uncovered flowers, and flower visitors of 47 taxonomically related plant species from such a hitherto data-scarce region are presented here.

4.4 Material and methods

4.4.1 Study site and plant material

The study site "Estación Científica San Fransisco" (03° 58´ S, 79° 04´ W; 1800 m to 3150 m a.s.l.) is located within the Eastern Cordillera of the southern Ecuadorian Andes, bordering the Podocarpus National Park, which is known as an outstanding biodiversity hotspot (Barthlott *et al.* 1996). Most parts are covered with undisturbed or slightly disturbed montane rain forest. Detailed information on the floristic composition of the study site is provided in Bussmann (2001), Paulsch (2002) and Homeier (2004). Mean annual temperatures range from 15.5° C in the lower areas to 9° C at higher elevations. Annual rainfall increases from about 2000 mm in lower areas to more than 5000 mm in higher areas (P Emck, University of Erlangen, Germany, unpubl. res.). Fieldwork was carried out from March to July 2000, September 2000 to February 2001, and from August to December 2001. All members of the order found at the study site were investigated except nine species of the subfamily Asclepiadoideae (Apocynaceae), which are treated in a separate paper, because their highly derived floral structure, their pollinia-forming habit demands and special pollination mechanisms. Gelsemiaceae and Loganiaceae did not occur at the study site. For Rubiaceae, the taxonomic classification of Andersson (1993) was followed and Gentianaceae

were classified following Struwe *et al.* (2002). Voucher specimens are housed at MO and UBT.

4.4.2 Characterization of flower syndromes and observation of flower visitors.

Considering the floral morphology of Gentianales, there is a great variability of floral displays (corolla size, color, scent) and nectar accessibility (corolla shape, corolla opening, tube length). The notion of pollination syndrome (Vogel 1969, Faegri and van der Pijl 1980, Proctor *et al.* 1996) was used to group the species. Classification was based on a set of morphological characteristics such as corolla shape, corolla color, scent, pattern of floral anthesis, and nectar secretion. Additionally, flower visitors were observed in the field. Each plant species considered to belong to the melittophilous, myiophilous or ornithophilous syndrome was observed for at least 12 hours from 06.00 h to 18.00 h in blocks of four hours. Night flowering species were observed during the day and from 18.00 h until midnight.

Species were classified as myiophilous when they were exclusively visited by diptera. The criteria for melittophily were: flowers open during the day, corolla white, cream, yellow or light blue, in some cases sweet diurnal scent emission (*Faramea coerulescens, F. glandulosa, Arcytophyllum macbridei*), small corolla tubes (< 15mm), and no visitation by hummingbirds. The criteria for ornithophily were: corolla or inflorescence branch red, yellow, blue, or violet, no scent, and frequent visitation by hummingbirds. "Sphingophily" is used as a generic term for all species morphologically adapted to pollination by lepidopterans (including psychophily: *Arachnothryx lojensis*). The criteria for sphingophily in the narrow sense were synchronized anthesis at night, corolla colored white to cream, very narrow corolla tube, sweet fragrance, and scent emission beginning or becoming more intense in the evening. Finally, chiropterophily was assigned by bell-shaped corolla, mushroom-like scent being more intensive during the night, and visitation by bats.

4.4.3 Nectar sampling and analysis

In order to measure the nectar volumes that legitimate flower visitors may obtain, nectar standing crop was sampled at 06.00 h, 10.00 h, 14.00 h, and 18.00 h for diurnal and nocturnal uncovered flowers, and at 18.00 h, 22.00 h, 02.00 h, and 06.00 h for nocturnal uncovered flowers. To determine the daily nectar production and nectar sugar concentrations, flowers were covered in bud stage. The nectar of bee-, fly-, and hummingbird flowers was sampled in the evening, and that of moth- and bat flowers was sampled in the early morning by inserting microcapillaries and then recording the nectar volume. An aliquot of 2 µL nectar (or less if flowers contained < 2µL nectar) was injected into Eppendorf® caps with 70%

ethanol for each flower. Nectar taken at the same time and from the same species was pooled. The samples were frozen until determination of nectar concentration and composition. For analysis, samples were dried in a vacuum centrifuge, diluted with 200 µL water, and filtered using a WATERS™ High Performance Carbohydrate Column to avoid contamination. The injection volume was 10 µL, and elution took place with an acetonitrile-water-mixture (71:29) at a flow rate of 1.4 $mL \cdot min^{-1}$ and a temperature of 35° C. Glucose, fructose, and sucrose were detected with a refraction index detector and quantified with the WATERS Millenium Software™ from WATERS. Concentrations were converted from $\mu g\ \mu L^{-1}$ to sucrose-equivalent, % weight per total weight, using table 63 in the 50th edition of the Handbook of Chemistry and Physics (Weast, 1969).

4.4.4 Statistical analysis

Data were tested for normality and homogeneity of variance. In order to meet these criteria, nectar volume of covered flowers was log (x+1) transformed, and sugar ratio was square root transformed. When data met the assumption for parametric statistics, ANOVA followed by Tukey-Kramer HSD for unequal N were used to test for differences of species means among classes of pollination. Because data on nectar standing crop violated the normality assumption for parametric statistics, the Kruskal-Wallis rank sums test followed by the Tukey-Kramer multiple comparison for non-parametric data were used to ascertain differences of species means among classes of pollination (Siegel and Castellan 1988). The Chord-Normalized Expected Species Shared (CNESS) distance index (Trueblood *et al.* 1994), ranging between 0 and the square root of 2, was used to determine differences between the sampled species' nectar sugar composition. CNESS is a metric version of Grassle and Smith`s (1976) NESS dissimilarity index, and both can be regarded as more generalized forms of the Morisita index (Morisita 1959). These are the most appropriate indices for analyzing quantitative data (Wolda 1981, Wolda 1983, Trueblood *et al.* 1994). Calculation of the CNESS index was performed using the updated version of the Combinatorial Polythetic Agglomerative Hierarchical Clustering (COMPAH 96) program (Boesch 1977) provided by Gallagher at UMASS/Boston *(http://www.es.umb.edu/edgwebp.htm*). Non-linear Multi-dimensional Scaling (NMDS) was used to visualize similarities among the species. Stress is a measurement that reflects the degree of deviation of NMDS distances from true matrix distances. According to Clarke (1993), stress values below 0.05 give an excellent representation with no prospect of misinterpretation. Sørensen Index, based on presence-absence data, was calculated for the floral visitors of each plant species. Euclidean distances

were calculated for the nectar volumes of covered and uncovered flowers, as well as for nectar sugar concentrations. Matrix correlation tests were used to associate distance matrices (Mantel 1967). For example, (1-Sørensen) the matrix of floral visitors can be directly compared to the dissimilarity (CNESS) matrix of nectar sugar composition, or nectar concentration (Euclidean) distances, or any other derived matrices (e.g., from nectar volume data). For the performance of matrix correlation tests, distance matrices were calculated for 46 plant species; *Palicourea* sp. was excluded because no floral visit was observed. Matrix correlation tests were performed by the program Primer™ Version 5 (Clarke and Gorley 2001). To test correlation, Pearson correlation was used for parametric data (sugar ratio versus dimension 1 of the NMDS), and Spearman rank order correlation R was used for non-parametric data (nectar volumes of uncovered versus covered flowers; mean versus standard deviation of nectar volumes in covered and uncovered flowers). The data analysis software, STATISTICA™, Version 7.0 from StatSoft, Inc. (2004) was used.

Table 4.1 Tribe, flower visitors of each species investigated, sample size N_f flowers, N_i individuals, N_s pooled nectar samples analyzed, nectar sugar concentration and composition, daily nectar production of covered flowers and nectar standing crop grouped according to their pollination syndrome. G for Gentianaceae, R for Rubiaceae, Tribal affiation is designated by the following numerals: G1 Gentianeae, G2 Helieae, R1 Psychotrieae, R2 Hedyotideae, R3 Coussareae, R4 Cinchoneae, R5 Hillieae, R6 Rondeletieae, R7 Coccocypseleae, R8 Condamineeae, R9 Isertieae, Flower visitors: G Glossophagidae, T Trochilidae, Hym Hymenoptera (mainly Apidae), Lep Lepidoptera except Sphingidae, Noctuideae, Geometridae, Sph Sphingidae, Noc Noctuidae and Geometridae, Dip Diptera, Col Coleoptera. Flower visitors were observed by the author except as noted [1]Matt (2001), [2]Dziedzioch (2001), [3]Paulsch (pers. comm.). *x* = mean, *s.d.* = standard deviation, *n* = number of flowers sampled for nectar volumes.

Species	Tribe	Flower visitors	N_f (N_i)	N_s	Conc. [%w /w] *x*± *s.d.*	% Fructose *x*± *s.d.*	% Glucose *x*± *s.d.*	% Sucrose *x*± *s.d.*	S/(F+G) *x*± *s.d.*	Nectar production 24 h covered *x*± *s.d.* [µL] (*n*)	Standing crop 18.00 - 6:00 h *x*± *s.d.* [µL] (*n*)	Standing crop 6:00 - 18:00 h *x*± *s.d.* [µL] (*n*)
Myiophily												
Arcytophyllum filiforme (Ruiz & Pav.) Standl.	R2	Dip	3 (1)	1	32.0	30.8	36.3	33.0	0.5	0.3 ± 0.2 (3)	-	0.1 ± 0.03 (7)
Dioicodendron dioicum (K. Schum. & K. Krause) Taylor	R8	Dip	13 (2)	1	31.0	20.0	34.1	46.0	0.9	no data	-	0.3 ± 0.1 (13)
Gentianella sp. 1	G1	Dip	15 (15)	1	59.0	45.8	54.2	0.0	0.0	0.7 ± 0.5 (6)	-	0.1 ± 0.1 (15)
Halenia sp. 1	G1	Dip	12 (10)	2	26.8 ± 14.5	44.1 ± 9.7	42.8 ± 8.9	13.1 ± 18.5	0.2	1.1 ± 0.4 (10)	-	0.1 ± 0.1 (12)
Psychotria aubletiana Steyerm.	R1	Dip	3 (1)	1	13.0	25.5	28.6	45.9	0.8	no data	-	0.5 ± 0.1 (3)
Psychotria sp. 1	R1	Dip	16 (6)	2	25.0 ± 8.5	16.2 ± 13.8	10.5 ± 8.1	73.3 ± 21.9	4.7 ± 4.6	0.7 ± 0.4 (9)	-	0.2 ± 0.2 (14)
Melittophily												
Faramea uniflora Dwyer & M. V. Hayden	R3	Hym, Dip	9 (6)	2	2.3 ± 2.5	0	0	100	not defined	0.2 ± 0.2 (7)	-	0.2 ± 0.2 (11)
Psychotria acuminata Benth.	R1	Hym, Dip	34 (6)	1	9.0	16.2	17.3	66.5	2.0	1.9 ± 0.8 (18)	-	0.8 ± 0.4 (19)
Psychotria tinctoria Ruiz & Pav.	R1	Hym	6 (4)	2	49.5 ± 3.5	18.3 ± 2.5	21.3 ± 3.0	60.4 ± 5.4	1.5 ± 0.3	4.9 ± 3.4 (6)	-	0.8 ± 0.7 (6)
Palicourea sp. nov. ined. C.M. Taylor	R1	Hym	78 (8)	3	24 ± 5.6	16.6 ± 1.4	16.5 ±1.3	66.9 ± 2.5	2.0 ± 0.2	5.8 ± 2.3 (78)	-	0.1 ± 0.3 (25)
Coccocypselum condalia Pers.	R7	Hym	27 (8)	2	13.7 ± 11.7	5.7 ± 5.2	6.8 ± 6.0	87.5 ± 11.2	4.5 ± 1.2	0.8 ± 0.4 (15)	-	0.3 ± 0.2 (27)

Table 4.1 continued

Species	Tribe	Flower visitors	N_f (N_i)	N_s	Conc. [%w /w] *x*± *s.d.*	% Fructose *x*± *s.d.*	% Glucose *x*± *s.d.*	% Sucrose *x*± *s.d.*	S/(F+G) *x*± *s.d.*	Nectar production 24 h covered *x*± *s.d.* [µL] (*n*)	Standing crop 18.00 - 6:00 h *x*± *s.d.* [µL] (*n*)	Standing crop 6:00 - 18:00 h *x*± *s.d.* [µL] (*n*)
Manettia sp. 2	R2	Hym	23 (3)	1	22.0	9.0	8.4	82.6	4.7	3.1 ± 1.4 (23)	-	0.8 ± 1.2 (23)
Rudgea ciliata (Ruiz & Pav.) Spreng.	R1	Hym	25 (4)	2	40.8 ± 14.5	12.7 ± 4.8	13.3 ± 7.6	73.9 ± 12.4	3.3 ± 2.0	2.1 ± 0.9 (11)	-	0.6 ± 0.5 (22)
Stilpnophyllum oellgaardii L. Andersson	R4	Hym	45 (7)	6	27.4 ± 4.5	4.9 ± 1.4	15.8 ± 1.2	79.2 ± 2.5	3.9 ± 0.6	3.9 ± 2.2 (62)	-	1.2 ± 0.9 (20)
Arcytophyllum macbridei Standl.	R2	Hym	27 (6)	3	20.8 ± 11.6	30.9 ± 14.7	30.9 ± 19.7	38.1 ± 34.2	0.6 ± 1.0	0.5 ± 0.2 (12)	-	0.3 ± 0.3 (35)
Arcytophyllum capitatum (Benth.) K. Schum.	R2	Hym, Col	40 (14)	5	38.3 ± 12.9	38.8 ± 3.3	44.0 ± 7.0	17.2 ± 10.2	0.2 ± 0.1	1.0 ± 0.5 (24)	-	0.2 ± 0.1 (40)
Arcytophyllum ciliolatum Standl.	R2	Hym, Col	45 (12)	6	41.5 ± 20.8	31.8 ± 5.9	32.0 ± 5.7	36.1 ± 11.5	0.6 ± 0.3	1.4 ± 0.8 (13)	-	0.6 ± 0.7 (31)
Arcytophyllum thymifolium (Ruiz & Pav.) Standl.	R2	Hym, Col	8 (4)	2	22 ± 21.2	44.8 ± 0.4	42.8 ± 1.5	12.4 ± 1.9	0.1 ± 0.02	0.4 ± 0.2 (7)	-	0.3 ± 0.2 (8)
Arcytophyllum vernicosum Standl.	R2	Hym, Col, Dip	12 (3)	2	22 ± 2.8	22.5 ± 2.1	24.0 ± 0.2	53.5 ± 2.2	1.2 ± 0.1	0.4 ± 0.4 (13)	-	0.4 ± 0.2 (12)
Notopleura vargasiana sp. nov. ined. C.M. Taylor	R1	Hym, Lep, Dip	21 (7)	3	26.5 ± 25.3	6.4 ± 4.3	5.6 ± 3.8	88.0 ± 8.0	13.6 ± 14.5	0.8 ± 0.3(10)	-	0.3 ± 0.3 (21)
Faramea cf. *glandulosa* Poepp. & Endl.	R3	Hym, Lep	8 (4)	2	41.5 ± 9.2	14.3 ± 9.8	13.6 ± 9.7	72.1 ± 19.5	3.8 ± 3.3	1.6 ± 0.8 (12)	-	0.4 ± 0.2 (12)
Psychotria reticulata Ruiz & Pav.	R1	Hym, Lep	19 (5)	3	25.3 ± 14.0	12.7 ± 0.5	16.4 ± 2.6	70.9 ± 2.1	2.4 ± 0.3	1.0 ± 0.5 (27)	-	0.3 ± 0.2 (12)
Faramea coerulescens K. Schum. & K. Krause	R3	Hym, Lep, T[3]	8 (3)	1	14.5	23.2	17.1	59.8	1.5	4.7 ± 2.6 (8)	-	0.9 ± 0.7 (10)
Ornithophily												
Palicourea angustifolia Kunth	R1	T, Hym, Lep	24 (6)	8	19.8 ± 5.9	14.4 ± 2.2	12.4 ± 2.7	73.2 ± 4.7	2.8 ± 0.7	7.7 ± 2.6 (24)	-	2.2 ± 1.7 (53)
Palicourea calycina Benth.	R1	T, Hym, Lep	44 (12)	9	24.9 ± 10.5	18.3 ± 5.9	16.0 ± 7.5	65.8 ± 13.2	2.2 ± 0.8	16.1 ± 5.1 (44)	-	1.1 ± 1.0 (49)
Palicourea canarina C.M. Taylor	R1	T, Hym, Lep	106 (14)	3	14.0 ± 2.0	29.3 ± 3.7	15.1 ± 3.8	55.6 ± 6.3	1.3 ± 0.3	14.4 ± 9.6 (106)	-	5.6 ± 7.0 (62)
Palicourea heterochroma K. Schum. & K. Krause	R1	T, Hym, Lep	21 (6)	13	15.3 ± 4.9	18.2 ± 5.4	14.4 ± 5.3	67.4 ± 10.6	2.3 ± 0.9	43.1 ± 16.3 (21)	-	4.0 ± 4.8 (29)

Table 4.1 continued

Species	Tribe	Flower visitors	N_f (N_i)	N_s	Conc. [%w /w] *x*± *s.d.*	% Fructose *x*± *s.d.*	% Glucose *x*± *s.d.*	% Sucrose *x*± *s.d.*	S/(F+G) *x*± *s.d.*	Nectar production 24 h covered *x*± *s.d.* [µL] (*n*)	Standing crop 18.00 - 6:00 h *x*± *s.d.* [µL] (*n*)	Standing crop 6:00 - 18:00 h *x*± *s.d.* [µL] (*n*)
Palicourea luteonivea C.M. Taylor	R1	T, Hym, Lep	26 (8)	16	15.9 ± 4.0	14.8 ± 4.0	10.8 ± 8.6	74.4 ± 12.5	3.3 ± 0.8	15.9 ± 7.1 (26)	-	2.8 ± 3.4 (92)
Palicourea subtomentosa (Ruiz & Pav.) DC.	R1	T², Hym, Lep	15 (4)	2	17.0 ± 5.7	14.2 ± 3.8	12.2 ± 4.8	73.6 ± 8.6	3.0 ± 1.3	1.6 ± 0.6 (15)	-	0.6 ± 0.5 (15)
Palicourea cf. *weberbaueri* K. Krause	R1	T, Hym, Lep	33 (10)	23	21.8 ± 8.4	20.4 ± 7.5	20.8 ± 11.2	58.9 ± 18.0	1.7 ± 0.8	7.5 ± 3.8 (45)	-	1.6 ± 2.9 (92)
Palicourea lobbii Standl.	R1	T, Hym	13 (5)	3	17.7 ± 2.1	11.8 ± 0.8	7.7 ± 0.7	80.5 ± 0.8	4.1 ± 0.2	10.6 ± 3.7 (15)	-	3.6 ± 4.0 (15)
Palicourea lyristipula Wernham	R1	T, Hym	49 (10)	14	26.1 ± 11.7	18.0 ± 6.6	17.6 ± 8.3	64.3 ± 14.7	2.1 ± 1.0	4.8 ± 2.0 (57)	-	0.8 ± 1.3 (154)
Palicourea thyrsiflora (Ruiz & Pav.) DC.	R1	T, Hym	35 (6)	13	10.6 ± 2.6	22.8 ± 6.7	17.7 ± 5.8	59.5 ± 12.3	2.1 ± 2.6	30.6 ± 15.7 (35)	-	2.8 ± 2.9 (51)
Manettia sp. 1	R2	T	12 (4)	4	8.3 ± 2.4	15.9 ± 6.5	7.3 ± 4.9	76.8 ± 11.3	4.1 ± 2.5	51.9 ± 7.1 (12)	-	1.7 ± 2.4 (38)
Symbolanthus calygonus (Ruiz & Pav.) Griseb. ex Gilg	G2	T	10 (5)	1	15.5	20.1	8	71.9	2.6	48.9 ± 16.5 (10)	-	6.8 ± 5.7 (15)
Palicourea sp. 1	R1	-	11 (1)	1	14	17.8	17.6	64.6	1.8	14.5 ± 4.5 (11)	-	0.5 ± 0.7 (11)
Psycho-Sphingo-Phalaenophily												
Arachnothryx lojensis Steyerm.	R6	Lep	17 (5)	11	15.5 ± 4.8	7.6 ± 3.6	3.0 ± 5.3	89.4 ± 8.3	10.3 ± 4.6	1.8 ± 0.9 (17)	-	0.6 ± 1.1 (248)
Ladenbergia sp. 1	R4	Noc, Sph, T	3 (2)	1	22	12.2	6.4	81.4	4.4	45.2 ± 9.7 (3)	41.3 ± 15.3 (3)	1.1 ± 1.0 (5)
Palicourea andrei Standl.	R1	Noc, Sph, T	5 (2)	1	17	16	20.2	63.7	1.8	14.7 ± 3.1 (5)	13.3 ± 3.3 (11)	1.5 ± 1.0 (4)
Isertia laevis (Triana) B. M. Boom	R9	Noc, Sph, T, Hym	28 (6)	18	17.9 ± 6.8	33.6 ± 9.0	31.3 ± 7.1	35.1 ± 12.2	0.7 ± 0.9	39.3 ± 19.6 (28)	18.5 ± 14.3 (163)	4.7 ± 7.2 (119)
Ladenbergia sp. 2	R4	Noc, Sph, Hym	10 (1)	1	9.5	14.5	17.6	67.9	2.1	5.4 ± 1.7 (10)	6.4 ± 1.9 (15)	0.2 ± 0.3 (15)
Hillia parasitica Jacq.	R5	Sph, Hym	4 (2)	1	16	2.5	3.6	93.9	15.5	41.0 ± 9.9 (3)	32 ± 11.9 (6)	24.3 (1)
Hillia wurdackii Steyerm.	R5	Sph	3 (2)	1	19.5	6.5	2.5	91	10.1	38.3 ± 15.2 (4)	36.7 ± 13.1 (7)	29.5 (1)

Table 4.1 continued

Species	Tribe	Flower visitors	N_f (N_i)	N_s	Conc. [%w /w] *x*± *s.d.*	% Fructose *x*± *s.d.*	% Glucose *x*± *s.d.*	% Sucrose *x*± *s.d.*	S/(F+G) *x*± *s.d.*	Nectar production 24 h covered *x*± *s.d.* [µL] (*n*)	Standing crop 18.00 - 6:00 h *x*± *s.d.* [µL] (*n*)	Standing crop 6:00 - 18:00 h *x*± *s.d.* [µL] (*n*)
Chiropterophily												
Macrocarpaea arborescens Gilg	G2	G[1], T, Hym	7 (4)	1	23	43.5	29.6	26.9	0.4	67.9 ±15.8 (7)	51.7 ± 18.7 (15)	1.6 ± 1.7 (14)
Macrocarpaea harlingii J. S. Pringle	G2	G[1], T, Hym, Noc	13 (6)	1	11	36.3	35.3	28.4	0.4	73.1 ± 27.8 (13)	60.6 ± 27.7 (18)	2.5 ± 2.5 (19)
Macrocarpaea noctiluca J. R. Grant & Struwe	G2	G[1], T, Hym, Spi, Noc, Lep, Dip	12 (6)	1	10.5	33.9	25.8	40.3	0.7	98.8 ± 28.3 (12)	58.7 ± 33.8 (32)	4.4 ± 12.6 (26)
Symbolanthus cf. sp. nov. ined.	G2	G[1], T, Hym, Spi, Noc	12 (5)	1	12	34.9	19.2	45.9	0.8	102.9 ± 42.1 (15)	83.9 ± 28.0 (12)	9.5 ± 6.2 (15)

4.5 Results

4.5.1 Nectar sugar composition and concentration

Floral nectars were sucrose-dominant in all flowers classified as ornithophilous, as well as in the majority of flowers classified as sphingophilous, with sugar ratios ranging from 1.3 to 15.5 (the only exception was *Isertia laevis* with 0.7; see Table 4.1). Sucrose/hexose ratios below 1 were found in bat-flowers all belonging to the tribe Helieae. Nectar sugar ratio ranged from 0.1 to 13.6 within the melittophilous syndrome. Sugar composition varied markedly among myiophilous species (Table 4.1) from hexose-dominant (*Gentianella* sp.) to hexose-rich *(Halenia* sp.) to sucrose-rich (*Arcytophyllum filiforme, Psychotria aubletiana, Dioicodendron dioicum*) and sucrose-dominant (*Psychotria* sp.). It is worth noting that the hexose-dominant and hexose-rich species occur at elevations above 3000 m (the only exception is *Macrocarpaea harlingii*).

There is a significant sugar ratio difference between sphingophilous and myiophilous species and between sphingophilous and chiropterophilous species (ANOVA with a following post hoc test; see Table 4.2).

Table 4.2 Means (x) and standard deviation ($s.d.$) of nectar sugar concentration and sugar ratio, nectar volume covered, nectar standing crop, of flowers in different pollination syndromes. Mann-Whitney U test of significant differences between covered nectar volumes and standing crop. n = number of species, [1]number of species nectar production: n = 4 myiophilous syndrome; [2]number of species sugar ratio: n = 16 melittophilous syndrome. [a,b,c] same letter indicates significantly different pairs after ANOVA with following Tukey-Kramer HDS post hoc test (α=0.05) or Kruskall-Wallis ANOVA with Tukey-Kramer post hoc test (α=0.05).

		Conc. [%w /w]	Sugar ratio S/(F+G)	Nectar volume 24 h covered [µL]	Standing crop 6:00 - 18:00 h [µL]	*Mann-Whitney U* Nectar volume covered *versus Standing crop*
	n	*x*± *s.d.*	*x*± *s.d.*	*x*± *s.d.*	*x*± *s.d.*	
Syndrome						
Myiophilous	6 [1]	31.1 ± 15.2	1.2 ± 1.8 [a]	0.7 ± 0.3 [a,b,c]	0.2 ± 0.2 [a,b,c]	z=2.2, p=0.025
Melittophilous	17[2]	25.9 ± 12.8	2.9 ± 3.2	2.0 ± 1.8 [e,g,h]	0.5 ± 0.3 [d,e]	z=2.3, p=0.021
Ornithophilous	13	17.0 ± 5.1	2.6 ± 0.9	20.6 ± 17.2 [a,d,e]	2.6 ± 1.9 [a,d]	z=3.4, p=0.000
Sphingophilous	7	16.8 ± 3.9	6.4 ± 5.6 [a,b]	26.5 ± 18.5 [b,f,g]	8.8 ± 12.5 [b]	z=3.8, p=0.000
Chiropterophilous	4	14.1 ± 5.9	0.6 ± 0.2 [b]	85.7 ± 17.7 [c,d,f,h]	4.5 ± 3.5 [c,e]	z=-2.2, p=0.025
ANOVA/ K-W ANOVA		$F_{4,42}$ =3.7 p=0.011	$F_{4,41}$ =4.3 p=0.005	$F_{4,40}$ =29.3 p=0.000	H(4, 7)=27.4 p=0.000	

Differing nectar compositions among species, based on the CNESS index are visualized using non-linear multidimensional scaling (stress=0.014; Figure 4.1). The dominant cluster was characterized by species belonging to the melitto-, ornitho-, and sphingophilous syndrome; only sphingophilous *Isertia laevis*, ornithophilous *Palicourea canarina* and melittophilous members of the genus *Arcytophyllum* are separated from this cluster. Species receiving visits exclusively from dipters are well separated from the main cluster (only myiophilous *Psychotia* sp. is located within the main cluster). Chiropterophilous species belonging to the tribe Helieae are further separated from the main cluster. There is a significant negative correlation (r=0.395, t=-2.8, p=0.007; Pearson) between dimension 1 and the sugar ratio.

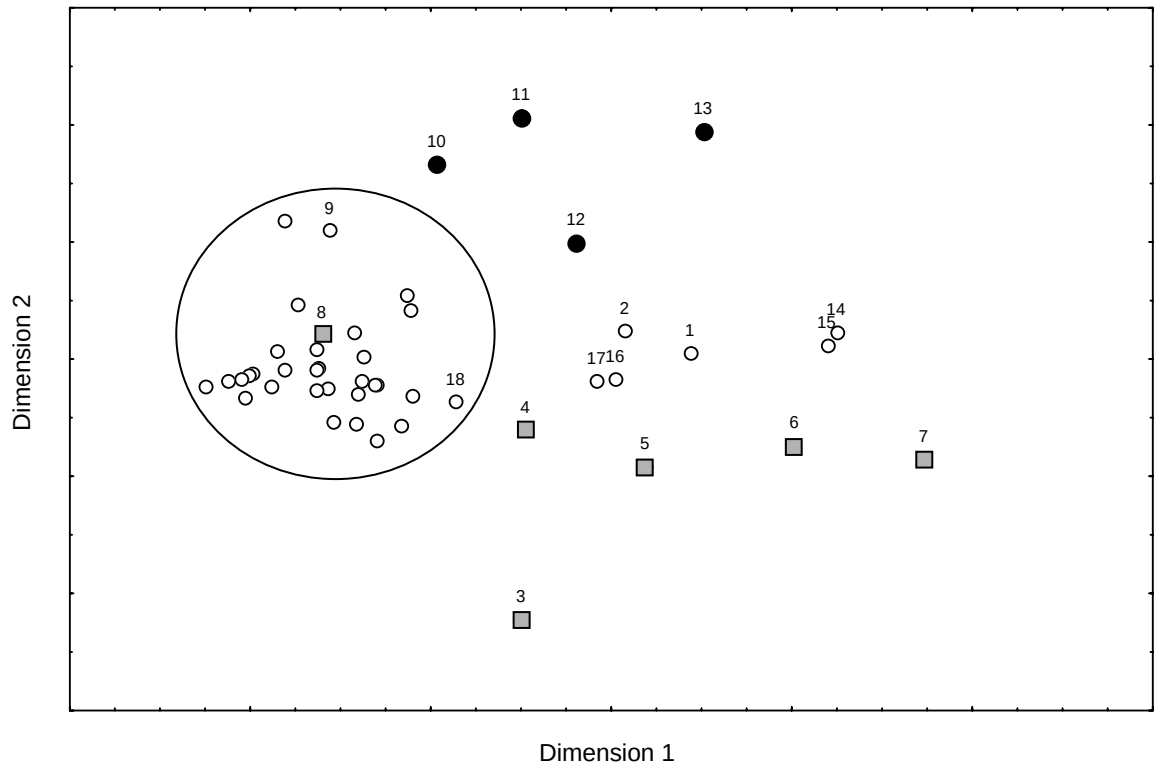

Figure 4.1 Non-linear multidimensional scaling (NMDS) of the nectar sugar composition of 47 species based on CNESS index. 1 *Isertia laevis*, 2 *Palicourea canarina*, 3 *Dioicodendron dioicum*, 4 *Psychotria aubletiana*, 5 *Arcytophyllum filiforme*, 6 *Halenia*, 7 *Gentianella*, 8 *Psychotria* sp. 9 *Symbolanthus calygonous*, 10 *Macrocarpaea harlingii*, 11 *Sym.* sp., 12 *M. noctiluca*, 13 *M. arborescens*, 14 *A. thymifolium*, 15 *A. capitatum*, *16 A. ciliolatum*, 17. *A. macbridei*, 19. *A. vernicosum*. Squares: myiophilous species, filled circles: chiropterophilous species.

Sucrose concentration averaged 149 µg µL^{-1} in species with melittophilous syndrome, compared to 50 µg µL^{-1} in those with chiropterophilous syndrome, whereas hexose concentration was similar 81 µg µL^{-1} in bee flowers and 98 µg µL^{-1} in bat flowers (Figure 4.2). Sugar proportions between ornithophilous and sphingophilous flowers are more or less equal, amounting to 98 µg µL^{-1} sucrose in the former and 114 µg µL^{-1} sucrose in the latter, while hexose concentration was 44 µg µL^{-1} in hummingbird flowers and 33 µg µL^{-1} in moth flowers (Figure 4.2). Within these two types of flowers, hexose proportion was clearly lower than in bee, bat and fly flowers. The highest hexose concentration of 146 µg µL^{-1} was found in flowers of the myiophilous syndrome, whose sugar proportions were inverse to those of the melittophilous syndrome. Considering hexose only, the proportion of fructose to glucose was more or less balanced across all species. Total sugar concentrations were variable among species (Table 4.1), and no significant differences could be detected among syndromes (ANOVA with following Tukey-Kramer post hoc test, Table 4.2).

Nectar sugar concentration was not significantly correlated with floral visitors (R=0.097, p=0.077, Matrix correlation). There was, however, a slight significant correlation between nectar sugar composition and floral visitors (R=0.197, p=0.043, Matrix correlation).

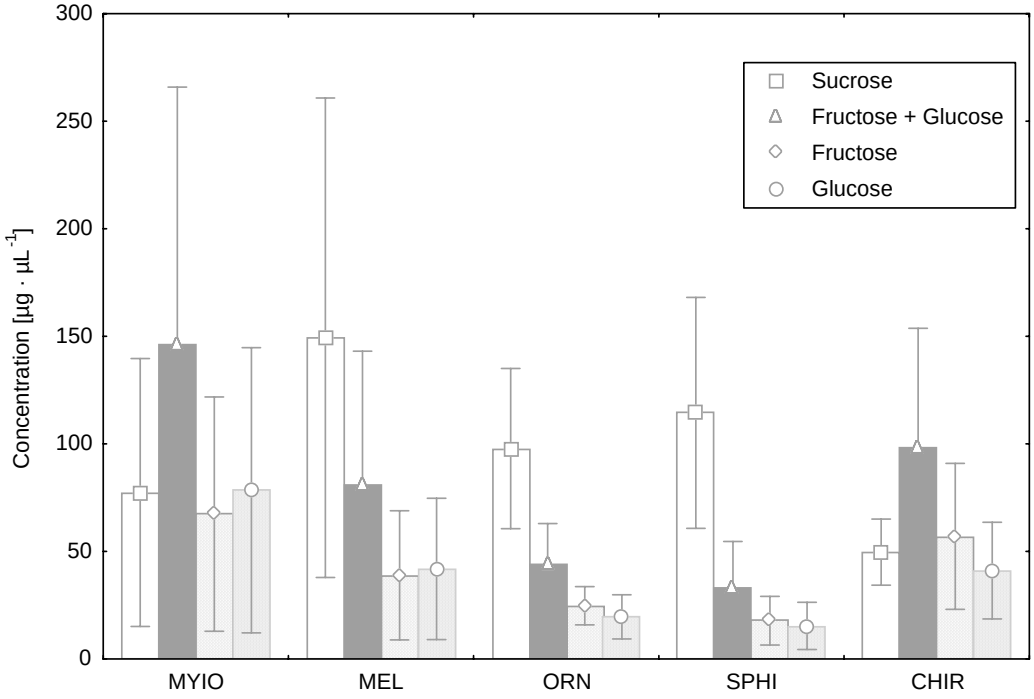

Figure 4.2 Mean proportion of sucrose and hexose (fructose + glucose) in the nectar of 47 Gentianales species arranged according to their pollination syndromes: MYIO, myiophilous syndrome (n= 6); MEL, melittophilous syndrome (n =17); ORN, ornithophilous snydrome (n =13); SPHI, sphingophilous syndrome (n = 7); CHIR, chiropterophilous syndrome (n = 4). Vertical bars represent standard deviation.

4.5.2 Nectar volume and standing crop

In covered flowers, nectar volumes varied markedly among the pollination syndromes and among species with the same syndrome, ranging from 0.3 to 1.1 µL in fly-, 0.2 to 5.8 µL in bee-, 1.6 to 51.9 µL in hummingbird-, 67.9 to 102.9 µL in bat-, and 1.8 to 45.2 µL in moth flowers (Table 4.1). Daily nectar production differed significantly among the pollination syndromes, except for ornithophilous versus sphingophilous and myiophilous versus melittophilous (ANOVA, with following Tukey-Kramer post hoc test; see Table 4.2). In uncovered flowers sampled during the day, nectar volumes among the pollination syndromes and among species with the same syndrome were less variable, and ranged from 0.1 to 0.5 µL in fly-, 0.1 to 1.2 µL in bee-, 0.5 to 6.8 µL in hummingbird-, 1.6 to 9.5 µL in bat-, and 0.2 to 29.5 µL in moth flowers (Table 4.1). Nectar standing crop measured during the day differed significantly between myiophilous flowers versus ornithophilous, sphingophilous, and chiropterophilous flowers, and between melittophilous flowers versus ornithophilous and chiropterophilous flowers (Table 4.2).

A significant corellation was found between the nectar volumes of covered flowers and floral visitors (R=0.228, p=0.007, Matrix correlation test). Standing crop and floral visitors were also significantly correlated (R=0.157, p=0.028, Matrix correlation test).

Nectar standing crop sampled during the day was significantly correlated with nectar volumes of covered flowers (Spearman coefficient R=0.83, p=0.000). Conversely, diurnal standing crop values differed significantly from covered nectar volumes in all syndromes (Mann-Whitney U-test, Table 4.2). Nectar standing crop of bat- and moth flowers sampled at night did not differ significantly (Mann-Whitney U-test: bat flowers z=-1.7, p=0.08, moth flowers z=0.8, p=0.42) from those of covered flowers, which indicates a low nocturnal visitation rate.

Among species, the distribution of variability of nectar volumes measured by the coefficient of variation (standard deviation mean^{-1}) is shown in Figure 4.3. Nectar volumes of uncovered flowers sampled during the day were more variable than the nectar volumes sampled at night and the nectar volumes of covered flowers. Coefficients of variation among nectar volumes in uncovered nocturnal flowers ranged within those for covered flowers, further indicating a low nocturnal visitation rate. There was a significant linear correlation between the means and standard deviations of nectar volumes of covered and uncovered flowers sampled during the day (Spearman coefficient R = 0.98, p=0.000, R = 0.94, p=0.000, respectively).

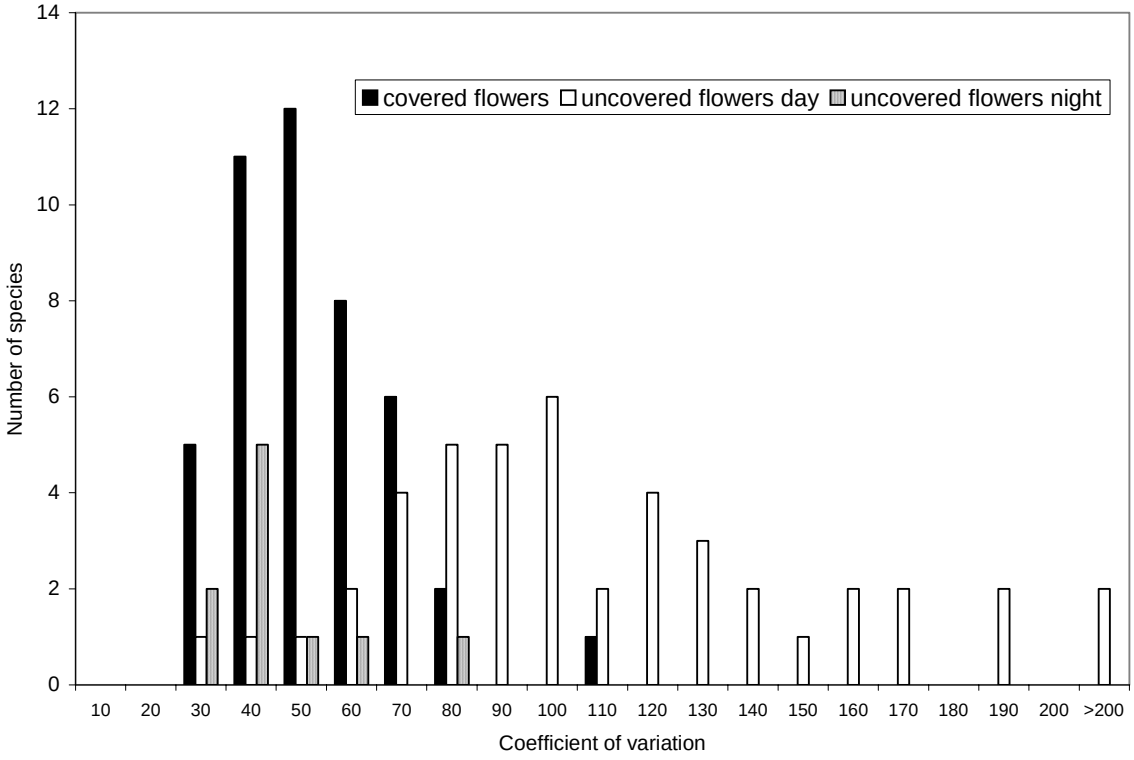

Figure 4.3 Distribution of variability, measured by the coefficient of variation of nectar volume of covered flowers (45 plant species) and uncovered flowers (10 species probed during the night, and 45 species probed during the day).

4.6 Discussion

4.6.1 Nectar sugar composition and concentration

The main goal of this study is to determine whether nectar features are related to the type of pollinator. Regarding the pollination syndromes, no nectar sugar concentration correlation was found. Regarding sugar composition, the only significant differences were found in chiropterophilous and myiophilous flowers, which had a significantly lower sugar ratio than sphingophilous flowers. This is further substantiated by the NMDS of the CNESS dissimilarity index based on nectar sugar compositions (Figure 4.1). Nectars from flowers visited by hummingbirds, bees, butterflies and moths formed one homogenous cluster, and nectar from flowers visited exclusively by flies (except *Psychotria* sp.) formed a second group. A third group included nectar from flowers visited by bats. Although sucrose is the predominant floral nectar sugar in 41 out of 47 investigated species, nectars from species

within each pollination syndrome tend to have characteristic sugar compositions (Figure 4.2). In this study, sucrose was the dominant constituent in all flowers of the ornithophilous syndrome. Ornithophilous flowers of several taxonomic groups have sucrose-dominant nectar (Baker and Baker 1983, Freeman *et al.* 1984, Gottsberger *et al.* 1984, Stiles and Freeman 1993, Dziedzioch 2001, Perret *et al.* 2001, Galetto and Bernardello 2003). Nectar of the investigated ornithophilous plants contained, on average, 68.2% sucrose, agreeing with the results of Nicolson and Fleming (2003), who showed that hummingbird nectars cluster around 64% sucrose. Considering the sugar concentration in nectar of Gentianales, values for hummingbird flowers (17.0%) were slightly lower than those found in the literature, citing a range from 21 to 26% (Baker 1975, Waser and Pyke 1981, Heyneman 1983, Stiles and Freeman 1993, Sazima *et al.* 1996, Kraemer 1998, Perret *et al.* 2001, McDade and Weeks 2004a). However, feeding experiments with hummingbirds show, that even more concentrated sugar solutions (31- 45%) are preferred (Pyke and Waser 1981, Kingslover and Daniel 1983, Tamm and Gass 1986, Roberts 1996, Nicolson and Fleming 2003).

This study's data on the sugar composition of sphingophilous Rubiaceae agree with the studies of several other families (Baker and Baker 1983, Haber and Frankie 1989, Schwerdtfeger 1996), showing sucrose-dominant nectar presence in the majority of sphingophilous flowers. Sphingophilous flowers produced less concentrated nectar (16.8%) than the reported mean of 21% (Haber and Frankie 1989), and 19% (hawkmoths) and 22% (settling moths) reviewed by Heyneman (1983).

The nectar of the majority (13 of 17 species) of melittophilous flowers is sucrose-dominant. This agrees with previous observations for melittophilous flowers of the Antirrhineae (Scrophulariaceae; Elisens and Freeman 1988), Iridaceae (Goldblatt *et al.* 1998), and Sinningieae (Gesneriaceae; Perret *et al.* 2001). Hexose-dominant to sucrose-rich nectar is found in four species of *Arcytophyllum* (Rubiaceae). According to the phylogeny of the genus *Arcytophyllum* provided by Andersson *et al.* (2002) and Wolff and Liede-Schumann, (unpubl. data), the most derived species of *A. macbridei* and *A. vernicosum* have higher sucrose proportions, whereas the basal *A. thymifolium* has a very low sucrose/hexose ratio. This suggests a tendency towards a higher percentage of sucrose in the genus. Nectar concentration of the flowers of the melittophilous syndrome studied here are lower (25.9%) than the corresponding values in the temperate and tropical regions reported by Pyke and Waser (1981: 36%) and Galetto *et al.* (1998: 48%), but are close to the value (29%) for melittophilous Gesneriaceae (Perret *et al.* 2001). Bees prefer very concentrated nectar to guarantee energetically profitable foraging (Bolten and Feinsinger 1978).

Distinctive nectar composition is associated with the chiropterophilous syndrome, in which particularly low sucrose production is responsible for hexose dominance (Figure 4.2). The high hexose proportion found in flowers of *Macrocarpaea* corresponds well with other bat flowers (Baker and Baker 1983, Baker *et al.* 1998, Perret *et al.* 2001). Nectar concentration (14%) of this study's bat pollinated Gentianaceae corresponds with the chiropterophilous Gentianaceae (10 – 15%) reported by Machado *et al.* (1998), as well as with the results from Sazima *et al.* (1999) who reported an average sugar concentration of bat-pollinated flower assemblages of 15% (lowland) and 18% (highland). These values are close to the median range of the frequency distribution reviewed by Helversen (1993) for 33 species of neotropical bat-pollinated flowers. Roces *et al.* (1993), however, showed in a series of dual choice tests that glossophagine bats preferred higher nectar concentrations up to 50%.

Flies prefer hexose-dominant and hexose-rich nectars (Baker and Baker 1983). High hexose proportions are found in flowers exclusively visited by flies. Sugar concentration (31%) varied markedly within the myiophilous syndrome (range 13 to 59%). Pombal and Morellato (1995) found very low sugar concentration (2%) in fly-pollinated Araliaceae. Machado and Loiola (2000) report 16% in *Cordia* (Boraginaceae) and 30% in *Borreria* (Rubiaceae).

Except for the nectar of *Psychotria aubletiana*, nectars analyzed of 21 species of tribe Psychotrieae are quite homogenous and sucrose-dominant, even though species are morphologically classified as ornithophilous, melittophilous, sphingophilous and myiophilous. In contrast, working with two other ornithophilous Ecuadorian species of the tribe, Bernardello *et al.* (1994) found hexose-rich and hexose-dominant nectar. The sugar compositions among Gentianales reported here indicate sucrose-dominant (53.5-100% sucrose) or sucrose-rich (33-46% sucrose) nectars predominate, even though flies, bees, beetles, diurnal and nocturnal butterflies, hummingbirds, and bats were the principal floral visitors. Only *Arcytophyllum capitatum, A. thymifolium*, *Macrocarpaea harlingii, M. arborescens, Halenia* sp. and *Gentianella* sp. had hexose-rich to hexose-dominant nectar (0-28.4% sucrose). The homogeneity of nectar sugar composition in the majority of species indicates that this is a conservative characteristic in the investigated Gentianales. The data support hypotheses of phylogenetic constraint on nectar sugar composition. Interestingly, similar results are found in different families if nectar sugar composition is compared to flower morphology and studied within small monophyletic groups (Elisens and Freeman 1988, Galetto *et al.* 1998, Torres and Galetto 2002, Perret *et al.* 2001).

In general, no correlation of floral visitors to nectar concentration was found (Matrix correlation). A weak significant correlation was found, however, between floral visitors and nectar sugar composition (Matrix correlation). It is likely that there has been some degree of an adaptive convergence of floral nectar compositions to principal pollinator type within the constraints set by phylogenetic history.

4.6.2 Nectar volume and standing crop

The nectar volumes of covered flowers are related to, and differ significantly among, pollination syndromes, with the exception of ornithophilous versus sphingophilous and myiophilous versus melittophilous flowers.

In this study, bat flowers contained about half of the average nectar volume found by Sazima *et al.* (1999: 151µL lowland, 167µL highland). Nectar volume of seven bat visited flowers studied by Tschapka (2004) varied from 100 to 21260µl. However, Perret *et al.* (2001) reported an average amount of 89µL for two chiropterophilous *Sinningieae* (Gesneriaceae), and Machado *et al.* (1998) reported for the Gentianaceae *Irlbachia* an average nectar amount of 43µL. Nevertheless, the bat flowers investigated here contained four times as much nectar as the studied hummingbird flowers. Mean nectar amounts from the ornithophilous flowers fell within the range of other neotropical bird-visited flowers, with 16.9 µL (Sazima *et al.* 1996), 28.9 µL (Kraemer 1998), 16.3 µL (Schmitt 2000), 18.4 µL (Perret *et al.* 2001), 38.5 µL (Dziedzioch 2001), and 8.8 to 72.7 µL (McDade and Weeks 2004a) being reported. Haber and Frankie (1989) observed highly variable nectar volumes among sphingophilous species with a mean of approximately 60 µL, which is twice the mean nectar volume found in this study. Low nectar volumes have generally been found in melittophilous flowers, however Perret *et al.* (2001) found more than ten times more nectar (15.4 µL) in flowers of bee-pollinated Gesneriaceae than in the bee flowers studied here. Mean nectar volumes below one µl were found in myiophilous species. In addition, there is a significant correlation between floral visitors and covered and uncovered nectar volumes (Matrix correlation).

The nectar volumes of covered flowers have little relation to the standing crop quantities actually offered to potential flower visitors (as this study shows by the significantly lower values in standing crop compared to the cumulative nectar of covered flowers, and by the results of McDade and Weeks 2004b), but even the standing crop nectar volumes differed among the syndromes. On the other hand, there is a positive correlation between nectar sampled during the day in covered and uncovered flowers. According to Zimmermann (1988),

there must be a significant relationship between nectar production and standing crop if pollinators are to exert any selective pressure on the rate of nectar production. The amount of nectar obtained by the pollinators from standing crop is determined by nectar production, as well as by depletion and by the morphological match between the pollinator and the flower (Rathcke 1992). Environmental factors such as temperature, relative humidity, and soil moisture also affect standing crop nectar. The data reveal great variability in the coefficients of variation (CV) for nectar volume among plant species, and even greater variability in the CV for diurnal nectar standing crop. Variability in nectar amount is quite common (Rathcke 1992, Cresswell 1998, Petanidou and Smets 1995, McDade and Weeks 2004a, b). Foragers are sensitive to the CV of the reward (review in Real and Caraco 1986, Kacelnik and Bateson 1996, Bateson 2002, Shafir *et al.* 2003). Among other things, a pollinator's behavior is influenced by the CV of nectar standing crop, i.e., the higher the CV, the stronger the risk-aversion (Bateson 2002, Shafir *et al.* 2003). The linear correlation between the mean and standard deviation of nectar volume and standing crop between plant species found in this study is in accordance with the findings of Petanidou and Smets (1995) and McDade and Weeks (2004a, b).

Nectar volume influence pollinators´ behavior, which governs pollen receipt and donation (see e.g. Ladio and Aizen 1999, Lasso and Naranjo 2003, Manetas and Petropoulou 2000, Wolff *et al.* in press). Effective pollination is guaranteed, when nectar reward is abundant enough to attract the pollinator, but small enough to force the pollinator to visit various individuals. Nectar volume production is therefore important in floral evolution and probably influenced by the most effective pollinator.

In summary, sucrose is the predominant floral nectar sugar in the order Gentianales. The homogeneity of nectar sugar composition in the majority of species indicates that this is a conservative characteristic in the investigated Gentianales. There is no correlation between sugar concentration and pollination syndromes. Nectar sugar composition does not differ significantly among the pollination syndromes (two exceptions being sphingophilous versus chiropterophilous and myiophilous nectars); only nectar volumes are related to pollination syndromes. Although certain nectar compositions and concentrations may be preferred by a given visitor, the results of the study show that various compositions and concentrations are accepted and tolerated by the visitor, not unlike the feeding behavior of other species, including our own. However, some degree of an adaptive convergence of floral nectar compositions to principal pollinator type within the constraints set by phylogenetic history is likely. The driving force to visitation appears to be the volume of nectar the visitor can expect

to consume. As the data on nectar volumes disclose, nectar production is important in floral evolution and influenced by the predominant pollinator.

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5 Phylogeny and Reproductive Biology of the distylous *Arcytophyllum* (Rubiaceae)

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Organisms Diversity & Evolution, in press, as "Evolution of flower morphology, pollen dimorphism, and nectar composition in *Arcytophyllum*, a distylous genus of Rubiaceae"

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5.1 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-ratio and seed set of ten species in eleven populations. Stigma and anther levels differed significantly between the morphs in the species/populations investigated except for *A. filiforme*. 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, both with regard to the number (long-style > short-style) and to the size (short-style > long-style) was observed in all taxa investigated except for *A. filiforme.* According to the pollen-ovule-ratios the breeding systems range from facultative autogamy to facultative xenogamy, independent of phylogenetic relationsship. The main floral visitors of the species studied were Hymenoptera, Diptera and Coleoptera. Seed production did not differ significantly between the morphs in eight of the eleven species/populations investigated. There is, however a tendency in all species/populations (except for *A. macbridei* Peru) that the short-styled morph

had a higher percentage of seeds per ovule indicating that the short-styled morphs display higher female reproductive success.

5.2 Key words

Arcytophyllum, floral morphology, heterostyly, ITS, nectar composition, P/O ratio, pollination, seed set, Spermacoceae.

5.3 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 occuring 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, and most *Arcytophyllum* species had originally been described under *Hedyotis* (Mena 1990). Terrell (1999) transferred another *Hedyotis* species, *H. serpyllacea* Schltdl., to *Arcytophyllum,* based on overall similarity. *Hedyotis* and its relatives had been placed traditionally 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) could show that *A. serpyllacea* is not monophyletic with other *Arcytophyllum* species (nor with any of the *Hedyotis* analyzed) and that 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- and short-styled flowers of the always distylous genus.

The flowers of *Arcytophyllum* are distylous. Individual plants produce either long-styled morphs (ls) with the stigma positioned above the anthers, or short-styled (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. These morphs are cross-compatible, but are often within-morph incompartible. We are not aware of a comparative study of nectar composition in heterostylous species and their

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 ten out of the 15 known *Arcytophyllum* species in the light of their phylogeny.

5.4 Material and Methods

5.4.1 Phylogeny

The original matrix of analysis 2 in Andersson *et al.* (2002), comprising 1788 positions of the *rps*16 intron and the *trn*L-F intron of cpDNA of eleven *Arcytophyllum* species, and its sister clade of 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 Gene Bank, ITS sequences of *H. longifolia* (as "*Hedyotis longifolia*") and *H. nigricans* could be retrieved. We originally sequenced 16 accessions (Table 5.1, see here also for authors of species) of *Arcytophyllum* for ITS using the primers P17F and 26S-82R and the sequencing primers P16F and P25R. Analysis 1 comprises these 16 samples; as outgroup the two sequences from Gene Bank were used.

Sequence comparison showed that there were no sequence differences between the long-styled and short-styled morphs of *A. capitatum* and *A. setosum*; therefore, only one of these sequences were used. The two populations of *A. filiforme* differed in one site, 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; as outgroup the two sequences from Gene Bank were used. 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. First, only the nine species available in both datasets were analyzed, second, gaps were added for the missing partial sequences 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).

Table 5.1 Voucher information.

Long styled (ls), short styled (ss), if not indicated, material sequenced was sterile.

Accession	Morph	Country. Province. Locality	Collection
A. aristatum Standl.	ls	Ecuador. Carchi. El Ángel	Wolff 25(MO, UBT)
A. capitatum (Benth) K. Schum.	ls	Ecuador. Loja. Cajanuma	Wolff 4 (MO, UBT)
A. capitatum (Benth) K. Schum.	ss	Ecuador. Loja. Cajanuma	Wolff 4 (MO, UBT)
A. ciliolatum Standl.	-	Ecuador. Zamora-Chinchipe. Valladolid	Wolff 36 (MO, UBT)
A. filifome (Ruiz & Pav.) Standl.	ss	Ecuador. Azuay. Cajas	Wolff 1 (MO, UBT)
A. filifome (Ruiz & Pav.) Standl.	ls	Peru. Amazonas. Chachapoyas. Leimebamba	Wolff 47 (MO, UBT)
A. lavarum K. Schum.	-	Costa Rica. Cartago. Cerro de la Muerte	Wolff 59 (MO, UBT)
A. macbridei Standl.	-	Peru. Amazonas. Chachapoyas. Leimebamba	Wolff 34 (MO, UBT)
A. cf. *macbridei* Standl.	ls	Ecuador. Loja. Cajanuma	Wolff 5 (MO, UBT)
A. muticum (Wedd.) Standl.	-	Costa Rica. Cartago. Cerro de la Muerte	Wolff 60 (MO, UBT)
A. rivetii Danguy & Cherm.	ss	Peru. Amazonas. Chachapoyas. Leimebamba	Wolff 35 (MO, UBT)
A. setosum (Ruiz & Pav.) Schltdl.	ss	Ecuador. Azuay. Cajas	Wolff 2 (MO, UBT)
A. setosum (Ruiz & Pav.) Schltdl.	ls	Ecuador. Azuay. Cajas	Wolff 2 (MO, UBT)
A. thymifolium (Ruiz & Pav.) Standl.	-	Peru. Lima. Yauyos-Laraos	Beltrán s.n. (UBT)
A. thymifolium (Ruiz & Pav.) Standl.	-	Ecuador. Imbabura. Cuicocha	Homeier s.n. (UBT)
A. vernicosum Standl.	ls	Ecuador. Loja. Cajanuma	Wolff 3 (MO, UBT)
Hedyotis nigricans (Lam.) Fosb.	-	USA. Eastern USA	Church 2003
Houstonia longifolia Gaertn.	-	USA. South and Central US Mexico. Northern	Church 2003

5.4.2 Flower morphology and pollen ovule ratio

Fresh floral material from both morphs was collected in the field and measured under a steromicroscope. Figure 5.1 illustrates the parameters measured. Morphological differences between the two floral morphs were compared with a one-way Anova. We determined the pollen-ovule 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 pollen-ovule ratios (P/O) for individual buds by dividing the number of pollen grains by the number of ovules. Then we calculated P/O-ss/ls as the ratio of averaged short-styled morph pollen grains to averaged ovule numbers of the long-styled morph and vice versa (P/O-ls/ss).

5.4.3 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 to avoid contamination on a WATERS High Performance Carbohydrate Column. The injection volume was 10 µl and the eluation took place with an acetonitrile-water-mixture (71:28), with 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 or 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).

5.4.4 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 by fixed periods of 15 min. All visits on flowers in the observed patches counted.

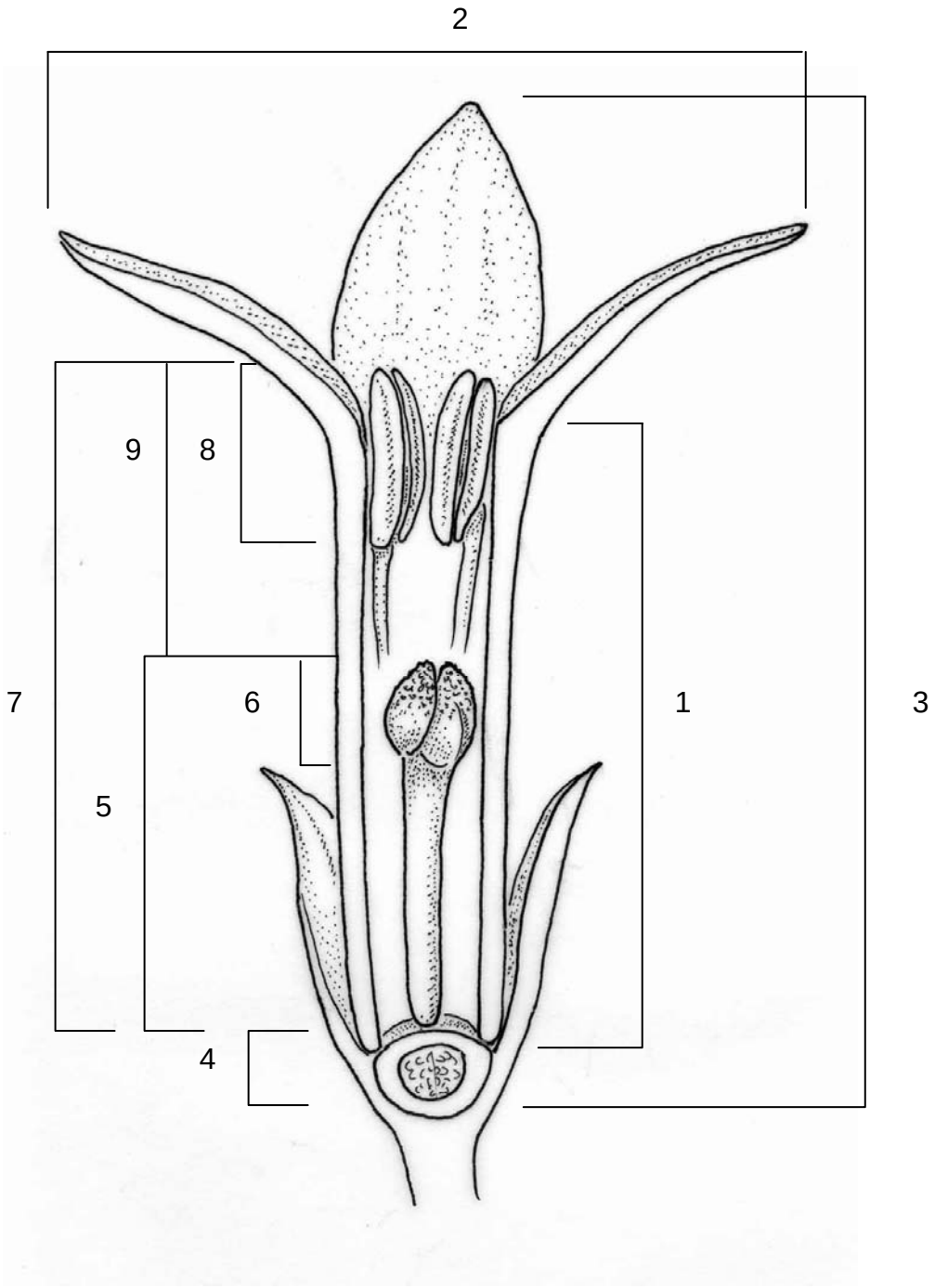


Figure 5.1 Schematic flower of *Arcytophyllum* (short styled morph, drawn from *A.* cf. *macbridei* Wolff 5), indicating the distances measured. Numbers correspond to the following measurements for both morphs. 1 corolla tube length, 2 corolla diameter, 3 total flower length, 4 ovar length, 5 stigma level, 6 stigma lobe length, 7 anther level, 8 anther length, 9 anther-stigma separation.

5.5 Results

5.5.1 Phylogeny

The ITS alignment, available on the website of SLS and TreeBase (Sanderson *et al.* 1994), comprised fifteen taxa and 587 characters. 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, and branch-and-bound search (Hendy and Penny 1989) results in four trees of 231 steps (CI=0.76, RI=0.79, RC=0.68). The strict consensus tree (Figure 5.2) confirms the position of *A. thymifolium* as sister to all other *Arcytophyllum* species with high bootstrap support. The sister group position of *A. aristatum* and *A. muticum* found by Andersson *et al.* (2002) is retrieved, albeit unsupported, and *A. ciliolatum* remains unresolved. *Arcytophyllum vernicosum* is placed in a group with *A. macbridei* and *A. setosum*, which is sister to *A. lavarum*. The two accessions of *A. filiforme* form a well-supported clade with *A. capitatum* and a second reasonably well supported clade, which is sister to the Central American *A. lavarum*, is formed by *A. vernicosum*, *A. macbridei* and *A. setosum*.

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, Figure 5.3). In the resulting tree, both the monophyly of *Arcytophyllum* and the basal position of *A. thymifolium* are strongly supported. Likewise strongly supported is the sister group relationship of *A. ciliolatum* and *A. rivetii* while 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 raises 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 (Figure 5.4), *A. capitatum* and *A. filiforme* form a well supported clade, *A. ericoides* is added to the *A. rivetii*/*A. ciliolatum* clade, and this trichotomy is also well supported. *A. nitidum* is sister to *A. setosum* with very good support, while the remaining relationships remain unchanged both in position and support.

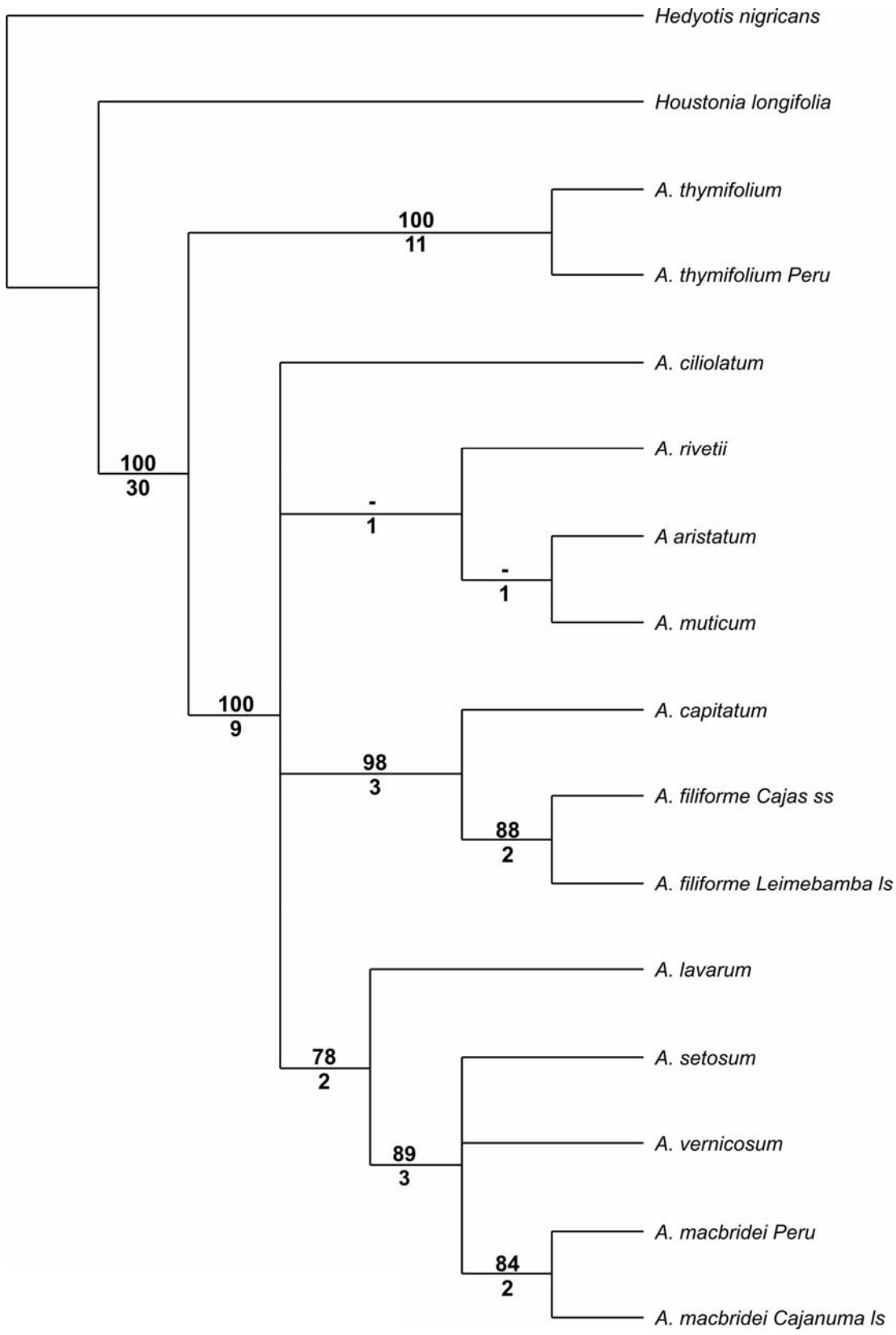


Figure 5.2 Strict consensus of the four most parsimonious trees retrieved from Branch-and-Bound analysis of the ITS dataset (l=231 steps, CI=0.76, RI=0.79, RC=0.68). Numbers above branches indicate bootstrap percentages, those below branches the decay indices (Bremer values).

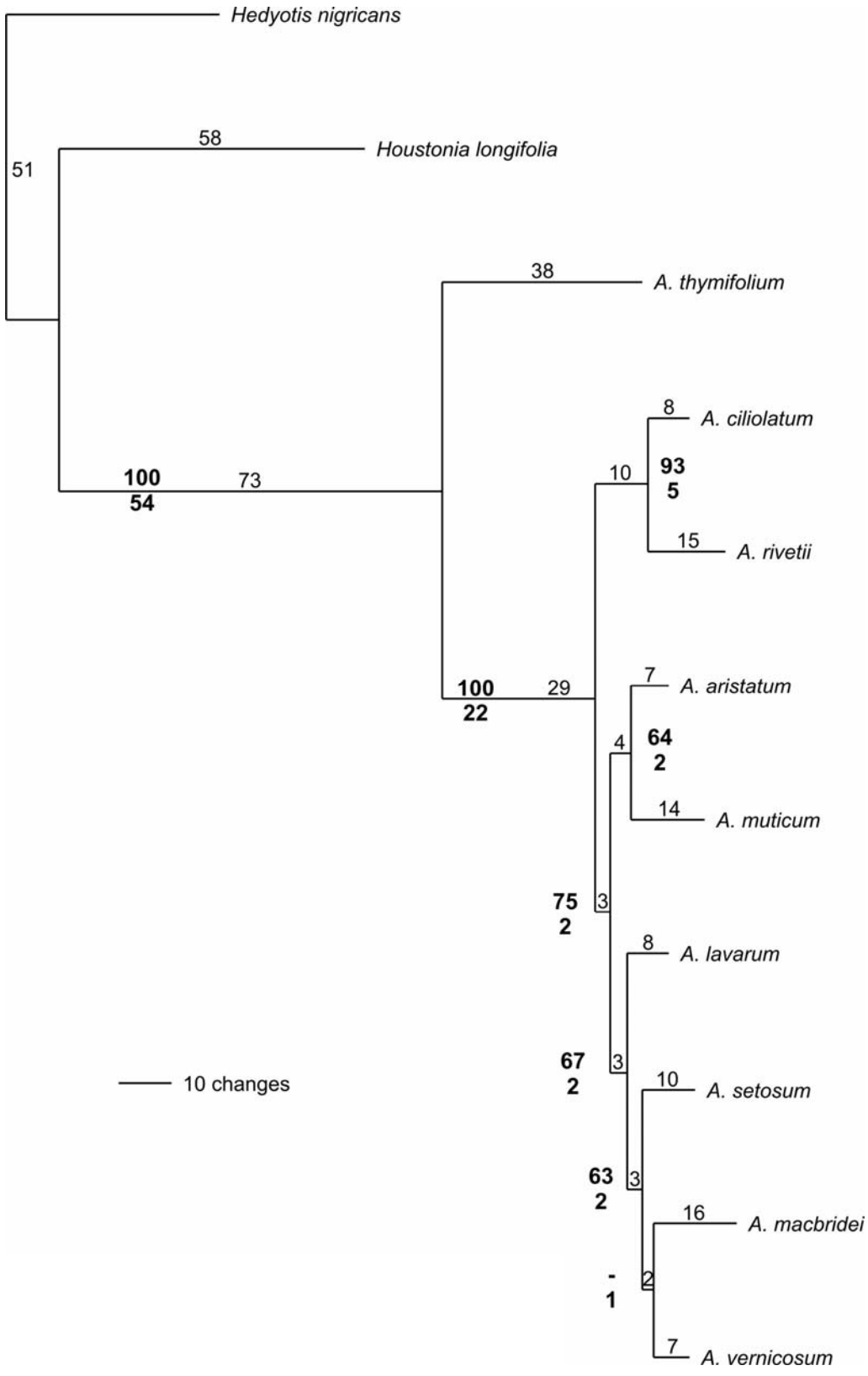


Figure 5.3 Single most parsimonious tree derived from from Branch-and-Bound analysis of the nine *Arcytophyllum* species for which both ITS and cpDNA data were available 359 steps (CI=0.79, RI=0.8, RC= 0.72). Numbers as in Figure 5.2.

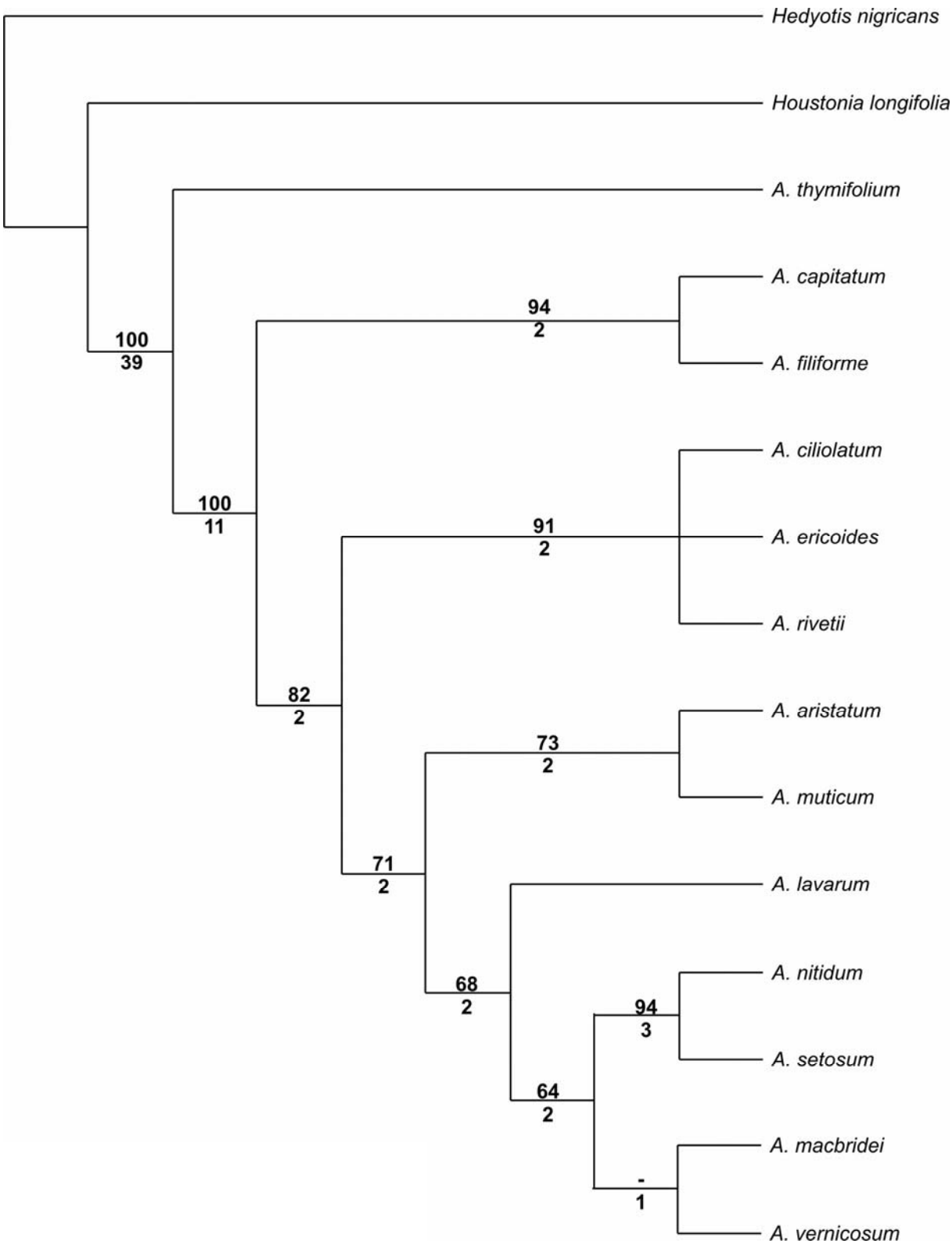


Figure 5.4 Strict consensus of the two most parsimonious trees resulting from analysis of cpDNA and ITS for all taxa, with missing partial sequences coded as "n" (l=378 steps, CI=0.78, RI=0.8, RC=0.71). Numbers as in Figure 5.2.

5.5.2 Floral morphology

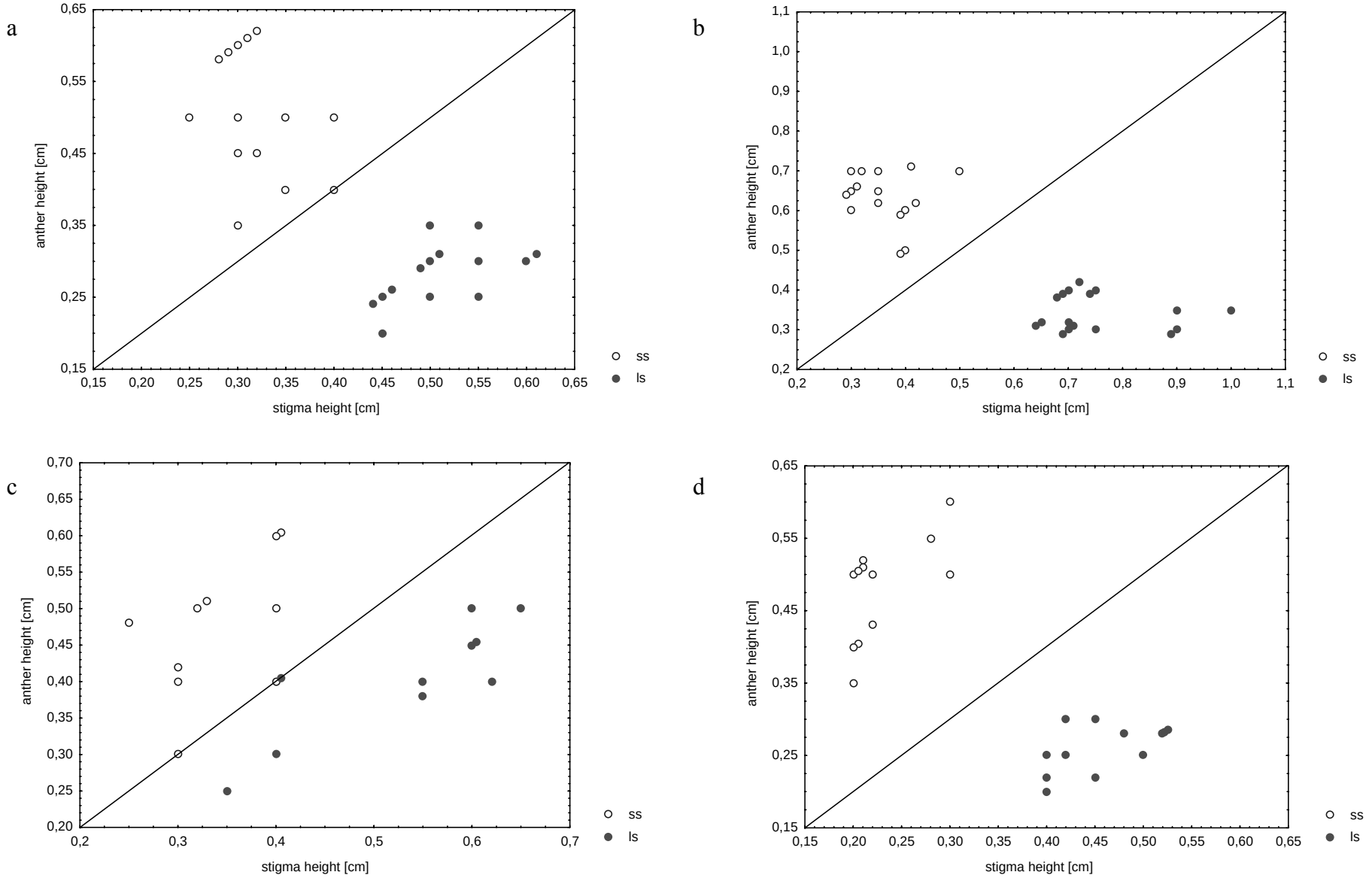
The flowers of *Arcytophyllum* are 5–13 mm long (Table 5.2), with the tube slightly longer than the four lobes (Figure 5.1). The flowers of all investigated *Arcytophyllum* species/populations are distylous with significant differences in morphology between the morphs (Tables 5.2, 5.3, Figure 5.5). In the short-styled morphs (Figure 5.1) the anthers are positioned at the mouth of the corolla tube overtopping the style and stigma whereas in the long-styled morphs, style and stigma overtop the anthers, which are sunken in the corolla tube. Figure 5.5 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. rivetii* and *A. vernicosum* studied, individuals with anthers and stigmas on the same levels occur (Figure 5.5). 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. Stigma level depends on the length of the style and size of the ovary (Richards and Barrett 1992). The ovar is significantly longer in long-styled flowers than in short-styled ones, with the exception of *A macbridei* (Peru), *A. ciliolatum, A. filiforme,* and *A. rivetii.* The averaged anther levels differ significantly between long- and short-styled morphs in all species studied except *A. filiforme* (Table 5.2). Short-styled flowers have significantly larger corolla tubes in *A. ciliolatum, A. thymifolium, A. capitatum, A. rivetii, A. lavarum* and *A. macbridei* (Peru). Stigma lengths are significantly shorter in long-styled morphs with the exception of *A. ciliolatum, A. lavarum, A. macbridei* (Peru), and *A. setosum*. Short-styled flowers have longer anthers in *A. capitatum, A. rivetii, A. macbridei* (Ec), *A. macbridei* (Peru) and *A. vernicosum*.

Table 5.2 Floral measurements in eleven *Arcytophyllum* species/populations [mm]. Abbreviations: long styled (ls), short styled (ss), *n* = number of measured flowers, *x* = mean, *s.d.* = standard deviation.

	Morph	*n*	Corolla tube, length	Corolla, diameter	Total flower, length	Ovar, length	Stigma, level	Stigma lobe, length	Anther, level	Anther, length	Anther-stigma separation
			x ± *s.d.*	*x* ± *s.d.*	*x* ± *s.d.*	*x* ± *s.d.*	*x* ± *s.d.*	*x* ± *s.d.*	*x* ± *s.d.*	*x* ± *s.d.*	*x* ± *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

Table 5.3 Results of one-way ANOVA tests for floral dimorphism in eleven *Arcytophyllum* species/populations.

	Corolla tube, length		Corolla, diameter		Total flower, length		Ovar, 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	**0.008****	1.17	0.292	24.57	**0.000*****	**0.30**	0.590	124.15	**0.000*****	9.65	**0.006****	205.40	**0.000*****	0.52	0.478	496.51	**0.000*****
A. capitatum	4.97	**0.033***	46.48	**0.000*****	5.84	**0.022***	**13.23**	**0.001****	177.14	**0.000*****	4.63	**0.039***	215.07	**0.000*****	6.44	**0.016***	335.60	**0.000*****
A. filiforme	0.75	0.397	0.54	0.472	0.20	0.660	**0.68**	0.419	25.27	**0.000*****	7.24	**0.015***	2.97	0.102	0.07	0.789	66.58	**0.000*****
A. ciliolatum	20.66	**0.000*****	2.64	0.118	0.68	0.419	**2.17**	0.155	152.53	**0.000*****	0.32	0.575	95.94	**0.000*****	0.10	0.758	486.04	**0.000*****
A. rivetii	8.69	**0.008****	33.44	**0.000*****	0.04	0.845	**6.98**	**0.016***	86.98	**0.000*****	13.87	**0.001****	44.14	**0.000*****	5.88	**0.025***	237.06	**0.000*****
A. aristatum	0.85	0.368	0.00	1.000	4.31	**0.049***	**1.69**	0.207	56.88	**0.000*****	166.38	**0.000*****	135.56	**0.000*****	0.36	0.556	232.01	**0.000*****
A. lavarum	9.51	**0.006****	0.14	0.713	8.35	**0.009****	**6.74**	**0.018***	114.86	**0.000*****	0.46	0.505	91.38	**0.000*****	0.96	0.339	417.46	**0.000*****
A. setosum	1.07	0.315	0.39	0.539	5.07	**0.037***	**1.01**	0.329	119.15	**0.000*****	2.19	0.156	37.33	**0.000*****	0.07	0.790	330.99	**0.000*****
A. macbridei (Peru)	50.15	**0.000*****	8.33	**0.007****	0.05	0.822	**11.55**	**0.002****	263.32	**0.000*****	0.05	0.821	174.37	**0.000*****	5.13	**0.031***	499.01	**0.000*****
A. macbridei (Ec)	1.05	0.309	22.04	**0.000*****	25.53	**0.000*****	0.76	0.387	396.62	**0.000*****	13.39	**0.001*****	183.35	**0.000*****	9.69	**0.003****	715.76	**0.000*****
A. vernicosum	0.47	0.499	0.00	1.000	8.28	**0.008****	**26.86**	**0.000*****	108.61	**0.000*****	15.54	**0.001*****	72.55	**0.000*****	13.38	**0.001****	169.97	**0.000*****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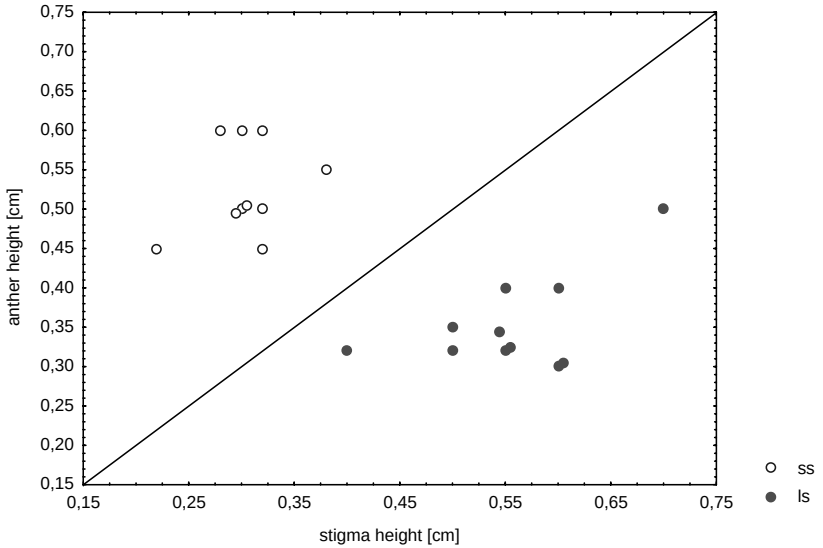
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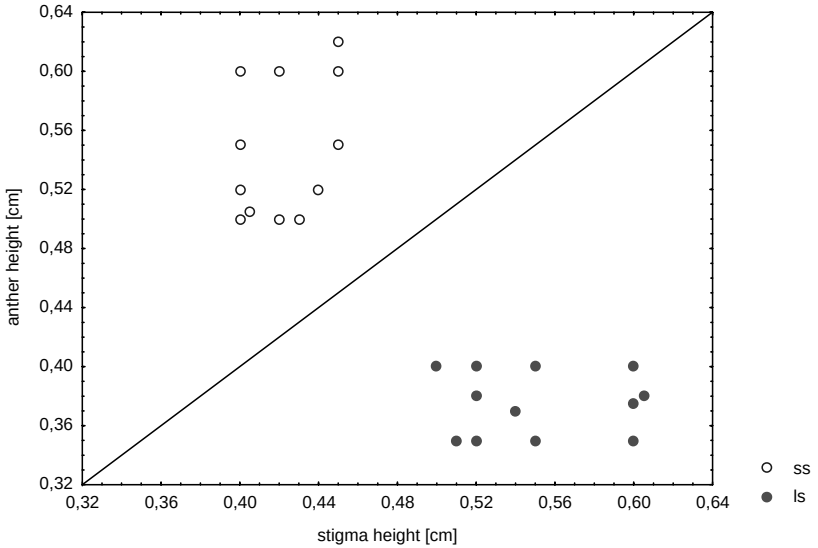
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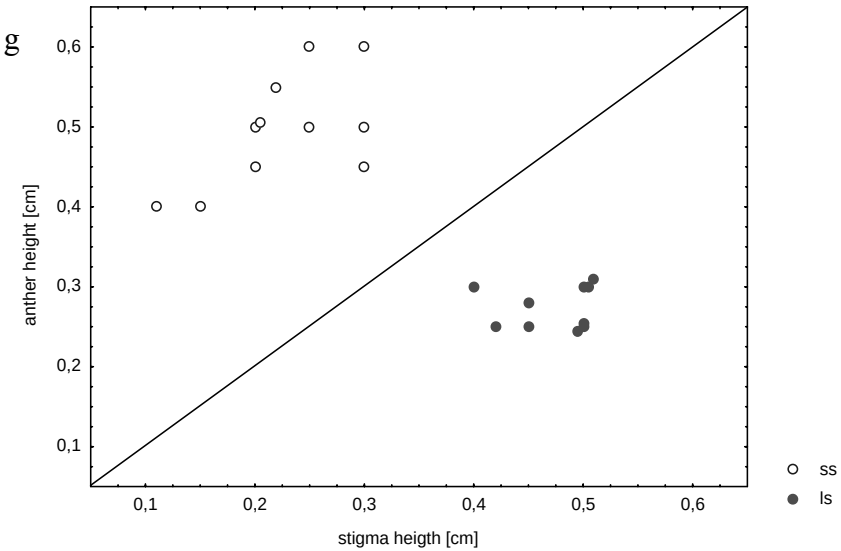
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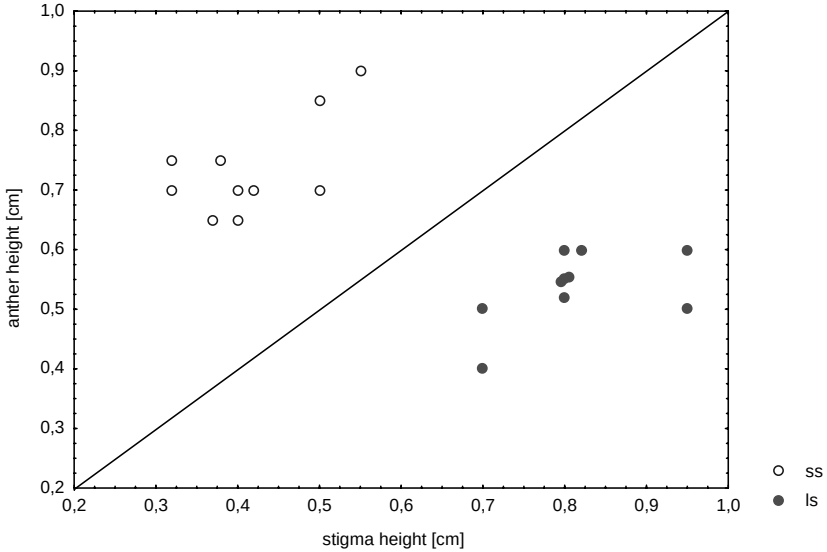
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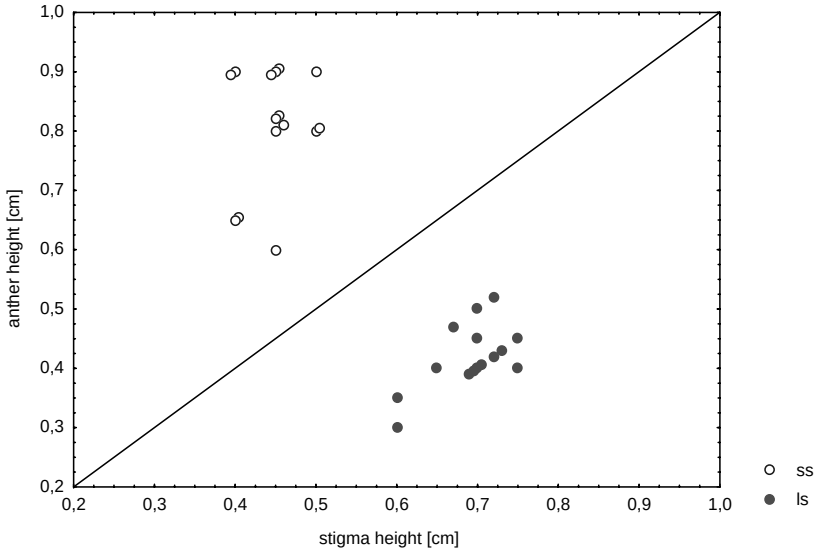

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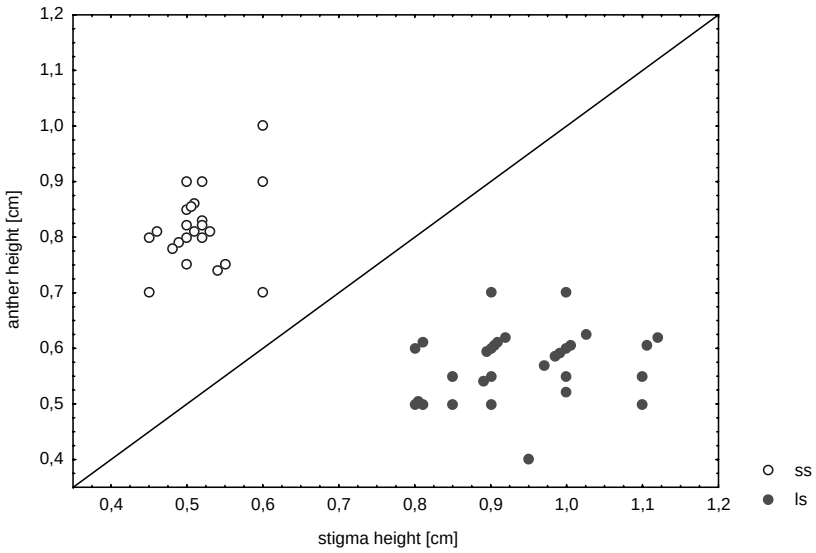

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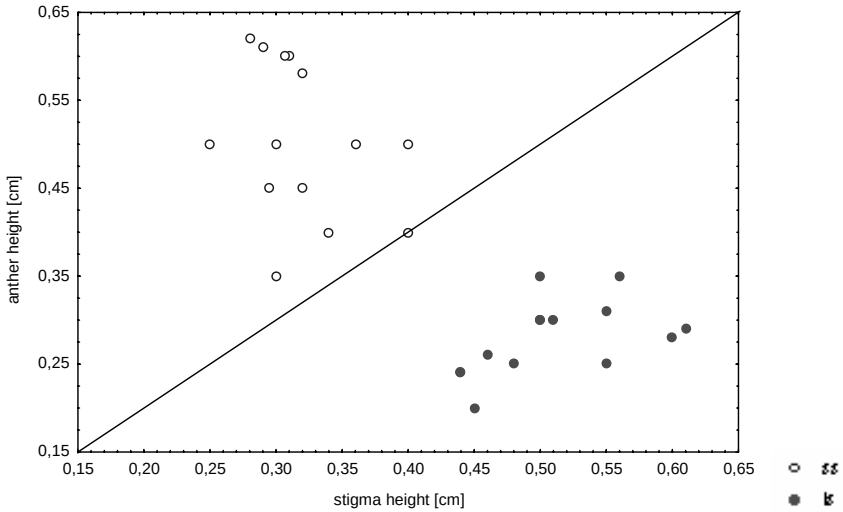

Figure 5.5: Anther-stigma separation. Arrangement of species according to the phylogeny of Figure 5.4. Circles = short styled morph, filled circles = long styled morph.

a = *A. 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*

5.5.3 Pollen dimorphism, pollen-ovule-ratio and seed set

Ovule number varied from about six in *A. rivetii* to 28 in *A. vernicosum* (short-styled morph). In six species both floral morphs had similar numbers of ovules (Table 5.4). In *A. thymifolium, A. macbridei* (Ec), and *A. macbridei* (Peru) ovule number was significantly higher in the long-styled morph, whereas in *A. vernicosum* and *A. lavarum* ovule number was significantly higher in the short-styled morph. Interspecific variations concerning the number of pollen grains were observed, with lowest records in *A. filiforme* (approx. 2800 grains per flower) and highest values in *A. macbridei* (Ec) (approx. 13000 grains per flower). Furthermore, a high intraspecific variation could be observed, with long-styled flowers presenting a significantly higher number of pollen grains than short-styled ones in all species investigated, except for *A. filiforme* (Table 5.5). The ratio of long-styled to short-styled pollen grain number ranges from 0.95 in *A. filiforme* to 1.7 in *A. thymifolium*. Pollen grain size in long-styled morphs was significantly smaller than in short-styled morphs, with exception of *A. filiforme*. The lowest P/O ratios of long-styled pollen to ovules of the short-styled flowers and vice versa were found in *A. vernicosum,* followed by *A. filiforme* and *A. aristatum*, while the highest ratios were found in *A. rivetii*. P/O ratios of long-styled pollen to ovules of the short-styled flowers exceeded those of the reverse type with the exception in *A. lavarum* and *A. setosum*.

Seed production (Table 5.6) in fruits of *A. aristatum, A. lavarum* and *A. vernicosum* was significantly higher in short-styled patches than in long-styled patches. In the other species, there was only a tendency that the percentage of mature seeds to ovules was always greater in short-styled than in long-styled flowers.

Table 5.4 Pearson correlation on corolla tube lenght and anther heights and corolla tube lenght and stigma heights of each morph for eleven *Arcytophyllum* species/populations.

	Morph	Corolla tube length *versus* anther heights				Corolla tube length *versus* stigma heights			
		r (X.Y)	r²	t	p	r (X.Y)	r²	t	p
A. thymifolium	ls	-0.18	0.03	-0.54	0.604	0.07	0.01	0.22	0.830
A. thymifolium	ss	**0.80**	**0.64**	3.79	**0.005****	0.24	0.06	0.70	0.506
A. capitatum	ls	0.04	0.00	0.15	0.883	-0.17	0.03	-0.66	0.520
A. capitatum	ss	**0.71**	**0.51**	3.79	**0.002****	-0.45	0.21	-1.91	0.077
A. filiforme	ls	**0.69**	**0.47**	2.67	**0.028***	0.61	0.37	2.18	0.061
A. filiforme	ss	0.31	0.10	0.92	0.382	0.44	0.19	1.38	0.205
A. ciliolatum	ls	0.34	0.12	1.16	0.275	0.13	0.02	0.42	0.680
A. ciliolatum	ss	**0.60**	**0.36**	2.38	**0.038***	0.48	0.23	1.72	0.117
A. rivetii	ls	0.43	0.18	1.41	0.191	0.10	0.01	0.29	0.775
A. rivetii	ss	**0.69**	**0.47**	2.69	**0.028***	0.31	0.09	0.91	0.389
A. aristatum	ls	-0.23	0.05	-0.74	0.473	**-0.72**	**0.52**	-3.27	**0.008****
A. aristatum	ss	-0.45	0.20	-1.59	0.143	-0.42	0.17	-1.45	0.179
A. lavarum	ls	0.18	0.03	0.52	0.618	0.36	0.13	1.09	0.308
A. lavarum	ss	**0.63**	**0.39**	2.42	**0.038***	0.58	0.34	2.15	0.060
A. setosum	ls	0.62	0.39	2.24	0.055	**0.69**	**0.47**	2.67	**0.029***
A. setosum	ss	**0.91**	**0.83**	6.33	**0.000*****	0.43	0.19	1.36	0.212
A. macbridei (Peru)	ls	0.49	0.24	2.04	0.063	0.28	0.08	1.05	0.314
A. macbridei (Peru)	ss	0.37	0.13	1.42	0.180	**0.76**	**0.58**	4.26	**0.001*****
A. macbridei (Ec)	ls	0.29	0.09	1.61	0.118	**0.54**	**0.29**	3.42	**0.002****
A. macbridei (Ec)	ss	**0.47**	**0.22**	2.43	**0.024***	0.37	0.14	1.83	0.081
A. vernicosum	ls	0.49	0.24	1.92	0.078	**0.63**	**0.39**	2.78	**0.017***
A. vernicosum	ss	**0.87**	**0.76**	6.11	**0.000*****	-0.38	0.14	-1.40	0.186

Table 5.5 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 pollen grain number ls/ss, pollen grain size, results of one-way ANOVA for differences in pollen grain size between morphs, ratio pollen grain size ss/ls, ovule number, results of one-way ANOVA for differences in ovule number between morphs, ratio ovule number ls/ss,and pollen-ovule-ratio: ratio ls/ss (pollen of ls to ovule of ss), ratio ss/ls (pollen of ss to ovule of ls), ratio ls/ls (pollen of ls to ovule of ls), ratio ss/ss (pollen of ss to ovule of ss) for eleven *Arcytophyllum* species/populations.

			Pollen grain number per flower				Pollen grain size [µm]				Ovule number			Pollen-ovule-ratios		
	Morph	n	*x* ± *s.d.*	F	p	Ratio ls/ss	*x* ± *s.d.*	F	p	Ratio ss/ls	*x* ± *s.d.*	F	p	Ratio ls/ss	Ratio ss/ls	Ratio ls/ls Ratio ss/ss
A. thymifolium	ls	10	5446 ± 545	66.55	**0.000*****	1.70	27.1 ± 0.6	193.04	**0.000*****	1.17	11.3 ± 1.3	8.81	**0.008****	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	**0.036***	1.13	27.6 ± 1.2	87.63	**0.000*****	1.15	8.9 ± 1.3	0.06	0.805	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	0.623	0.95	22.2 ± 0.5	1.65	0.215	1.02	9.3 ± 2.7	2.90	0.106	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	**0.002****	1.52	26.2 ± 0.9	19.75	**0.000*****	1.07	14.3 ± 2.5	0.04	0.836	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	**0.001****	1.28	24.2 ± 0.7	26.97	**0.000*****	1.12	6.3 ± 0.8	3.53	0.077	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	**0.028***	1.17	23.6 ± 1.5	56.47	**0.000*****	1.13	13.0 ± 2.7	0.01	0.928	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	**0.040***	1.17	27.5 ± 1.1	43.87	**0.000*****	1.15	10.8 ± 1.6	9.20	**0.007****	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	**0.034***	1.20	33.0 ± 1.7	17.43	**0.000*****	1.07	15.1 ± 3.7	0.15	0.696	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	**0.006****	1.29	29.3 ± 0.6	39.66	**0.000*****	1.08	22.9 ± 3.0	4.95	**0.039***	609.5	419.5	540.3
A. macbridei (Peru)	ss	10	9608 ± 760				31.6 ± 1				20.3 ± 2.2					473.3
A. macbridei (Ec)	ls	10	13866 ± 1180	4.39	**0.050***	1.10	27.1 ± 0.6	100.82	**0.000*****	1.14	24.9 ± 2.6	14.89	**0.001****	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	**0.009****	1.28	25.3 ± 1.3	33.44	**0.000*****	1.10	24.3 ± 3.7	6.29	**0.021***	286.9	259.5	332.5
A. vernicosum	ss	10	6292 ± 1741				27.9 ± 0.5				28.1 ± 3.4					223.9

Table 5.6 Number of seeds per fruit, results of one-way ANOVA for differences in number of seeds per fruits between long-styled (ls) and short-styled (ss) morphs, and percent seed set of eleven *Arcytophyllum* species/populations.

	Morph	Seeds per fruit			Seed set
		$x \pm s.d.$	F	p	[%]
A. thymifolium	ls	9.3 ± 2.4 (26)	1.71	0.198	82.3
A. thymifolium	ss	8.4 ± 2.5 (25)			97.7
A. capitatum	ls	3.9 ± 2.1 (21)	0.54	0.467	43.8
A. capitatum	ss	4.4 ± 2.1 (21)			50.0
A. filiforme	ls	4.7 ± 2.4 (21)	0.57	0.454	50.5
A. filiforme	ss	5.2 ± 2.1 (21)			69.3
A. ciliolatum	ls	8.3 ± 4.1 (23)	1.11	0.298	58.0
A. ciliolatum	ss	9.5 ± 3.5 (21)			65.1
A. rivetii	ls	5.4 ± 1.4 (19)	0.36	0.550	85.7
A. rivetii	ss	5.0 ± 1.6 (21)			89.3
A. aristatum	ls	7.0 ± 3.2 (17)	8.60	**0.006****	53.8
A. aristatum	ss	10.2 ± 3.2 (17)			79.1
A. lavarum	ls	9.5 ± 2.3 (26)	12.16	**0.001*****	88.0
A. lavarum	ss	12.7 ± 4.2 (29)			90.1
A. setosum	ls	12.5 ± 5.2 (22)	0.21	0.648	82.8
A. setosum	ss	13.1 ± 4.6 (23)			84.5
A. macbridei (Peru)	ls	12.4 ± 5.1 (20)	0.78	0.381	54.1
A. macbridei (Peru)	ss	11.0 ± 5.1 (22)			54.2
A. macbridei (Ec)	ls	12.5 ± 6.3 (26)	0.15	0.700	50.2
A. macbridei (Ec)	ss	13.2 ± 5.8 (27)			65.0
A. vernicosum	ls	15.2 ± 5.2 (20)	32.56	**0.000*****	62.6
A. vernicosum	ss	24.1 ± 4.8 (21)			85.8

5.5.4 Scent, Flower Color and Nectar

The flowers of *A. macbridei* (Ec) emitted a very intensive, sweet, perfumed fragrance while the flowers of *A. macbridei* (Peru) were odorless for human noses. The flowers of *A. lavarum* were also odorous but less intensively. All other species did not produce a scent recognizable for humans. The corolla color was pink in *A. macbridei* (Ec), while *A. macbridei* (Peru), as all other species, had a white corolla.

Arcytophyllum species showed a large variability in nectar sugar composition (Table 5.7). 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 of *A. macbridei* is sucrose rich and sucrose dominant in *A. vernicosum.* Corolla

tube length and sugar ratio was 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 5.7). Nectar volume of unbagged flowers was similar within the morphs, only in *A. lavarum* and *A. ciliolatum* significant differences were found. Nectar concentration averaged 28.5 ± 10.2 % [w/total w] calculated from 20 nectar samples of both morphs in the ten investigated species/populations.

Table 5.7 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 eleven *Arcytophyllum* species/ populations.

	Morph	Fructose [%]	Glucose [%]	Sucrose [%]	Sugar ratio	Nectar conc. [%w/w]	Nectar volume x ± 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	0.180
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	0.419
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	0.675
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	**0.000*** **
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	0.642
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	0.284
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	**0.000*** **
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	0.284
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	0.054
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	0.295
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	0.795
A. vernicosum	ss	20.9	24.0	55.1	1.23	20	0.55 ± 0.47 (15)		

5.5.5 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 with less than one record per hour was observed in *A. aristatum, A. rivetii* and *A. filiforme*, while the flowers of *A. setosum* received on average six visits per hour (Table 5.8). Insect activity was very irregular, and dependent on good weather conditions. Highest visitor frequency was observed in dry weather in the late morning.

Table 5.8 Visits per 15 minutes, visits per hour, and observed flower visitors of eleven *Arcytophyllum* species/populations.

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

5.6 Discussion

5.6.1 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 is possible to identify its members by means of a morphological key, now made available by Mena (1990).

This paper records a range extension for *A. macbridei*, which was so far 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). Additionally the flowers emit a sweet scent, while 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 in the two populations of *A. thymifolium* may be due to the wide geographical and altitudinal range of the species (Columbia to Peru, 400 m to 4000 m; Mena 1990). Mena (1990) described this species as certainly the most variable of all *Arcytophyllum* species. However, a larger sample of *A. thymifolium* populations would be necessary in order suggest a subdivision of the species. *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 (Figure 5.4) confirming the results of Andersson *et al.* (2002). The *A. capitatum*/*A. filiforme* clade, as well as the *A. nitidum*/*A. setosum* clade, are both formed by one northern and one southern species each. *Arcytophyllum capitatum* occurs in Columbia and Ecuador, while *A. filiforme* ranges from Ecuador to Bolivia; *A. nitidum* exists in Venezuela and Columbia, whereas *A. setosum* is distributed from Columbia to Bolivia (Mena 1990). It might be hypothesized that both the *A. capitatum*/*A. filiforme* and the *A. nitidum*/*A. setosum* pair of sister species may be descendents of a common ancestor each, from which speciation occurred due to geographical separation.

5.6.2 Floral morphology

As in most other heterostylous species (Ganders 1979) *Arcytophyllum* flowers are sympetalous with the stamens are adnate to corolla tube. Unfortunately, we did not measure filament insertion position, which determined anther level in *Hedyotis caerulea* (L.) Hook. (Ornduff 1980) but there is a significant positive correlation between corolla length and anther position in short-styled flowers in eight of the eleven investigated species/populations whereas in long-styled flowers a correlation of corolla length and stigma level are found in four species/populations (Tables 5.2, 5.3). Irregularities were found in *A. macbridei* (Peru) where corolla length of the short-styled morphs was significantly correlated with stigma position and in *A. filiforme*, in which corolla length was significantly correlated with anther position in long-styled flowers. Anther level mainly depends on corolla tube length in other Rubiaceae, e.g., *Gaertnera vaginata* Lam. (Pailler and Thompson 1997), *Bouvardia ternifolia* (Cav.) Schltdl., and *Psychotria chiapensis* Standl. (Faivre 2000). The fact that stigma lengths are shorter in long-styled morphs and that short-styled flowers have longer anthers is similar to many other distylous species, e.g. *Palicourea padifolia* (Willd. ex Roem. & Schult.) C.M. Taylor & Lorence (Contreras and Ornelas 1999). Longer corolla tubes on short-styled flowers, observed in six out of eleven 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; Riveros *et al.* 1995; Stone 1995; Pailler and Thompson 1997; Ree 1997; Contreras and Ornelas 1999; Passos and Sazima 1995; Faivre and McDade 2001). Larger 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 long-styled flowers of *A. capitatum, A. rivetii,* and *A macbridei* (both populations). Many heterostylous Rubiaceae show similar patterns of differences between short-styled and long-styled flowers as discussed above. The distribution of heterostyly in the family indicates that it is unlikely that heterostyly has evolved only once in all taxa that share these characteristics (Anderson 1973; Faivre and McDade 2001). Within *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, while the floral morphs of *A. filiforme* show significant differences only in stigma height, stigma length and stigma-anther separation. Likewise, in the well-supported trichotomy *A. ciliolatum*, *A. rivetii*, and *A. ericoides* (not studied), the morphs of *A. rivetii* show significant differences in all investigated features (except ovar length), while the morphs of *A. ciliolatum* differ only in corolla tube length, stigma height, anther height, and stigma-anther separation. In the two populations of *A. macbridei,* corolla tube length and total length were not significantly different in long- and short-styled morphs of the Ecuadorian population, while they were significantly different in the Peruvian population. On the other hand, ovar and stigma length did not differ significantly between long-styled and short-styled morphs in the Peruvian population, but 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.

5.6.3 Pollen dimorphism, pollen-ovule-ratio and seed set

Pollen dimorphisms in number (long-style > short-style) and/or size (short-style > long-style) have been observed in several Rubiaceae: *Hedyotis* L. (Ornduff 1980; Riveros *et al.* 1995), *Palicourea* Aubl. (Sobrevila *et al.* 1983; Contreras and Ornelas 1999). The ratio of long-styled to short-styled pollen grain number (0.95 in *A. filiforme* to 1.7 in *A. thymifolium*) fits well into the range reported by Ganders (1979) with values between 1.13 and 3.12. The

ratio of short-styled to long-styled morph pollen size in *Arcytophyllum* ranges from 1.02 to 1.17 which is similar to *Hedyotis caerulea* (1.2, Ornduff 1977) and *Hedyotis salzmannii* (DC.) Steud. (1.31, Riveros *et al.* 1995) but at the lower end of the distylous species reviewed by Dulberger 1992 (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*. This shows that neither pollen number and quality, nor ovule number seem to be under phylogenetic constraints. 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; Lehtilä and Strauss 1999; Quesada *et al.* 1995; Aizen and Raffaele 1998).

It is noteworthy that individuals with stigmas and anthers at the same level (homosytylous) occurred in populations of *A. filiforme* and *A. vernicosum* (lowest P/O-ratios), and of *A. rivetii* (highest P/O-ratio), so that P/O ratio is obviously independent from the degree of heterostyly (Table 5.5). However, these three species occur in separate clades, so that a phylogenetic tendency toward homostyly cannot be assumed. It might be hypothezised, though, that a tendency toward homostyly – from an originally heterostylous condition – is latent in *Arcytophyllum.* 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. Pollinator limitation may favour self-compatibility, a tendency found in particular in *A. filiforme* concluding from its low P/O ratio.

Interestingly, *A. thymifolium*, the most basal species, is at the same time the most widespread one and the one with the highest seed set (98%) in the short-styled morph. The tendency in all species/populations (except for *A. macbridei* Peru) that the short-styled morph had a higher percentage of seeds per ovules confirms the results of Garcia-Robledo and Mora-Kepfer (2004) in *A. lavarum* from Costa Rica, that the short-styled morphs display higher female reproductive success.

5.6.4 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 more derived species of the genus, with the most derived *A. macbridei* and *A. vernicosum* showing

high sucrose proportions, whereas the basal *A. thymifolium* has a very low sucrose/hexose ratio. No relationship, however, seems to exist between nectar sugar composition and observed floral visitors, questioning the adaptive value of the considerable variability in nectar sugar composition. Beside the possible phylogenetic interpretation of sugar contents in the nectar, the morphological features associated with heterostyly do not show any phylogenetically interpretable pattern, neither does the pollen-ovule ratio (Figure 5.4, Tables 5.3, 5.5).

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

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6 Synopsis and Perspectives

The question of how closely plants and pollinators are tied to each other seems fundamental to the understanding of temperate and tropical ecosystems. However, this issue has been very little explored, even though close flower-pollinator interactions have been considered for more than a century. Major morphological features of flowers have been selectively adapted by specialization into suits of canalized characteristics, forming "pollination syndromes" that are adapted to specific classes of pollen vectors (Vogel 1969, Faegri and van der Pijl 1980). Likewise, some pollinators exhibit extreme specialization in the flowers they attempt to exploit for critical floral resources.

Waser and Price (1990) and Waser *et al.* (1996) criticize the pollination syndrome concept, indicating the consequences of generalization for broader ecological interactions (mainly in temperate ecosystems), and raise a general caveat against assuming specialization as the inevitable outcome of selection among interacting species. This study confirms their results for an Andean montane forest by showing that nature is very flexible, and that pollinators commonly show a plastic behavior by visiting a variety of plant species without as much regard for their floral traits as might be expected. For example, *Isertia laevis* (Rubiaceae) possesses flowers with traits typical for the sphingophilous pollination syndrome, however, observation of floral visitors showed that nine different species of hummingbirds and one flower piercer were frequent diurnal visitors, while nocturnal visitation frequency by the expected sphingids was very low.

Observations of floral visitors provided in this thesis (for a total of 47 species) further showed that pollination syndromes cannot serve to automatically characterize the pollinator spectrum of a given plant, and any visitor at any time may act as a pollen carrier (Baker 1961, Herrera 1988). From the point of flower visitors, it does not matter from what flowers they gather a reward, as long as the reward is obtainable and palatable. From the plant's point of view, however, it is vital to distinguish pollinators from mere visitors (nectar and/or pollen robbers). Flowers of *Isertia laevis* visited by hummingbirds showed a high fruit set but a low seed set, indicating less effective pollination by diurnal visitors. Nocturnal pollen vectors, on the other hand, resulted in a high seed set, indicating a better adaptation of the flowers of *Isertia laevis* to sphingids than to hummingbirds as pollinators. Regarding relative reproductive success (i.e., efficiency of pollination defined as fruit set x seed set), both diurnal

and nocturnal pollinators are equally successful. The plant gains from generalization, since frequent visitation by not-so-effective pollinators contribute substantially to seed production, when expected pollinators are scarce.

The complex floral morphology found in the Asclepiadoideae (Apocynaceae) have often been proposed as a general trend toward specialization, however, information on specific pollination processes and pollinators is rare, and only a few field observations of Latin American species were published (Skutch 1988, Kunze and Liede 1991, Liede 1994, Krings 1999, Vieira and Shepherd 1999a). All these works typically deal with comparatively large flowered, rather easily accessible plants. Small flowered neotropical species of the tribe Asclepiadeae as presented here, however, have never been the subject of a pollination study before. Numerous kinds of visitors of the flowers of nine Asclepiadeae species were observed, but pollinaria were carried by only four insect species. The flowers showed a comparatively low pollinaria removal rate, and an even lower average was recorded for the pollinia insertion rate. The percentage of inserted pollinia to removed pollinaria, however was comparatively high, showing that if an insect achieved pollinia transfer, it performed the function very effectively. This suggests limited specialization with some degree of generalization. One would expect to find similar patterns of plant-pollinator interactions in other geographical regions in which such Asclepiadoideae occur, and (upon closer examination) that numerous, seemingly generalized species in the montane rain forest exhibit some degree of specialization toward a particular pollinator guild.

Floral nectar is one of the most important rewards offered by plants to pollinators (Simpson and Neff 1983). Therefore, nectar chemistry is an important component of floral biology. The evolution of nectar traits can be seen as a result of plant adaptation to preferences, digestive abilities, or sugar intake efficiencies of specific pollinators (Hainsworth and Wolf 1976, Stiles 1976, Pyke and Waser, 1981, Zimmermann 1983, Galen and Plowright 1985, Tamm and Gass 1986, Cresswell and Galen 1991, Martínez del Rio *et al.* 1992, Hodges 1995, Meléndez-Ackerman *et al.* 1997, Schemske and Bradshaw 1999). On the other hand, there is great evidence for homogeneity of nectar sugar composition among phylogenetically related taxa over various pollination syndromes (Galetto *et al.* 1998, Perret *et al.* 2001, Galetto and Bernardello 2003), questioning the adaptive values of the considerable variability in nectar sugar composition.

Field investigations, as presented in this thesis, are necessary to determine the role of nectar features in the interaction between plants and flower visitors. Sucrose is the predominant floral nectar sugar in the Gentianales studied, suggesting that nectar sugar

composition is a conservative characteristic. However, some degree of an adaptive convergence of floral nectar compositions to principal pollinator type within the constraints set by phylogenetic history is likely. The only significant differences regarding nectar sugar composition were found in chiropterophilous and myiophilous flowers, which had a significantly lower sugar ratio than sphingophilous flowers. This separation is further substantiated by non-metric multidimensional scaling using CNESS index of dissimilarity based on nectar sugar compositions. A weak significant correlation exists between floral visitors and nectar sugar compositions in Gentianales. Regarding the subfamily Asclepiadoideae, which has been treated separately from the other members of the order because of its highly derived floral structure including pollinia formation demanding special pollination mechanisms exhibit no association between nectar sugar composition and a pollinator´s sugar preferences in the eight species investigated. In the Gentianales, nectar sugar concentration did not differ significantly among the pollination syndromes, and matrix testing revealed no correlation between observed floral visitors and nectar concentrations.

The nectar volumes of covered and uncovered flowers are related to, and differ significantly among pollination syndromes. Matrix tests revealed a correlation between floral visitors and the nectar volume of covered flowers and (to a lesser extent) uncovered flowers. From an outcrossing plant´s perspective, flowers are most likely to be effectively pollinated when the nectar reward is abundant enough to attract the pollinator, but small enough to force the pollinator to make numerous plant to plant visits (Heinrich and Raven 1972, Heinrich 1975, Baker 1975). Therefore, the driving force to visitation appears to be the volume of nectar the visitor can expect to consume. This thesis shows further that nectar dynamics of the sphingophilous *Isertia laevis* are adapted to nocturnal pollination. Nectar volumes increased during the first part of the night and reached their maximums after midnight. Other such nocturnal pollination dynamics examples are provided by Willson *et al.* (1979), Willmott and Búrquez (1996), Matt (2001), Wolff *et al.* (in press).

The Ecuadorian provinces of Loja and Zamora-Chinchipe, including the Podocarpus National Park, represent the areas of greatest species diversity in *Arcytophyllum*; eight out of 15 species occur in the region (Mena 1990), stimulating this study's investigation on the phylogeny of the genus. According to this study's findings, and those of Andersson *et al.* (2002), the most derived species, *A. macbridei* and *A. vernicosum*, have higher sucrose proportions, whereas the basal *A. thymifolium* has a very low sucrose/hexose ratio. There is a tendency toward a higher percentage of sucrose in the more derived members of the genus. This shows the importance of information emerging from a well resolved phylogeny in order

to interpret data of nectar sugar composition. The expression of heterostyly in the genus was compared with phylogenetic relationships and inter- and intraspecific variations in floral morphology, nectar, pollen-ovule ratio, and seed set of ten species in eleven populations that were analyzed. Stigma and anther levels, as well as pollen grain size and number differed significantly among the morphs in all species/populations investigated except for one species. But the expression of heterostyly in ancillary features varied among closely related species and among populations, showing a great variability of floral morphology.

The reasons for the high diversity of Asclepiadoideae species in the Ecuadorian Andes are little understood. The nine species studied are mostly limited to a narrow geographical range, comprising southern Ecuador and northern Peru. It is not known whether the ranges of the pollinators correspond thereto. As recent phylogenetic studies have shown (Liede-Schumann *et al.* 2005), all species under consideration here belong to a large, morphologically very diverse, exclusively New World clade. Rapini and Van den Berg (2005) attribute a relatively recent origin to this clade, and state that it is still in active speciation and radiation. Montane forests in many areas of the tropical Andes occur on very steep slopes, subject to frequent natural landslides. Such gaps are often preferred habitats for twining species such as the Asclepiadoideae investigated, and are important factors in the dynamics of forest regeneration. Frequent formation of these habitats might serve as an important factor supporting the rapid radiation of small-flowered, ecologically undemanding, adaptable and diverse groups such as *Scyphostelma*, which still contains a large, unknown number of recognized and (so far) undescribed species (Liede-Schumann and Meve, unpubl.). The long flowering period of *Scyphostelma*, together with its effective pollination and high fruit set, are valuable prerequisites for the successful adaptive radiation observed. The observation of limited specialization coincides with the understanding of the American Asclepiadeae as a still actively radiating and rapidly evolving branch of the Apocynaceae.

Most Andean rainforests have already been destroyed, and the remaining habitat islands are threatened by fire, road construction, anthropogenously caused land slides, agriculture, and timber logging (Hamilton *et al.* 1995). The current reduction of biodiversity seems to exceed that of the great natural catastrophes at the end of the Paleozoic and Mesozoic eras, in which most of the plants survived, though animal diversity was reduced (Wilson 1988). Now, for the first time, plant diversity is sharply declining (Knoll 1984). Since the greatest diversity exists in the tropical areas, conservation efforts in countries such as Ecuador have been made very difficult by social, economic, and political problems. The Republic of Ecuador suffers the highest deforestation rate of all South American countries

(FAO 2003) with a concomitant loss of the majority of its biodiversity. Nevertheless, there are first steps toward conservation in the country, and Ecuador must be congratulated by already placing a considerable percentage of its territory under protection. Notwithstanding ecological protectionism, there is no prospect that the necessary scientific research tasks will be completed before a large portion of the species vanishes (Wilson 1988). The number of professional systematists competent to deal with the great number of species found in the humid tropic forests has been reduced due to decreased professional opportunities, reduced funding for research, and the assignment of higher priorities to other disciplines. Without a thorough understanding of ecosystems, it is highly unlikely they will be adequately preserved.

Our permanent study site in southern Ecuador allows investigatation of important community ecological and evolutionary questions. Gentianales is a suitable model group, since it members are very abundant at the study site and include the major life forms such as trees, shrubs, herbs, and vines, and the flowers show morphological adaptation to ornithophily, melittophily, myiophily, chiropterophily, and sphingophily. Investigation of phylogenetically related sympatrically occurring taxa are rarely performed, but urgently needed in order to provide basic information for an understanding of these threatened ecosystems.

The investigation and study of Gentianales as a floral biology model group could lead in several directions. The diversity and occurrence of members of the order Gentianales in an altitudinal gradient should be recorded, to determine if a spatial and temporal variability in floral nectar resources exists. Further reproductive phenology over a time period of several years is necessary. Detailed comparisons among populations of the plants investigated by this study are important. For example, investigations at lower elevations are needed to test if the scarcity of sphingid visits to *Isertia laevis* is an altitudinal effect. A highly speculative, but testable hypothesis is that the variable *I. laevis* might shift between favoring hummingbirds and sphingids over time, depending on altitude, e.g., by a shift in nectar production towards daytime and/or by starting with flower anthesis in the early afternoon. The permanent site in Ecuador should allow *Isertia* to be studied again in future years, to determine if such a shift occurs.

Since this study is one of the first to analyze small flowering, neotropical, twining, inhabitants of forests and forest margins, the uniqueness of the observed patterns is uncertain, compelling further investigations of twining, small flowering species in tropical montane forests targeting the Asclepiadoideae and other Angiosperm families. As the results of the *Arcytophyllum* paper show, the expression of heterostyly varies widely among

phylogenetically related taxa. Heterostylous species should be sampled in a variety of genera (e.g., *Arachnothryx, Faramea, Notopleura, Palicourea*, *Psychotria*, *Rudgea*, *Stilpnophyllum*) and pollination syndromes (e.g., ornithophilous, melittophilous, sphingophilous, myiophylous flowers), because pollinators play a direct role in the selection of morphology; differences in pollinator relationships might explain variations in the expression of heterostyly. Such data are important to understand the patterns of variation in heterostyly expression across the Rubiaceae. Comparison of two populations of *Arcytophyllum macbridei* showed variability in floral morphology and different features in heterostyly expression, suggesting that studies on various populations are needed to describe species. Such a description must comprise not only floral morphology, but also morph ratios within populations, self-compatibility experiments, detailed observations on pollen carryover, stigmatic pollen loads, and controlled pollination experiments. This thesis is a first promising step towards an understanding of plant-pollinator interactions, the evolution and ecology of Gentianales in a tropical montane forest.

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7 Summary

The pollination and reproductive biology of mostly small flowered, taxonomically related species of the monophyletic order Gentianales was investigated in a tropical montane forest in southern Ecuador. This study is part of an interdisciplinary project on the diversity and functioning of a montane ecosystem, and provides important results with respect to pollination biology. Such data are rarely collected for such a large number of taxa in a way that permits asking questions concerning both community ecology and evolution.

This thesis investigates the contribution of diurnal floral visitors to the reproductive success of the night flowering "sphingophilous" *Isertia laevis* (Rubiaceae), and discusses the value of pollination syndromes in characterizing the pollinator spectrum of a given plant. *Isertia laevis* possesses flowers that are morphologically adapted to pollination by sphingids, but diurnal flower observation showed that nine different hummingbirds (*Trochilidae)* and one flower piercer (*Coerebidae)* were frequent visitors. Hummingbird activity with the flowers peaked in the morning hours. At night, only a few sphingid individuals were observed. Nectar volumes in covered, as well as in uncovered flowers, reached their peaks (27 µl) at night (2.00 h). Accumulated nectar, due to low nocturnal floral visitation rates, was a rich nectar source to diurnal floral visitors the next morning. After frequent visitation by hummingbirds in the early morning, nectar volumes decreased to very low levels. Pollination experiments showed that flowers exclusively presented to nocturnal pollinators had a low fruit set (14%) but a high seed set (59%). Flowers accessible only to diurnal floral visitors showed a high fruit set of 63%, but a low seed set of 14%. Efficiency of pollination (fruit set X seed set) was equal for both diurnal and nocturnal pollinators. This shows that frequently occurring, but not very effective pollinators contribute substantially to seed production when the expected pollinators are scarce.

This study is one of the first examining the pollination biology of Apocynaceae-Asclepiadoideae, tribe Asclepiadeae (other than the temperate genus *Asclepias*), providing basic information on a plant's reproductive biology, focusing on animal-plant interactions of these mostly twining, highly scattered inhabitants of forests and forest margins, possessing very small, inconspicuous flowers. The relatively large numbers of flowers, blooming simultaneously in many species of the Asclepiadoideae, were important for insect attraction. Floral longevity varied from three to five days, and was shortened by successful pollinia

insertion. A large variety of floral visitors was observed on the flowers, however, pollinaria were carried by only four insect species. The flowers showed a comparatively low pollinaria removal rate with an average of 0.32 ± 0.13, and an even lower average was recorded for the pollinia insertion rate at 0.13 ± 0.07. The percentage of inserted pollinia to removed pollinaria was comparatively high with an average of 42.7% ± 22.3%. This shows that if an insect achieves pollinia transfer, it does so very effectively. The derived floral structure and pollinating system in the Apocynaceae-Asclepiadoideae has often been characterized as a very close flower-pollinator interaction. The present study, however, suggests limited specialization, with some degree of generalization.

Sucrose-rich or -dominant nectars were found in 49 out of 55 species of Gentianales (including Apocynaceae-Asclepiadoideae, Gentianaceae, and Rubiaceae). Sucrose is the predominant floral nectar sugar in the order Gentianales (although the order possesses flowers morphologically adapted to ornithophily, sphingophily, chiropterophily, melittophily, and myiophily), suggesting that nectar sugar composition is a conservative characteristic. Even though the specialized Apocynaceae-Asclepiadoideae are visited by both Hymenoptera and Diptera, nectar of seven out of eight species studied was sucrose-dominant, and nectar of the one remaining species was sucrose-rich. Focusing on Gentianaceae and Rubiaceae only, nectar sugar concentration did not differ significantly among the aforementioned pollination syndromes. The only significant differences in sugar composition were found in chiropterophilous and myiophilous flowers, which had a significantly lower sugar ratio than sphingophilous flowers. A separation of chiropterophilous and myiophilous flowers from the other pollination syndromes was further substantiated by non-metric, multidimensional scaling using the CNESS index of dissimilarity, based on nectar sugar compositions. Further, matrix tests revealed no correlation of observed floral visitors to nectar concentrations, whereas a weak significant correlation was found between floral visitors and nectar sugar compositions. Therefore, some degree of adaptive convergence of floral nectar compositions to principal pollinator type within the constraints set by phylogenetic history is likely. Matrix tests revealed a correlation between floral visitors and nectar volume of covered flowers, and to a lesser extent, of uncovered flowers. The nectar volumes of covered and uncovered flowers were related to, and differ significantly among, pollination syndromes. Therefore, the driving force to visitation appears to be the volume of nectar the visitor can expect to consume.

A phylogenetic study of *Arcytophyllum* (Rubiaceae) based on Internal Transcribed Spacer (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 exclusively Central American *A. lavarum* was well embedded among South American species. To understand the expression of heterostyly in the genus, we analyzed the inter- and intraspecific variation in floral morphology, nectar, pollen-ovule ratio, and seed set of ten species in eleven populations. Stigma and anther levels differed significantly between the morphs in the species/populations investigated except in *A. filiforme*. Different expressions of heterostyly in *Arcytophyllum* seemed independent of phylogenetic relationships, however, there was a tendency toward a higher percentage of sucrose in the more derived species of the genus: the most derived, *A. macbridei* and *A. vernicosum*, showed high sucrose proportions, whereas the basal *A. thymifolium* had a very low sucrose/hexose ratio. Nectar sugar composition was similar between the morphs. There was a significant positive correlation between corolla tube length and the proportion of sucrose. Pollen dimorphism, both with regard to number (long-styled > short-styled) and to size (short-styled > long-styled) was observed in all taxa investigated except for *A. filiforme*. According to the pollen-ovule ratios, the breeding systems ranged from facultative autogamy to facultative xenogamy, independent of phylogenetic relationships. Besides the possible phylogenetic interpretation of sugar contents in the nectar, the morphological features associated with heterostyly did not show any phylogenetically interpretable pattern, and neither did the pollen-ovule ratio. This shows that both features in the expression of heterostyly and the reproductive system may vary among closely related species. The main floral visitors of the species studied were Hymenoptera, Diptera, and Coleoptera. Seed production did not differ significantly between the morphs in eight of eleven species/populations investigated. There was, however, a tendency in all species/populations (except for *A. macbridei* Peru) that the short-styled morph produced a higher percentage of seeds per ovules, indicating a greater female reproductive success.

8 Zusammenfassung

Die Bestäubungs- und Reproduktionsbiologie der meist kleinblütigen Arten der monophyletischen Ordnung Gentianales wurde in einem tropischen Bergregenwald in Südecuador untersucht. Die Studie wurde im Rahmen einer interdisziplinären Forschergruppe, die die Diversität und funktionale Zusammenhänge in einem montanen Ökosystem untersucht, durchgeführt und hat wichtige neu Ergebnisse zur Bestäubungsbiologie erbracht. Bislang wurden Daten, die sowohl eine ökologische als auch eine evolutionsbiologische Fragestellung erlauben, für eine solch große Artenzahl nur selten erhoben.

Der erste Teil der vorliegenden Dissertation untersucht den Beitrag tagaktiver Blütenbesucher zum Reproduktionserfolg der nachtblütigen "sphingophilen" *Isertia laevis* (Rubiaceae) und diskutiert den Wert von Bestäubungssyndromen zur Charakterisierung des Bestäuberspektrums einer bestimmten Pflanze. *Isertia laevis* besitzt Blüten, die morphologisch an die Bestäubung von Sphingiden angepasst sind, jedoch zeigten Bestäuberbeobachtungen während des Tages, dass neun verschiedene Kolibriarten (*Trochilidae*) und ein Hakenschnabel (*Coerebidae*) die Blüten regelmäßig besuchten. Die Aktivität der Kolibris erreichte in den Morgenstunden ein Maximum. Während der Nacht wurden lediglich einige Sphingiden-Individuen beobachtet. Das Nektarvolumen sowohl abgeschirmter als auch nicht abgeschirmter Blüten erreichte während der Nacht (2.00 h) ein Maximum (27µl). Der aufgrund geringen nächtlichen Blütenbesuchs angesammelte Nektar stellte eine reichliche Nektarquelle für tagaktive Blütenbesucher am nächsten Morgen dar. Nach häufigem Kolibri-Besuch in den früher Morgenstunden nahm das Nektarvolumen ab und erreichte ein niedriges Niveau. Bestäubungsexperimente zeigten, dass Blüten, die ausschließlich nachtaktiven Besuchern präsentiert wurden, bei geringem Fruchtansatz (14%) einen hohen Samenansatz (59%) aufwiesen. Dagegen wiesen Blüten, die nur tagaktiven Blütenbesuchern zugänglich waren, einen hohen Fruchtansatz von 63% auf, zeigten jedoch einen niederen Samenansatz von lediglich 14%. Die Bestäubungseffizienz (definiert als Fruchtansatz x Saatansatz) war sowohl für tagaktive als auch für nachtaktive Bestäuber gleich. Dies zeigt, dass häufige, aber nicht sehr effiziente Bestäuber substanziell zur Samenproduktion beitragen, wenn die erwarteten Bestäuber selten sind.

Die vorliegende Studie ist eine der wenigen, die die Bestäubungsbiologie von Apocynaceae-Asclepiadoideae, Tribus Asclepiadeae untersucht (außer der in gemäßigten Breiten lebenden Gattung *Asclepias*). Sie enthält grundlegende Informationen zur Reproduktionsbiologie, wobei der Schwerpunkt auf Tier-Pflanzen Interaktionen der hauptsächlich windenden, sehr vereinzelt vorkommenden und kleinblütigen Besiedler von Wäldern und Waldrändern gelegt wurde. Für die Anlockung von Insekten war die Signalwirkung, die von der relativ große Zahl synchron blühender Blüten vieler Arten ausgeht, von Bedeutung. Die untersuchten Arten wiesen eine Blühdauer von drei bis fünf Tagen auf, die durch erfolgreiche Pollinien-Einführung verkürzt wurde. Es wurde eine große Zahl verschiedener Blütenbesucher beobachtet, jedoch wurden Pollinarien nur von vier Insektenarten transportiert. Die Blüten zeigten eine vergleichsweise geringe Pollinarien-Entnahmerate von durchschnittlich 0.32 ± 0.13, wobei die durchschnittliche Pollinien-Einführungsrate mit 0.13 ± 0.07 noch geringer war. Der Prozentsatz eingeführter Pollinien zu entnommenen Pollinarien ist aber verhältnismäßig hoch mit durchschnittlich 42.7% ± 22.3%. Wenn also ein Insekt Pollinien-Übertragung erfolgreich durchführen kann, dann tut es das sehr effektiv. Die komplexe Blütenmorphologie innerhalb der Apocynaceae-Asclepiadoideae ist oft mit einem generellen Trend zur Spezialisierung in Verbindung gebracht worden, doch weist die vorliegende Untersuchung auf eingeschränkte Spezialisierung mit wenigstens einen gewissen Grad von Generalisierung hin.

Saccharose-reicher oder -dominanter Nektar wurde in 49 von 55 Arten der Ordnung Gentianales (einschließlich Apocynaceae-Asclepiadoideae, Gentianaceae und Rubiaceae) gefunden. Saccharose ist der Hauptzucker des Blütennektars der Ordnung Gentianales (obwohl die Ordnung Blüten besitzt, die morphologisch an Ornithophilie, Sphingophilie, Chiropterophilie, Melittophilie und Myiophilie angepasst sind), was zeigt, dass die Nektarzuckerzusammensetztung ein konservatives Merkmal ist. Obwohl die spezialisierten Apocynaceae-Asclepiadoideae sowohl von Hymenopteren als auch von Dipteren besucht wurden, war der Nektar von sieben der acht untersuchten Arten Saccharose-dominant, und der der letzten Art Saccharose-reich. Konzentriert man sich ausschließlich auf die Familien Gentianaceae und Rubiaceae, zeigt die Nektarzuckerkonzentation keine signifikanten Unterschiede zwischen allen oben genannten Bestäubungssyndromen. Der einzige signifikante Unterschied bezüglich der Zuckerzusammensetzung wurde in chiropterophilen und myiophilen Blüten gefunden, die eine signifikant niedrigere Zuckerratio aufwiesen als sphingophile Blüten. Die Trennung der chiropterophilen und myiophilen Blüten von den übrigen Bestäubungssyndromen wurde außerdem durch die nichtlineare, multidimensionale

Skalierung durch den CNESS Unähnlichkeitsindex, basierend auf der Nektarzucker-zusammensetzung, bestätigt. Außerdem konnten Matrixtests keine Korrelation zwischen beobachteten Blütenbesuchern und den jeweiligen Nektarkonzentrationen nachweisen, es wurde hingegen eine schwache signifikante Korrelation zwischen Blütenbesuchern und Nektarzuckerzusammensetzung gezeigt. Daraus lässt sich ein gewisser Grad an Adaptation der Zusammensetzung von Blütennektar an bestimmte Bäustäubertypen innerhalb bestimmter phylogenetischen Grenzen vermuten. Matrixtests wiesen Korrelationen zwischen Blüten-besuchern und Nektarvolumina abgeschirmter und - in geringerem Maß - nicht abgeschirmter Blüten auf. Nektarvolumina abgeschirmter und nicht abgeschirmter Blüten korrelierten mit und unterschieden sich signifikant zwischen den einzelnen Bestäubungssyndromen. Die Hauptursache, die einen Besucher veranlasst, bestimmte Blüten zu besuchen, scheint das Nektarvolumen zu sein, das ein Besucher dort vorzufinden erwartet.

Es wurde eine molekulare phylogenetische Untersuchungen an *Arcytophyllum* (Rubiaceae) basierend auf dem Internal Transcribed Spacer (ITS) durchgeführt und mit einer früheren Studie basierend auf cpDNA verglichen. Die Stellung der weitverbreiteten *A. thymifolium* als Schwestergruppe zu allen anderen Arten wurde bestätigt ebenso einige gut-unterstützte "Clades". Die ausschließlich in Zentralamerika vorkommende *A. lavarum* blieb zwischen südamerikanischen Arten eingebettet. Um die Bedeutung der Heterostylie in der Gattung zu verstehen, wurden inter- und intraspezifische Variationen bezüglich Blütenmorphologie, Nektar, Pollen-Ovula-Ratio und Samenansatz von zehn Arten in elf Populationen untersucht. Die Höhe von Stigma- und Antherenposition unterschied sich signifikant zwischen den beiden Blütentypen in den untersuchten Arten/Populationen mit Ausnahme von *A. filiforme*. Unterschiede im Grad der Heterostylie scheinen in der Gattung *Arcytophyllum* von den phylogenetischen Beziehungen unabhängig zu sein. Lediglich in der Nektarzusammensetzung zeichnete sich eine Tendenz in Richtung eines prozentual höheren Saccharoseanteils in den abgeleiteten Arten der Gattung ab, wobei die am stärkesten abgeleiteten Arten *A. macbridei* und *A. vernicosum* einen hohen Saccharoseanteil aufwiesen, während der Nektar der basalen *A. thymifolium* eine sehr geringe Saccharose/Hexose-Ratio zeigte. Die Nektarzuckerzusammensetzung zwischen den beiden Blütenmorphen war ähnlich. Die Blütenkronlänge war mit dem Saccharoseanteil signifikant positiv korreliert. Betrachtet man sowohl die Anzahl der Pollenkörner (langgriffelig > kurzgriffelig) als auch ihre Größe (kurzgriffelig > langgriffelig), so wurde in allen untersuchten Arten mit Ausnahme von *A. filiforme* ein Pollendimorphismus beobachtet. Entsprechend der Pollen-Ovula-Ratio wurde das Reproduktionssystem als fakultativ autogam bis fakultativ xenogam klassifiziert, und

zwar unabhängig von den jeweiligen phylogenetischen Beziehungen. Abgesehen von der möglichen phylogenetischen Interpretation der Muster bezüglich der Nektarzucker-zusammensetzung zeigten morphologische Merkmale im Hinblick auf Heterostylie keine phylogenetisch interpretierbaren Muster, dies war auch bei der Pollen-Ovula-Ratio nicht der Fall. Die vorliegende Studie zeigt, dass sich Heterostyliegrad und Reproduktionssystem zwischen nahverwandten Arten deutlich unterscheiden. Die hauptsächlichen Blütenbesucher der untersuchten Arten waren Hymenoptera, Diptera und Coleoptera. Die Samenproduktion unterschied sich in acht der elf untersuchten Arten/Populationen nicht signifikant zwischen den beiden Blütenmorphen. Es gab jedoch eine Tendenz in allen Arten/Populationen mit Ausnahme von *A. macbridei* (Peru) in Richtung eines höheren Prozentsatzes von Samen zu Eianlagen in den kurzgriffeligen Blütenmorphen, die auf einen größeren weiblichen Reproduktionserfolg der kurzgriffeligen Morphen hinweist.

9 Resumen

La biología polinización y reproductiva en la mayoría de las flores pequeñas y en las especies taxonomicalmente relacionadas del monofilético orden Gentianales ha sido investigada en un bosque tropical del montaña en el sur de Ecuador. Este estudio es parte de un proyecto interdisciplinario sobre la diversidad y la funcionalidad de este ecosistema montañoso en el sur de Ecuador y pone a disposición resultados importantes de la biología reproductiva. Este tipo de datos son raramente reunidos en un número tan grande de taxa y de una manera que permita preguntarse sobre la ecología y la evolución.

La presente tesis investiga la contribución de diurnos visitantes florales y su éxito reproductivo en *Isertia laevis* (Rubiaceae), que exhibe flores nocturnas y esfingófilias. La tesis estudia el valor del síndrome de polinización para caracterizar el espectro de los polinizadores de una planta. Las flores de *Isertia laevis* exhiben las características algo típicas del síndrome de polinización por mariposas nocturnas, pero observaciones diurnas florales demostraron nueve diferentes colibríes (*Trochilidae*) y un picaflor (*Coerebidae*) como visitantes frecuentes. La actividad de los colibríes en las flores fue muy alta en la madrugada. Durante la noche se observaron solo pocos individuales de esfíngidos. El volumen del néctar en flores cubiertas y no cubiertas llego a su máximo (27 µl) en la noche (2.00 h). El néctar acumulado por la baja frecuencia de visitantes nocturnos florales fue una rica fuente para los visitantes diurnos a la mañana siguiente. Después de la frecuente visita de los colibríes en la madrugada, el volumen del néctar decreció a un nivel muy bajo. Los experimentos de polinización demostraron que las flores exclusivamente presentadas a polinizadores nocturnos tenían una baja relación fruto/flor (0.14) pero una alta relación semilla/óvulo (0.59). Las flores accesibles sólo por visitantes diurnos florales demostraron una alta relación fruto/flor de 0.63 pero una baja relación semilla/óvulo de 0.14. La eficiencia de la polinización (relación fruto/flor x relación semilla/óvulo), fue igual por ambos polinizadores diurnos y nocturnos. Frecuentemente los abundantes polinizadores de baja eficiencia contribuyen substancialmente a la producción de semillas en el caso en que el polinizador esperado sea raro.

Este estudio es uno de los primeros que investiga la biología de polinización de Apocynaceae-Asclepiadoideae, tribu Asclepiadeae, poniendo a disposición la información básica de la biología reproductiva de estas plantas trepadoras. La investigación está enfocada en la interacción animal-planta de estos esporádicos habitantes del bosque y sus alrededores que exhiben muy pequeñas y poco llamativas flores. La sincronización de floración (el

número relativamente largo de flores que florecen simultáneamente) en muchas especies de Asclepiadoideae es muy importante para la atracción de insectos. Las flores permanecen abiertas de tres a cinco días y las flores marchitan después de la implantación exitosa de polinaria. Varios tipos de visitantes florales fueron observados en las flores, pero la polinaria fue transportada sólo en cuatro especies de insectos. Las flores demostraron una polinaria alejar ratio baja con un promedio de 0.32 ± 0.13, y un promedio aún más bajo fue registrado por la polinia implantar ratio 0.13 ± 0.07. El porcentaje de implantadas polinias á las alejadas polinarias esta alto con un promedio de 42.7% ± 22.3%. Esto demuestra que cuando un insecto alcanza una transferencia lo hace de manera muy efectiva. La estructura floral derivada y el sistema de polinización en las Apoynaceae-Asclepiadoideae fueron muchas veces entendidos como una muy relacionada interacción de flor-polinizador. El presente estudio propone sin embargo una especialización limitada, con un cierto grado de generalización.

El néctar rico en sucrosa y dominado de sucrosa se encontró en 49 de 55 especies de Gentianales (Apocynaceae-Asclepiadoideae, Gentianaceae, Rubiaceae). La sucrosa es el azúcar predominante del néctar floral en el orden Gentianales, aunque exhibe flores morfológicamente adaptadas a ornitófilia, esfingófilia, quiroterófilia, melitófilia y miófilia, proponiendo que la composición del azúcar del néctar sea una característica conservada. Aunque las flores especializadas de Apocynaceae-Asclepiadoideae fueron visitadas de Himenóptera y Díptera, el néctar en siete de ocho especies investigadas fue dominado de sucrosa y el néctar de la otra especie fue rico en sucrosa. Enfocado sólo en Gentianaceae y Rubiaceae, la concentración del azúcar en el néctar no fue significantemente diferente entre los síndromes de polinización mencionados arriba. Considerándose la composición del azúcar, las únicas diferencias significantes se encontraron en las flores quiropterófilias y miófilias, las cuales tenían una ratio de azúcar significativamente más baja que las flores esfingófilias. Una separación de las flores quiropterófilias y miófilias de las de otros síndromes de polinización está además mantenida de la non-metrico escalización multidimensional usando el índice de disimilaridad basado en la composición del azúcar del néctar. Adicionalmente la prueba matriz no demostró ninguna correlación de los visitantes florales observados con las concentraciones del néctar, sin embargo se encontró una correlación significativamente débil entre los visitantes florales y las composiciones del néctar. Es por eso que un cierto grado de convergencia adoptiva de la composición del néctar floral al tipo del polinizador principal dentro de las fuerzas de la historia filogenética es probable. Las pruebas matrices demostraron una correlación entre los visitantes florales y el

volumen del néctar de flores cubiertas, y en una escala más baja, de flores no cubiertas. El volumen del néctar de flores cubiertas y no cubiertas está correlacionado con y es significativamente diferente entre los síndromes de polinización. Por eso la frecuencia de las visitas suele ser el volumen del néctar que un visitante puede esperar consumir.

Un estudio filogenético del género *Arcytophyllum* (Rubiaceae) basando en ITS fue conducido y comparado con un precedente estudio basando en cpDNA. La posición de la corriente *A. thymifolium,* como hermana de todos los otros grupos fue confirmada y otros bien apoyados "clades"podrían ser revalorados. La especie *A. lavarum* de Centro América está bien colocada entre las especies suramericanas. Para entender la expresión de heterostilia en el género, se analizaron inter e intraespecíficas variaciones en la morfología floral, en el néctar, en la tasa polen-óvulo y en la relación semilla/óvulo de diez especies en once poblaciones. Los niveles del estigma y anteras fueron significantemente diferentes entre los morfos de las especies/poblaciones investigadas con excepción de *A. filiforme*. Diferentes expresiones de heterostilia en *Arcytophyllum* suelen ser independientes de las relaciones filogenéticas. Aunque hay una tendencia de un porcentaje más alto de sucrosa en el néctar de las especies más derivadas del género. Con las especies más derivadas *A. macbridei* y *A. vernicosum* se mostraron una alta proporción de sucrosa, mientras que las especies basal *A. thymifolium* tienen una ratio de sucrosa/hexose muy baja. La composición del azúcar en el néctar fue igual entre los morfos. Hay una importante y positiva correlación entre el largo del tubo de la corola y la proporción de la sucrosa. El dimorfismo del polen, considerado doblemente el número (estilo-largo > estilo-corto) y el tamaño (estilo-corto > estilo-largo) fue observado en todas las tasas investigadas, con excepción de *A. filiforme*. Según la tasa polen-óvulo, el sistema reproductivo se situa entre facultativo autogama y facultativo xenogama, independiente de la relación filogenética. Aparte de la posible interpretación filogénetica de contenido del azúcar en el néctar, las características asociadas con la heterostilia no demostraron ningún patrón filogénetico que se pueda interpretar, ni hace este la tasa polen-óvulo. El presente estudio propone que la expresión de heterostilia y el sistema reproductivo es muy variable entre especies relacionadas. Los principales visitantes florales en las especies estudiadas fueron Himenóptera, Díptera y Coleóptero. La producción de semillas no fue significativamente diferente entre los morfos en ocho de las once especies/poblaciones investigadas. Hay una tendencia en todas las especies/poblaciones (excepto *A. macbridei* Perú) que los morfos estilo cortos tienen un porcentaje más alto de la relación semilla/óvulo indicando que las morfos estilo-cortos tienen un éxito femenino más alto.

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Darstellung des Eigenanteils

Wolff D., Braun M., Liede S. 2003. Nocturnal versus diurnal pollination success in *Isertia laevis* (Rubiaceae): a sphingophilous plant visited by hummingbirds. *Plant Biology* **5**: 71-78.

Das Versuchsdesign, der überwiegende Teil der Feldarbeit, die Laborarbeiten, die Auswertung, Darstellung, Interpretation und Diskussion der Ergebnisse wurden von D. Wolff durchgeführt. M. Braun assistierte bei der Beobachtung der Blütenbesucher sowie bei der Nektarentnahme, die über mehrere Tage in zwei-stündigen Intervallen erfolgte.

Wolff D., Meve U., Liede-Schumann S.: Observations on pollination in Asclepiadoideae (Apocynaceae) of southern Ecuador

Die Feldarbeiten, Laborarbeiten, Auswertung und Darstellung der Ergebnisse wurden ausschließlich von D. Wolff geleistet. U. Meve stellte die Blütenzeichnung (Fig: 3.1), sowie die rasterelektronenmikroskopischen Aufnahmen (Fig. 3.2 und 3.3) zur Verfügung. In die Diskussion flossen die neuesten Ergebnisse aus der systematischen Arbeit von S. Liede-Schumann und U. Meve ein.

Wolff D.: Nectar sugar composition and volumes of 47 species of Gentianales from a southern Ecuadorian montane forest

Die in diesem Manuskript dargestellten Daten wurden ausschließlich von D. Wolff erhoben, analysiert, ausgewertet, dargestellt, interpretiert und diskutiert.

Wolff D. and Liede-Schumann S.: Phylogeny and Reproductive Biology of the distylous *Arcytophyllum* (Rubiaceae)

Alle Daten, die die Blüten- und Reproduktionsbiologie der Gattung betreffen, wurden allein von D. Wolff erhoben, analysiert, ausgewertet, dargestellt, interpretiert und diskutiert. Die der Erstellung des Cladograms zugrunde liegende Laborarbeit wurde von D. Wolff mit technischer Unterstützung von A. Täuber durchgeführt. Die phylogenetischen Analysen (Cladogramme) hat S. Liede-Schumann durchgeführt.

Appendix

A1 Species list

List of all species collected in a montane rainforest in southern Ecuador. Additionally species of the genus *Arcytophyllum* collected by D. Wolff in other Ecuadorian Provinces, Peru and Costa Rica are added. Herbarium acronyms following Index Herbariorum, ed. 8 (*http://207.156.243.8/emu/ih/index.php*)

Species/Genus	Voucher	Locality	Altitude [m]	Date	Lifeform	Deter-mination	Herbarium
Apocynaceae-Asclepiadoideae							
"Cynanchum" harlingii Morillo	167	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, QM	2400	18.12.2001	Vine	U. Meve, S. Liede	B, UBT
***Ditassa* R.Br.**							
D. anderssonii Morillo var. nov. ined.	s.n.	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, ABW	2100	13.01.2001	Vine	U. Meve, S. Liede	UBT
D. endoleuca Schltr.	s.n.	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2250	24.03.2000	Vine	U. Meve, S. Liede	UBT
***Jobinia* E. Fourn.**							
Jobinia sp.	s.n.	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, AKZ	1950	14.09.2001	Vine	U. Meve, S. Liede	UBT

***Orthosia* Decne.**							
Orthosia ellemannii (Morillo) ined. = "*Cynanchum" ellemannii* Morillo	48	Ecuador, Zamora-Chinchipe, ECSF, Secondary Vegetation	1850	28.01.2001	Vine	U. Meve, S. Liede	B, UBT
***Oxypetalum* R. Br.**							
Oxypetalum sp.	s.n.	Ecuador, Zamora-Chinchipe, ECSF, Secondary Vegetation	1850	01.06.2000	Vine	U. Meve, S. Liede	UBT
***Scyphostelma* Baillon**							
Scyphostelma sp. A	58	Ecuador, Zamora-Chinchipe "El Tiro", Subpáramo	2700	14.01.2001	Vine	U. Meve, S. Liede	B, UBT
Scyphostelma sp. B	117	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2200	01.11.2001	Vine	U. Meve, S. Liede	UBT
Scyphostelma sp. C	s.n.	Ecuador, Loja, "Cajanuma" Páramo	3000	16.11.2000	Vine	U. Meve, S. Liede	UBT
Gentianaceae							
***Centaurium* Hill**							
C. erythraea Rafn	113	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	1840	01.01.2001	Herb	D. Wolff	UBT
***Gentianella* Moench**							
Gentianella gilioides (Gilg.) Fabris	31	Ecuador, Loja, "Cajanuma" Páramo	3000	20.06.2000	Herb	D. Wolff	UBT

***Halenia* Borck.**							
Halenia sp. 1	34	Ecuador, Loja, "Cajanuma" Páramo	3350	20.06.2000	Herb	D. Wolff	UBT
Halenia weddelliana Gilg	32	Ecuador, Loja, "Cajanuma" Páramo	3350	20.06.2000	Herb	D. Wolff	UBT
***Macrocarpaea* Gilg**							
M. apparata J.R. Grant & Struwe	144	Ecuador, Zamora-Chinchipe, "Valladolid" Páramo	3100	14.12.2000	Shrub	D. Wolff, F. Matt	UBT
M. arborescens Gilg	56	Ecuador, Loja, "Cajanuma" Páramo	3000	23.01.2001	Shrub	D. Wolff, F. Matt	UBT
M. bubops J.R. Grant & Struwe	99	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, ABW	2400	24.01.2001	Shrub	D. Wolff, F. Matt	UBT
M. harlingii J. S. Pringle	118	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2200	07.11.2001	Shrub	D. Wolff, F. Matt	UBT
M. jensii J.R. Grant & Struwe	49	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2300	21.01.2001	Shrub	D. Wolff, F. Matt	UBT
M. luna-gentiana J.R. Grant & Struwe	s.n.	Ecuador, Loja, "Cajanuma" Páramo	3100	23.01.2001	Shrub	D. Wolff, M. Matt	UBT
M. noctiluca J. R. Grant & Struwe	98	Ecuador, Loja, "Cajanuma" Páramo	2700	23.01.2001	Shrub	D. Wolff, F. Matt	UBT
M. subsessilis Weaver & J.R. Grant	143	Ecuador, Zamora-Chinchipe, "Valladolid" Páramo	3100	16.10.2001	Shrub	D. Wolff F. Matt	UBT

***Symbolanthus* G. Don**							
S. calygonus (Ruiz & Pav.) Griseb. ex Gilg	114	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2200	01.01.2001	Shrub	D. Wolff	UBT
S. calygonus (Ruiz & Pav.) Griseb. ex Gilg	s.n.	Ecuador, Loja, "Cajanuma" Páramo	3000	23.01.2001	Shrub	D. Wolff	UBT
S. cf. sp. nov. indet.	117	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, ABW	1900	01.01.2001	Shrub	D. Wolff	UBT
***Tapeinostemum* Benth.**							
T. zamoranum Steyerm.	33	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2200	20.05.2000	Herb	D. Wolff	UBT
Rubiaceae							
***Arachnothryx* Planch.**							
A. lojensis Steyerm.	15	Ecuador, Zamora-Chinchipe, ECSF, Secondary Vegetation KW	1800	15.06.2000	Tree	D. Wolff	MO, UBT
A. lojensis Steyerm.	162	Ecuador, Zamora-Chinchipe, ECSF, Secondary Vegetation KW	1800	22.11.2000	Tree	D. Wolff	MO, UBT
***Arcytophyllum* Willd. ex Roem. & Schult.**							
A. aristatum Standl.	25	Ecuador, Carchi, "El Ángel" Páramo	3800	04.05.2000	Herb	D. Wolff	MO, UBT
A. capitatum (Benth.) K. Schum.	40	Ecuador, Zamora-Chinchipe, "Valladolid" Páramo	2900	14.12.2000	Shrub	D. Wolff	MO, UBT

A. capitatum (Benth.) K. Schum.	120	Ecuador, Zamora-Chinchipe, "Valladolid" Páramo	2900	16.10.2001	Shrub	D. Wolff	MO, UBT
A. capitatum (Benth.) K. Schum.	121	Ecuador, Loja, "Cajanuma" Páramo	3350	05.10.2001	Shrub	D. Wolff	MO, UBT
A. capitatum (Benth.) K. Schum.	4	Ecuador, Loja, "Cajanuma" Páramo	3400	05.10.2000	Shrub	D. Wolff	MO, UBT
A. ciliolatum Standl.	36	Ecuador, Zamora-Chinchipe, "Valladolid" Páramo	2900	14.12.2000	Shrub	D. Wolff	MO, UBT
A. ciliolatum Standl.	26	Ecuador, Loja, "Cajanuma" Cloudforest	2750	04.11.2001	Shrub	D. Wolff	MO, UBT
A. ciliolatum Standl.	123	Ecuador, Zamora-Chinchipe, "Valladolid" Páramo	2900	16.10.2001	Shrub	D. Wolff	MO, UBT
A. filiforme (Ruiz & Pav.) Standl.	47	Peru, Amazonas, Chachapoyas, near Balsas Páramo	3100	07.01.2001	Herb	D. Wolff	MO, UBT
A. filiforme (Ruiz & Pav.) Standl.	125	Ecuador, Zamora-Chinchipe, "Valladolid" Páramo	3441	19.11.2001	Herb	D. Wolff	UBT
A. filiforme (Ruiz & Pav.) Standl.	1	Ecuador, Azuay, "Cajas" Páramo	3850	23.10.2000	Herb	D. Wolff	MO, UBT
A. filiforme (Ruiz & Pav.) Standl.	124	Ecuador, Azuay, "Cajas" Páramo	3850	26.11.2001	Herb	D. Wolff	MO, UBT
A. lavarum K. Schum.	59	Costa Rica, Cartago, "Cerro de la Muerte" Páramo	3400	04.02.2001	Shrub	D. Wolff	MO, UBT
*A.*cf. *macbridei* Standl.	5	Ecuador, Loja, "Cajanuma" Páramo	3350	05.10.2000	Shrub	D. Wolff	MO, UBT
*A.*cf. *macbridei* Standl.	126	Ecuador, Loja, "Cajanuma" Páramo	3350	05.10.2001	Shrub	D. Wolff	MO, UBT
A. macbridei Standl.	34	Peru, Amazonas, Chachapoyas, near Balsas Páramo	3100	07.01.2001	Shrub	D. Wolff	MO, UBT

A. muticum (Wedd.) Standl.	60	Costa Rica, Cartago, "Cerro de la Muerte" Páramo	3400	04.02.2001	Shrub	D. Wolff	MO, UBT
A. rivetii Danguy & Cherm.	35	Peru, Amazonas, Chachapoyas, near Balsas Páramo	3850	07.01.2001	Shrub	D. Wolff	MO, UBT
A. setosum (Ruiz & Pav.) Schltdl.	122	Ecuador, Azuay, "Cajas" Páramo	3850	26.11.2001	Shrub	D. Wolff	MO, UBT
A. setosum (Ruiz & Pav.) Schltdl.	2	Ecuador, Azuay, "Cajas" Páramo	3850	23.10.2000	Shrub	D. Wolff	MO, UBT
A. thymifolium (Ruiz & Pav.) Standl.	119	Ecuador, Zamora-Chinchipe, ECSF, Páramo	3200	28.10.2001	Shrub	D. Wolff	MO, UBT
A. vernicosum Standl.	3	Ecuador, Loja, "Cajanuma" Páramo	3000	05.10.2000	Shrub	D. Wolff	MO, UBT
A. vernicosum Standl.	127	Ecuador, Loja, "Cajanuma" Páramo	3000	12.01.2001	Shrub	D. Wolff	MO, UBT
A. vernicosum Standl.	128	Ecuador, Zamora-Chinchipe Páramo	3441	19.11.2001	Shrub	D. Wolff	MO, UBT
***Bertiera* Aubl.**							
B. sp.	289	Ecuador, Zamora-Chinchipe, ECSF, Secondary Vegetation KW	2250	14.01.2001	Shrub	D. Wolff	UBT
***Borreria* G. Mey**.							
B. assurgens (Ruiz & Pav.) Griseb.	7	Ecuador, Zamora-Chinchipe, ECSF, Secondary Vegetation KW	1850	15.06.2000	Herb	C.M. Taylor	MO, UBT
B. prostrata (Aubl.) Miq.	62	Ecuador, Zamora-Chinchipe, ECSF, Secondary Vegetation KW	1850	19.09.2001	Herb	C.M. Taylor	MO, UBT

***Coccocypselum* Sw.**							
C. condalia Pers.	71	Ecuador, Zamora-Chinchipe, ECSF, Secondary Vegetation, KW	1900	22.09.2001	Herb	C.M. Taylor	MO, UBT
***Dioicodendron* Steyerm.**							
D. dioicum (K.Schum. & K.Krause) Taylor	72	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2350	28.09.2001	Tree	D. Wolff	MO, UBT
***Elaeagia* Wedd.**							
E. sp.1	154	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2200	22.10.2001	Tree	C.M. Taylor	MO, UBT
E. sp.2	171	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2300	22.10.2001	Tree	D. Wolff	MO, UBT
***Faramea* Aubl.**							
F. coerulescens K. Schum. & K. Krause	55	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2300	18.01.2001	Tree	D. Wolff	MO, UBT
F. cf. *glandulosa* Poepp. & Endl.	109	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, Q2	1900	28.10.2001	Tree	D. Wolff	MO, UBT
F. cf. *glandulosa* Poepp. & Endl.	149	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, Q2	2000	17.12.2000	Tree	D. Wolff	MO, UBT
F. cf. *glandulosa* Poepp. & Endl.	150	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, Q2	2200	16.11.2001	Tree	D. Wolff	MO, UBT
F. cf. *glandulosa* Poepp. & Endl.	151	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, Q2	1950	19.12.2001	Tree	D. Wolff	MO, UBT

F. uniflora Dwyer & M. V. Hayden	108	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, AKZ	1900	28.10.2001	Shrub	D. Wolff	MO, UBT
F. uniflora Dwyer & M. V. Hayden	21	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, Q2	2000	17.11.2000	Shrub	D. Wolff	MO, UBT
***Galium* L.**							
G. hypocarpium (L.) Endl. ex Griseb.	14	Ecuador, Zamora-Chinchipe, ECSF, Secondary Vegetation, KW	1900	15.06.2000	Herb	D. Wolff	MO, UBT
G. sp.	s.n.	Ecuador, Zamora-Chinchipe, ECSF, Secondary Vegetation, KW	1900	28.10.2001	Herb	D. Wolff	UBT
***Hillia* Jacq.**							
H. parasitica Jacq.	160	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, AKZ	1850	13.10.2001	Liana	D. Wolff	MO, UBT
H. wurdackii Steyerm.	161	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2250	23.10.2001	Liana	D. Wolff	MO, UBT
***Isertia* Schreb.**							
I. laevis (Triana) B.M. Boom	s.n.	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, KW	1900	01.04.2000	Tree	D. Wolff	UBT
***Joosia* Karst.**							
J. dielsiana Standl.	159	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, QM	2900	16.11.2001	Tree	C.M. Taylor	MO, UBT

***Ladenbergia* Klotzsch**							
L. sp. 1	158	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2300	15.10.2000	Tree	C.M. Taylor	MO, UBT
L. sp. 1	85	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2250	09.10.2001	Tree	C.M. Taylor	MO, UBT
L. stenocarpa (Lamb.) Klotzsch	74	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, AKZ	1900	05.10.2001	Tree	C.M. Taylor	MO, UBT
***Manettia* Mutis ex L.**							
M. sp.1	17	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, AKZ	1800	28.10.2001	Vine	C.M. Taylor	MO, UBT
M. sp.2	77	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	3200	13.10.2001	Vine	C.M. Taylor	MO, UBT
***Nertera* Banks ex Gaertn.**							
N. granadensis (Mutis ex L.f.) Druce var. *granadensis*	172	Ecuador, Zamora-*Chinchipe*, ECSF, Secondary Vegetation, KW	1850	20.12.2001	Herb	D. Wolff	MO, UBT
N. granadensis (Mutis ex L.f.) Druce var. *tetrasperma* (Kunth) L. Andersson	s.n.	Ecuador, Loja, "Cajanuma" Páramo	3000	16.11.2000	Herb	D. Wolff	UBT
***Notopleura* (Benth. & Hook.f) Bremek.**							
N. vargasiana sp. nov. C.M. Taylor ined	163	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2400	17.06.2000	Vine	C.M. Taylor	MO, UBT
N. vargasiana sp. nov. C.M. Taylor ined	164	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2300	22.10.2001	Vine	C.M. Taylor	MO, UBT

***Palicourea* Aubl.**							
P. andrei Standl.	51	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, QM	3000	27.01.2001	Tree	D. Wolff	MO, UBT
P. angustifolia Kunth	16	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, KW	1800	07.03.2000	Tree	D. Wolff	MO, UBT
P. angustifolia Kunth	133	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2000	22.11.2000	Tree	D. Wolff	MO, UBT
P. canarina C.M. Taylor	129	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, QM	3000	27.01.2001	Tree	C.M. Taylor	MO, UBT
P. calycina Benth.	79	Ecuador, Loja, "Cajanuma" Cloud Forest	2720	05.10.2001	Shrub	D. Wolff	MO, UBT
P. heterochroma K. Schum. & K. Krause	42	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, Q2	2100	17.12.2001	Shrub	D. Wolff	MO, UBT
P. jaramilloi C.M. Taylor	139	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2400	16.11.2001	Tree	D. Wolff	MO, UBT
P. lobbii Standl.	76	Ecuador, Loja, "Cajanuma" Páramo	3050	13.10.2001	Shrub	D. Wolff	MO, UBT
P. lobbii Standl.	130	Ecuador, Loja, "Cajanuma" Cloud Forest	2750	23.01.2001	Shrub	D. Wolff	MO, UBT
P. luteonivea C.M. Taylor	12	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, AKZ	2000	15.10.2000	Tree	D. Wolff	MO, UBT
P. lyristipula Wernham	81	Ecuador, Loja, "Cajanuma" Cloud Forest	2720	05.10.2001	Shrub	D. Wolff	MO, UBT
P. subtomentosa (Ruiz & Pav.) DC.	132	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2000	08.03.2000	Shrub	D. Wolff	MO, UBT

P. thyrsiflora (Ruiz & Pav.) DC.	135	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, KW	1800	22.09.2001	Tree	D. Wolff	MO, UBT
P. cf. *weberbaueri* K. Krause	19	Ecuador, Loja, "Cajanuma" Cloud Forest	2850	16.11.2000	Tree	D. Wolff	MO, UBT
P. cf. *weberbaueri* K. Krause	78	Ecuador, Loja, "Cajanuma" Cloud Forest	2720	05.10.2001	Tree	D. Wolff	MO, UBT
P. cf. *weberbaueri* K. Krause	41	Ecuador, Loja, "Cajanuma" Páramo	3300	14.12.2000	Tree	D. Wolff	MO, UBT
P. sp. nov. C.M. Taylor ined.	22	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2400	22.11.2000	Shrub	C.M. Taylor	MO, UBT
P. sp. nov. C.M. Taylor ined.	66	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2200	10.09.2001	Shrub	C.M. Taylor	MO, UBT
***Psychotria* L.**							
P. acuminata Benth.	45	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2100	15.01.2001	Shrub	C.M. Taylor	MO, UBT
P. acuminata Benth.	180	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2000	15.11.2001	Shrub	C.M. Taylor	MO, UBT
P. acuminata Benth.	131	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	1900	23.01.2001	Shrub	C.M. Taylor	MO, UBT
P. aubletiana Steyerm.	173	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, QM	2500	16.11.2001	Shrub	D. Wolff	MO, UBT
P. reticulata Ruiz & Pav.	70	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2200	28.09.2001	Tree	C.M. Taylor	MO, UBT
P. reticulata Ruiz & Pav.	88	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2100	27.09.2001	Tree	C.M. Taylor	MO, UBT

P. reticulata Ruiz & Pav.	140	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	1800	18.01.2001	Tree	C.M. Taylor	MO, UBT
P. ottonis Standl.	157	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, QM	3000	28.01.2001	Shrub	D. Wolff	MO, UBT
P. tinctoria Ruiz & Pav.	44	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, ABW	1800	15.01.2001	Tree	D. Wolff	MO, UBT
P. tinctoria Ruiz & Pav.	67	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, ABW	1950	14.09.2001	Tree	D. Wolff	MO, UBT
P. subg. *Heteropsychotria* Steyerm. sp.	39	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2400	18.11.2000	Shrub	C.M. Taylor	MO, UBT
P. subg. *Heteropsychotria,* Steyerm. sp.	148	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2450	13.12.2001	Shrub	C.M. Taylor	MO, UBT
P. subg. *Psychotria* sp.1	50	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, Q3	2000	17.12.2000	Tree	D. Wolff	MO, UBT
P. subg. *Psychotria* sp.1	68	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, Q3	1900	22.09.2001	Tree	D. Wolff	MO, UBT
P. subg. *Psychotria* sp.2	165	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, Q2	1900	28.10.2001	Tree	D. Wolff	MO, UBT
***Rudgea* Salisb.**							
R. ciliata (Ruiz & Pav.) Spreng.	153	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, QM	3000	19.09.2001	Tree	C.M. Taylor	MO, UBT
R. ciliata (Ruiz & Pav.) Spreng.	152	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, AKZ	1800	02.10.2001	Tree	C.M. Taylor	MO, UBT

R. poeppigii K. Schum. ex Standl.	46	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T3	1900	14.01.2001	Tree	D. Wolff	MO, UBT
***Schradera* Vahl**							
S. subandina K. Krause	156	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, QM	3000	16.11.2001	Liana	D. Wolff	MO, UBT
S. sp.	155	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	3100	22.10.2001	Vine	D. Wolff	MO, UBT
***Stilpnophyllum* Hook.f.**							
S. oellgaardii L. Andersson	63	Ecuador, Zamora-Chinchipe, ECSF, Montane Forest, T2	2300	14.09.2001	Tree	D. Wolff	MO, UBT

A2 ITS alignment

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A._aristatum                aaaa-ctcaacacgatcgaagtcgcggccgacaccggcaaaggaactgaataa-gaccccggagc-ttcgggcc--tcgaccatgac
A._capitatum_ls             aaaa-ctcaacacgatcgaagtcgcggccgacacttgcaaaggaactgaataa-gaccccggagc-ttcgggcc--tcgaccatgac
A._ciliolatum               aaaa-ctcaacacgatcgaagtcgcggccgacaccggcaaaggaactgaacaa-gaccccggagc-ttcgggcc--tcgaccatgac
A._filiforme_Cajas_ss       aaaa-ctcaacacgatcgaagtcgcggccgacacttgcaaaggaactgaataa-gaccccggagc-ttcgggcc--tcgaccatgac
A._filiforme_Leimebamba_ls  aaaa-ctcaacacgatcgaagtcgcggccgacacttgcaaaggaactgaataa-gaccccggagc-ttcgggcc--tcgaccatgac
A._lavarum                  aaaa-ctcaacacgatcgaagtcgcggccgacaccggcaatggaactgaataa-gaccccggagc-ttcgggcc--tcgaccatgac
A._macbridei_Peru           aaac-ctcaacacgatcgaagtcgcggctgacaccggcaaaggaactgaataa-gaccctggagc-ttcgggcc--tcgaccatgac
A._macbridei_Cajanuma_ls    aaac-ctcaacacgatcgaagtcgcggccgacaccggcaaaggaactgaataa-gaccctggagc-ttcgggcc--tcgaccatgac
A._muticum                  aaaa-ctcaacacgatcgaagtcgcggccgacaccggcaaaggaactgaataa-gaccccggagc-ttcgggcc--tcgaccatgac
A._rivetii                  aaaa-ctcaacacgatcgaagttgtggccgacaccgtcaaaggaactgaataa-gaccccggagc-ttcgggcc--tcgaccatgac
A._setosum_Cajas_ls         aaac-ctcaacacgatcgaagtcgcggccgacaccggcaaaggaactgaataa-gaccctggagc-ttcgggcc--tcgaccatgac
A._thymifolium              aaaatctcaactcgatcgaagtcgcgaccgagacgggcgcaggtacttaaaaaagaccccggagc-ttagggcc--tcgaccatgac
A._thymifolium_Peru         aaaatctcaactcgatcgaagtcgcggccgagacgggcacaagtacttaaaaa-gaccccggagc-ttagggcc--tcgaccatgac
A._vernicosum               aaac-ctcaacacgatcgaagtcgcggccgacacctgcaaaggcactgaataa-gaccctggagc-ttcgggcc--tcgaccatgac
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A3 ITS alignment in combined data set

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Hedyotis_nigricans   AATCATTGTCGAATCCTGCAAACAACCGCGAACTCGTTACATAAAA-CAT-C-GGGTGCTA--AGGCAACCGCCCAACGGCCCCGAATCTAACAAAACTA
Houstonia_longifolia GATCATTGTCGAATCCTGCAAACCACCGCGAACATGTTATATAAAA-CCT-C-AGGTGCTTGAGGGCAATTGCCTAACGGCCCTGAAACTAACAAAACTT
A._aristatum         GATCATTGTCGAATCCTGCAAACCACCGCGCACACGTTTC-TAAAA-TATTC-GGGTGCGGTCGGACAGCCGTCTGCCTGCCCCGGATCCAACAAAACTT
A._ciliolatum        GATCATTGTCGAATCCTGCAAACCACCGCGCACACGTTTC-TAAAA-TATTC-GGGTGCGGTCGGACAACCGTCTGCCTGCCCCGGACCCAACAAAACTT
A._lavarum           GATCATTGTCGAATCCTGCAAACCACCGTGCACACGTTTC-TAAAA-TATTC-GGGTGCAGTCGGACAGCCGTCTGCCTGCTCCGGACCCAACAAAACTT
A._macbridei         GATCATTGTCGAATCCTGCAAACCACCGCGCACACGTTTC-TAAAA-TATTC-GGGTGCAGTCGGACAGCCGTCTGCCTGCCCCGGACCCAACAAAACTT
A._muticum           GATCATTGTCGAATCCTGCAAACCACCGTGCACATGTTTC-TAAAA-TATTC-GGGTGCGGTCGGACAACCGTCTGCCTGCCCCGGACCCAACAAAACTT
A._rivetii           GATCATTGTCGAATCCTGCAAACCACCGCGCACACGTTTC-TAAAA-TATTC-GGGAGCGGTCGGACAGCCGTCTGCCTACCCCGGACCAAACAAAACTT
A._setosum           GATCATTGTCGAATCCTGCAAACCACCGCGCACACGTTTC-TAAAA-TATTC-GGGTGCAGTCGGACAGCTGTCTGCCTGCCCCGGACCCAACAAAACTT
A._thymifolium       GATCATTGTCGAATCCTGCAAACCACCGCGAACACGTTTA-TAAAAAYMTYCRGGGTGCGGGCGGACTCCCGTCTGCCGGCCCCGGACCSAACAAAACWT
A._vernicosum        GATCATTGTCGAATCCTGCAAACCACCGCGCACACGTTTA-TAAAA-TATTC-GGGTGCAGTCGGACAGCCGTCTGCCTGCCCCGGACCCAACAAAACTT


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Hedyotis_nigricans   CCGGCGCGGATTGCGTCAAGTACTACTCAAAACGGATCGCCCGCACTCCCCCGCGGCTTCCGCGGGACGTGTGCGGCGCGTCTGAATCGTAACCAATACG
Houstonia_longifolia CCGGCGCGAATTGTGCCAAGGACTACT-AAAACGGATCGTCTGCACTACCCCGCGGCTTCCGCGGTAAGGGTGCAGTGCGTCTGAATCGTAACCAATACG
A._aristatum         CAGGCGCGGAAAGCGCCAAGGACTACT-AAAACGGATCGTCCGTGTCCTCTTGCGGTTTCCGCGGGACGGATGCGGCACGTCTGAATCGTAACCAATACG
A._ciliolatum        CCGGCGCGGAAAGCGCCAAGGACTACT-AAAACGGATCGTCCGTGTCCTCTTGCGGTTTCCGCGGGACGGATGCGGTGCGTCTGAATCGTAACCAATACG
A._lavarum           CCGGCGCGGAAAGCGCCAAGGACTACT-AAAACGGATCGTTCGTGTCCTCTTGCGGTTTCCGCGGGACGGATGCGGCGCGTCTGAATCGTAACCAATACG
A._macbridei         CCGGCGCGGAAAGCGCCAAGGACTACT-AAAACGGATCGTTCGTGTCCTCTTGCGGTTTCCGCAGGACGGATGCGGCGTGTCTGAATCGTAACCAACACG
A._muticum           CAGGCGCGGAAAGCGCCAAGGAATACT-AAAACGGATCGTCCGTGTCCTCTTGCGGTTTCCGCGGGACGGATGCGGCACGTCTGAATCGTAACCAATACG
A._rivetii           CCGGCGCGGAAAGCGCCAAGGACTACT-AAAACGGATCGTCCGTGTCCTCTTGCGGTTTCCGCGGGACGGATGCGGCACGTCTGAATCGTAACCAATACG
A._setosum           CCGGCGCGGAAAGCGCCAAGGACTACT-AAAACGGATCGTTCGTGTCCTCTTGCGGTTTCCGCGGGACGGATGCGGCACGTCTGAATCGTAACCAACACG
A._thymifolium       CCGGCGCGRAAAGCGCCAAGGACTACT-AAAACGGATCGTCCGCRTCCGKCYTCGGCTTCCG-GGGACGGGYGCGGTGCGTCTGAATCGTAACCAAYACG
A._vernicosum        CCGGCGCGGAAAGCGCCAAGGACTACT-AAAACGGATCGTTCGTGTCCTCTTGCGGTTTCCGCGGGACGGATGCGGCGCGTCTGAATCGTAACCAACACG
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Hedyotis_nigricans   ACTCT-----------------------------------------------------------------------------------------ATCGAGTTTTT
Houstonia_longifolia ACTCT-----------------------------------------------------------------------------------------ATCGAGTTTTT
A._aristatum         ACTCTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTT
A._ciliolatum        ACTCTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTT
A._lavarum           ACTCTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTT
A._macbridei         ACTCTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTT
A._muticum           ACTCTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTT
A._rivetii           ACTCTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTT
A._setosum           ACTCTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTT
A._thymifolium       ACTCTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTT
A._vernicosum        ACTCTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTT

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Hedyotis_nigricans   GAACGCAAGTTGCGCCCGAAGCCACTAGGCCGAGGGCACATCTGCCTGGGCGTCACGCATCGTCGCCACCCTCCTAGCATA-C----GT--GCCTTGCGT
Houstonia_longifolia GAACGCAAGTTGCGCCCGAAGCCTTTAGGCCGAGGGCACATCTGCCTGGGCGTCACGCATCGTCGCCACCC-CCAAGCACATC-----------TTG---
A._aristatum         GAACGCAAGTTGCGCCCGAAGCCATTAGGCTGAGGGCACATCTGCCTGGGCGTCACGCATCGTCGCCACCCCCCTCGCATATCGAAAGTACGCAGAGCGG
A._ciliolatum        GAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACATCTGCCTGGGCGTCACGCATCGTCGCCACCCCCCTCGCATATCGAAAGTACGCAGAGCTG
A._lavarum           GAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACATCTGCCTGGGCGTCACGCATCGTCGCCACCCCCCTCGCATATCGAAAGTACGCAGAGCGG
A._macbridei         GAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACATCTGCCTGGGCGTCACGCATCGTCGCCACCCCCCTCGCATATCGAAAGTACGCAGAGCGG
A._muticum           GAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACATCTGCCTGGGCGTCACGCATCGTCGCCACCCCCCTCGCATATCGAAAGTACGCAGAGCGG
A._rivetii           GAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACATCTGCCTGGGCGTCACGCATCGTCGCCACCCCCCTCGCATATTCAAAGTACGCAGAGCGG
A._setosum           GAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACATCTGCCTGGGCGTCACGCATCGTCGCCACCCCCCTCGCATATCGAAAGTACGCAGAGCGG
A._thymifolium       GAACGCAAGTTGCGCCCGAAGCCATTCGGCCGAGGGCACATCTGCCTGGGCGTCACGCATCGTCGCCACCCCMCTCCCRCA-CGCAAGTGCGGYGAGCGR
A._vernicosum        GAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACATCTGCCTGGGCGTCACGCATCGTCGCCACCCCCCTCGCATATCGAAAGTACGCAGAGCGG
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Hedyotis_nigricans    -GGGTGGCGGAAATTGGCCTCCCGTGTCAAGCCCAAGCGGCGACGCGGTTGGCCTAAATCTGATCCTACACT-CGGGAGAGTCACGACTAGTGGTGGTTG
Houstonia_longifolia AGAGTGGCGGAAATTGGTCTCCCGTGTAAAGTCCCAGCGGCAACGCGGTTGGCCTAAATTGAATCCTCTATTT-GGGAAAATCACGACAAGTGGTGGTTG
A._aristatum          AGGGTGACGGAATTTGGCCTCCCGTGTGAAGCGTTTGCGCCGACGCGGCTGGCCTAAATCGAGTCCTCTGCAACGGGGGAGTCACGACAAGTGGTGGTTG
A._ciliolatum         AGGGTGACGGAATTTGGCCTCCCGTGTGAAGCGTTAGCGCCGACGTGGCTGGCCTAAATCGAGTCCTCTGCAACGGGGGAGTCACGACAAGTGGTGGTTG
A._lavarum            AGGGTGACGGAATTTGGCCTCCCGTGTGAAGCGTTAGCACCGACGCGGCTGGCCTAAATCGAGTCCTCTGCAACGGGGGAGTCACGACAAGTGGTGGTTG
A._macbridei          AGGGTGACGGAATTTGGCCTCCCGTGTGAAGCGTTAGYGCCGACGCGGCTGGCCTAAATCGAGTCCTCTGCAACGGGGGAGTCACGACAAGTGGTGGTTG
A._muticum            AGGGTGACGGAATTTGGCCTCCCGTGCGAAGCGTTAGCGCCGACGCGGCTGGCCTAAATCGAGTCCTCTGCAACGGGGGAGTCACGACAAGTGGTGGTTG
A._rivetii            AGGGTGACGGAATTTGGCCTCCCGTGTGAAGCGTTAGCGCCGACGCGGCTGGCCTAAATCGAGTCCTCTGCAACGGGGGAGTCACGACAAGTGGTGGTTG
A._setosum            AGGGTGACGGAATTTGGCCTCCCGTGTGAAGCGTTAGCGCCGACGCGGCTGGCCTAAATTGAGTCCTCTGCAACGGGGGAGTCACGACAAGTGGTGGTTG
A._thymifolium        CGGGTGACGGAAGCTGGCCTCCCGTGCCRGGCSYTWTSGCCGACGCGGCTGGCCTAAATCGAGTCCTCTGCY-CGGGGGAGTCACGACAAGTGGTGGTTG
A._vernicosum         AGGGTGACGGAATTTGGCCTCCCGTGTGAAGCGTTAGCGCCAACGCGGCTGGCCTAAATCGAGTCCTCTGCAACGGGGGAGTCACGACAAGTGGTGGTTG


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Hedyotis_nigricans    AAACCCTCAACTCGATCGAAGTCGTGGCCG-CAACGCGAAAGGATATTAA-AC-GACCCTGGAGCCTAAGGGCCCCTCGACTATGAC
Houstonia_longifolia AAAAGTTCAACTCGATCGAAGTCGTGGACGTACCGCATAAAGGAGATGAA-AA-GACCCAAGAGCC-----GCC--ATAGTGTTGGC
A._aristatum          AAAA-CTCAACACGATCGAAGTCGCGGCCGACACCGGCAAAGGAACTGAATAA-GACCCCGGAGC-TTCGGGCC--TCGACCATGAC
A._ciliolatum         AAAA-CTCAACACGATCGAAGTCGCGGCCGACACCGGCAAAGGAACTGAACAA-GACCCCGGAGC-TTCGGGCC--TCGACCATGAC
A._lavarum            AAAA-CTCAACACGATCGAAGTCGCGGCCGACACCGGCAATGGAACTGAATAA-GACCCCGGAGC-TTCGGGCC--TCGACCATGAC
A._macbridei          AAAC-CTCAACACGATCGAAGTCGCGGCYGACACCGGCAAAGGAACTGAATAA-GACCCTGGAGC-TTCGGGCC--TCGACCATGAC
A._muticum            AAAA-CTCAACACGATCGAAGTCGCGGCCGACACCGGCAAAGGAACTGAATAA-GACCCCGGAGC-TTCGGGCC--TCGACCATGAC
A._rivetii            AAAA-CTCAACACGATCGAAGTTGTGGCCGACACCGTCAAAGGAACTGAATAA-GACCCCGGAGC-TTCGGGCC--TCGACCATGAC
A._setosum            AAAC-CTCAACACGATCGAAGTCGCGGCCGACACCGGCAAAGGAACTGAATAA-GACCCTGGAGC-TTCGGGCC--TCGACCATGAC
A._thymifolium        AAAATCTCAACTCGATCGAAGTCGCGRCCGAGACGGGCRCARGTACTTAAAAAAGACCCCGGAGC-TTAGGGCC--TCGACCATGAC
A._vernicosum         AAAC-CTCAACACGATCGAAGTCGCGGCCGACACCTGCAAAGGCACTGAATAA-GACCCTGGAGC-TTCGGGCC--TCGACCATGAC
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A4 cpDNA alignment adapted from Andersson *et al.* 2002

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Hedyotis_nigricans   ATTGTGGATTCCTTTCTTTTATCCACCACTTTCTATTTCTATATTTTCCTATATAAGTGAAGGTGCTCGTGACTCGACATCAG-ATGGTAATGGAAATAG
Houstonia_longifolia ATTGTGGATTCCTTTCTTTTATCCACCACTTTCTATTTCTATATTTTCCTATATAAGTGAAGGTGCTCGTGACTTGACATCAG-ATGGTAATGGAAATAG
A._aristatum         TTTGTGGATT-ATTTCTTTTATCCACCACTTTCCATTTC---------ATATATAAGTGAGGGTGCTCGCGACTCGACATCAGTCTGGTAATGGAAATAG
A._ciliolatum        TTTGTGGATT-ATTTCTTTTATCCACCACTTTCCATTTC---------ATATATAAGTGAGGGTGCTCGCGACTCGACATCAGTCTGGTAATGGAAATAG
A._lavarum           ??????????????????????TCCACCACTTTCCATTTC---------ATATATAAGTGAGGGTGCTCGCGACTCGACATCAGTCTGGTAATGGAAATAG
A._macbridei         ????????????????????????????????????????????????????????????????????????????TCGACATCAGTCTGGTAATGGAAATAG
A._muticum           TTTGTGGATT-ATTTCTTTTATCCACCACTTTCCATTTC---------ATATATAAGTGAGGGTGCTCGCGACTCGACATCAGTCTGGTAATGGAAATAG
A._rivetii           TTTGTGGATT-ATT---------CACCACTTTC-ATTTC---------ATATATAAGTGAGGGTGCTCGCGACTTGACATCAGTCTGGTAATGGAAATAG
A._setosum           TTTGTGGATT-ATTTCTTTTATCCACCACTTTCCATTTC---------ATATATAAGTGAGGGTGCTCGCGACTCGACATCAGTCTGGTAATGGAAATAG
A._thymifolium       TTTGTGGATTCCTTTCTTTTATCCACCACTTTCTATTTC---------ATATATAAGTGAGGGTGCTCGCGACTCGACATCAGTCTGGTAATGGAAATAG
A._vernicosum        TTTGTGGATT-ATTTCTTTTATCCACCACTTTCCATTTC---------ATATATAAGTGAGGGGGCTCGCGACTCGACATCAGTCTGGTAATGGAAATAG

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Hedyotis_nigricans   CCCCTGGTGGAGCTCGAGTCGAGTGTATTAATTCATTTTTCGGAGTAAGAATCTAGGGTTAATGCAAATCAATAAATTAGAACAACTTCGTGAATATCTC
Houstonia_longifolia CCCATGGTGGAGCTCGAGTCGAATGTATTAATTCATTTTTCGGAGCAAGAATCTAGGGTTAATGCAAATCAATAAATTAGAATAACTTCGTGAATATCTC
A._aristatum         CCTATGGTGGAGCTCGAGTAGAACGTATTAATTCATTTTTCGGAGCAAGAATCTAGGGTTAATGCAAATCAATAAATTGGAACAACTTCGTGAATATCTC
A._ciliolatum        CCCATGGTGGAGCTCGAGTAGAACGTATTAATTCATTTTTCGGAGCAAGAATCTAGGGTTAATGCAAATCAATAAATTGGAACAACTTCGTGAATATCTC
A._lavarum           CCTATGGTGGAGCTCGAGTAGAACGTATTAATTCATTTTTCGGAGCAAGAATCTAGGGTTAATGCAAATCAATAAATTGGAACAACTTCGTGAATATCTC
A._macbridei         CCTATGGTGGAGCTCGAGTAGAACGTATTAATTCATTTTTCGGAGCAAGAATCTAGGGTTAATGCAAATCAATAAATTGGAACAACTTCGTGAATATCTC
A._muticum           CCTATGGTGGAGCTCGAGTAGAACGTATTAATTCATTTTTCGGAGCAAGAATCTAGGGTTAATGCAAATCAATAAATTGGCACAACTTCGTGAATATCTC
A._rivetii           CCCATGGTGGAGCTCGAGTAGAACGTATTAATTTATTTTTCGGAGCAAGAATCTAGGGTTAATGCAAATCAATAAATTGGAACAACTTCGTGAATATCTC
A._setosum           CCTATGGTGGAGCTCGAGTAGAACGTATTAATTCATTTTTCGGAGCAAGAATCTAGGGTTAATGCAAATCAATAAATTGGAACAACTTCGTGAATATCTC
A._thymifolium       CCCATGGTGGAGCTCGAGTAGAACGTATTAATTCATTTTTCGGAGCAAGAATCTAGGGTTAATGCAAATCAATAAATTGGAACAACTTCGTGAATATCTC
A._vernicosum        CCTATGGTGGAGCTCGAGTAGAACGTATTAATTCATTTTTCGGAGCAAGAATCTAGGGTTAATGCAAATCAATAAATTGGAACAACTTCGTGAATATCTC
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.....................0.....................................................................................0
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Hedyotis_nigricans   TTAGATATAAAAATCGAAAGGATTTGATTCGAGCAAATTCTCCAGTCAAAGGGAAAATTGGTTGGAGTTAATCAAAAATTTTCGATCTAAAGTGTATTGC
Houstonia_longifolia TTAGATATAAAAATCGAAAGGATTTGATTCGACCAAATTCTTCAGTCAAAAGGAAAATTGCTTGGAGTTAATCAAAAATTTTCGATCTAAAGTGTATTGC
A._aristatum         TTAGATATAAAAATCGAAAGGATCTGATTCGAGCAAATTCTCCAGTAAAAAGGAAAATGGGTTGGAATTAATCAAAAAATTTCGATCTAAAGTGTATCAC
A._ciliolatum        TTAGATATCCAAATCGAAAGGATCTGATTCGAGCAAATTCTCCAGTAAAAAGGAAAATGGGTTGAAATTAATCAAAAAATTTCGATCTAAAGTGTATCGC
A._lavarum           TTAGATATAAAAATCGAAAGGATCTGATTCAAGCAAATTCTCCAGTAAAAAGGAAAATTGGTTGGAATTAATCAAAAAATTTCGATCTAAAGTGTATCAC
A._macbridei         TTAGATATAAAAATCGAAAGGATCTGATTCGAGCAAATTCTCCAGTAAAAAGGAAAATGGGTTGGAATTAATCAAAAAATTTCGATCTAAAGTGTATCGC
A._muticum           TTAGATATAAAAATTGGAAGGATCTGATTCGAGCAAATTCTCCAGTAAAAAGGAAAATGGGTTGGAATTAATCAAAAAATTTCGATCTAAAGTGTATCAC
A._rivetii           TTAGATATCAAAATCGAAAGGATCTGATTCGAGCAAATTCTCCAGTAAAAAGGAAAATTGGTTGAAATTAATCAAAAAATTTCGATCTAAAGTGTATCGC
A._setosum           TTAGATAGAAAAATCGAAAGGATCTGATTCGAGCAAATTCTCCAGTAAAAAGGAAAATTGGTTGGAATTAATCAAAAAATTTCGATCTAAAGTGTATCAC
A._thymifolium       TTAGATATAAAAATCGAAAGGATCTGATTCGAGCAAATTCTCCAGTAAAAAGGAAAATTGGTTGGAATTAATCAAAAATTTTCGATCTAAAGTGTATCGC
A._vernicosum        TTAGATATAAAAATCGAAAGGATCTGATTCGAGCAAATTCTCCAGTAAAAAGGAAAATGGGTTGGAATTAATCAAAAAATTTCGATCTAAAGTGTATCAC


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Hedyotis_nigricans   ACGGGAATCAATCGTTCGTAAGATTCTTGGATAGAAGGAAATCCAAAATAAGGGGTATGTTGCTACCATTTTTAAAGGATTAAGAAGCACCGAAGCAACG
Houstonia_longifolia ACGGGAATCAATCGTTCGTAAGATTCTTGGATAGAAGGAAATCCAAAATAAGGGGTATGTTGCTACCATTTTTAAAGGATTAAGAAGCACCGAAGCAATA
A._aristatum         ACGGGAATCAATCGTTCGTAAGATTCTTGGATAGAAGGAAATCCAAAATAAGGGGTATGTTGCTACCTTTTTTAAAGGATTAAGAAGCACCGAAGCAATG
A._ciliolatum        ACGGGAATCAATCGTTCGTAAGATTCTTGGATAGAAGGAAATCCAAAATAAGGGGTATGTTGCTACCATTTTTAAAGGATTAAGAAGCACCGAAGCAATG
A._lavarum           ACGGGAATCAAGCGTTCGTAAGATTCTTGGATAGAAGGAAATCCAAAATAAGGGGTATGTTGCTACCATTTTTAAAGGATTAAGAAGCACCGAAGCAATG
A._macbridei         ACGGGAATCAATCGTTCGTAAGATTCTTGGATAGAAGGAAATCCAAAATAAGGGGTATGTTGCTACCATTTTTAAAGGATTAAGAAGCACCGGAGCAATG
A._muticum           ACGGGAATCAATCGTTCGTAAGATTCTTGGATAGAAGGAAATCCAAAATAAGGGGTATGTTGCTACCCTTTTTAAAGGATTAAGAAGCACCGAAGCAATG
A._rivetii           ACGGGAATCAATCGTTCGTAAGATTCTTGGATAGAAGGAAATCCAAAATAAGGGGTATGTTGCTACCATTTTTAAAGGATTAAGAAGCACCGAAGCAATG
A._setosum           ACGGGAATCAATCGTTCGTAAGATTCTTGGATAGAAGGAAATCCAAAATAAGGGGTATGTTGCTACCATTTTTAAAGGATTAAGAAGCACCGAAGCAATG
A._thymifolium       ACGGGAATCAATCGTTCGTAAGATTCTTGGATAGAAGGAAATCCAAAATAAGGGGTATGTTGCTACCATTTTGAAAGGATTAAGAAGCACCGAAGCAATG
A._vernicosum        ACGGGAATCAATCGTTCGTAAGATTCTTGGATAGAAGGAAATCCAAAATAAGGGGTATGTTGCTACCATTTTTAAAGGATTAAGAAGCACCGAAGCAATG
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Hedyotis_nigricans   TCTAAACCTAATGATTGA------TAAAGGATCCCAAAACAAGGAAATACCGTCTCAAGAACTGCTTGCCCAGATTTAAAAATCTAAATCTAGTTTTTAT
Houstonia_longifolia TCTAAACCTAATGATTGA------TAAAGGATCCCAAAACAAGGAAATACCGTCTCAAGAACTGCTTGCCCAGATTTAAAAATCGAAATCTAGTTTTTAT
A._aristatum         TCTAAACCTAATGATTGA------TAAAGGATCCCAAAACAAGGAAATACCGTCTCAAGAACTGCTTGCCCAAATTTAAGAATCCAAATCTAGTTTTTAT
A._ciliolatum        TCTAAACCTAATGATTGA------TAAAGGATCCCAAAACAAGGAAATACCGTCTCAAGAACTGCTTGCCCAAATTTAAGAATCCAAATCTAGTTTTT--
A._lavarum           TCTAAACCTAATGATTGA------TAAAGGATCCCAAAACAAGGAAATACCGTCTCAAGAACTGCTTGCCCAAATTTAAGAATCCAAATCTAGTTTTTAT
A._macbridei         TCTAAACCTAATGATTGA------TAAAGGATCCCAAAACAAAGAAATAACGTCTCAAGAACTGCTTGCCCAAATTTAAGAATCCAAATCTAGTTTTTAT
A._muticum           TCTAAACCTAATGATTGA------TAAAGGATCCCAAAACAAGGAAATACCGTCTCAAGAACTGCTTGCCCAAATTTAAGAATCCAAATCTAGTTTTTAT
A._rivetii           TCTAAACCTAATGATTGA------TAAAGGATCCCAAAACAAGGAAATACCGTCTCAAGAACTGCTTGCCCAAATTTAAGAATCCAAATCTAGTTTT---
A._setosum           TCTAAACCTAATGATTGA------TAAAGGATCCCAAAACAAGGAAATACCGTCTCAAGAACTGCTTGCCCAAATTTAAGAATCCAAATCTAGTTTTT-T
A._thymifolium       TCTAAACCTAATGATTGA------TAAAGGATCCCAAAACAAGGAAATACCGTCTCAAGAACTGCTTGCCCAAATTTAAGAATCCAAATCTAGTTTATAT
A._vernicosum        TCTAAACCTAATGATTGA------TAAAGGATCCCAAAACAAGGAAATACCGTCTCAAGAACTGCTTGCCCAAATTTAAGAATCCAAATCTAGTTTTTAT


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Hedyotis_nigricans   TTTT--AAATGAGAGACAAACGAAATGGCGGTTGTAAAGACCACTCATCAATAAATGAAATTGCCTAACTATT------GTTCCTTGGGCTATTTAAGAG
Houstonia_longifolia TTTT--AAATGAGAGACAAACGAAATGGCGGTTGTAAAGACCACTC---AATAAATGAAATTGCCTAACTATT------GTTCCTTGGGCTATTTAAGAG
A._aristatum         TTTT--AAACGAGAGACAAACCAAATGGCGGTTGTAAAGACCACTC---AATAAATGAAATTGCCTAACTATT------GTTCCTTGGGCTATTTAAGAG
A._ciliolatum        TTTT--AAACGAGAGACAAACGAAATGGCGGTTGTAAAGACCACTC---AATAAATGAAATTGCCTAACTATT------GTTCTTTGGGCTATTTAAGAG
A._lavarum           TTTT--AAACGAGAGACAAACGAAATGGCGGTTGTAAAGACCACTC---AATAAATGAAATTGCCTAACTATT------GTTCCTTGGGCTATTTAAGAG
A._macbridei         TTTT--AAACGAGAGACAAACGAAATGGCGGTTGTAAAGACCACTC---AATAAATGAAATTGCCTAACTATT------GTTCCTTGGGCTATTTAAGAG
A._muticum           TTTT--AAACGAGAGACAAACGAAATGGCGGTTGTAAAGACCACTC---AATAAATGAAATTGCCTAACTATT------GTTCCTTGGGCTATTTAAGAG
A._rivetii           TTTT--AAACGAGAGACAAACGAAATGGCGGTTGTAAAGACCACTC---AATAAATGAAATTGCCTAACTATT------GTTCTTTGGGCTATTTAAGAG
A._setosum           TTTT--AAACGAGAGACAAACGAAATGGCGGTTGTAAAGACCACTC---AATAAATGAAATTGCCTAACTATT------GTTCCTTGGGCTATTTAAGAG
A._thymifolium       TTTT--AAACGAGAGACAAACGAAATGGCGGTTGTAAAGACCACTC---AATAAATGAAATTGCCTAACTATTGCTATTGTTCCTTGGAGTATTTAAGAG
A._vernicosum        TTTT--AAACGAGAGACAAACGAAATGGCGGTTGTAAAGACCACTC---AATAAATGAAATTGCCTAACTATT------GTTCCTTGGGCTATTTAAGAG
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Hedyotis_nigricans   TTATTTAATTTGAGTTATGAGTCCAAATTTTCTCTTTT-TTTTATTTTCAAGAA----------ACGAAGAAGAAAAAAA-----GAGAAAA----AAAA
Houstonia_longifolia TTATTTAATTTGAGTTATGAGTCCGAATTTTCTCTTTTTTTTTATTTTCAAGAA----------ACGAAGAAGAAAAAAA-----GAGAAAA-----AAA
A._aristatum         TTATTTAATTTGAGTTATGAGTCCGAATTATCTCTTTT-TGTCATTTTCAAGAA----------ACGAAGAAGAAAAAAAA----GATAAAA------AA
A._ciliolatum        TTATTTAATTTGAGTTATGAGTCCGAATTATCTCTTTT-TGTCATTTTCAAGAA----------ACGAAGAAGAAAA--------------------AA
A._lavarum           TTATTTAATTTGAGTTATGAGTCCGAATTATCTCTTTT-TGTCATTTTCAAGAA----------ACGAAGAAGAAAAAAAA----GATAAAA------AA
A._macbridei         TTATTTAATTTGAGTTATGAGTCCGAATTATCTCTTTT-TGTCATTTTCAAGAA----------ACGAAGAAGAAAAAAAA----GATAAAA------AA
A._muticum           TTATTTAATTTGAGTTATGAGTTCGAATTATCTCTTTT-TGTCATTTTCAAGAATTTTCAAGAACCGAAGAAGAAAAAAAA----GATAAAA------AA
A._rivetii           TTATTTAATTTGAGTTATGAGTCCGAATTATCTCTTTT-TGTCATTTTCAAGAATTTTCAAGAACCGAAGAAGAAAAAAAA----GAT---------AAA
A._setosum           TTATTTAATTTGAGTTATGAGTCCGAATTATCTCTTTT-TGTCATTTTCAAGAATTTTCAAGAACCGAAGAAGAAAAAAAA----GATAAAA-----AAA
A._thymifolium       TTATTTAATTTGAGTTATGAATCCGAATTATCTCTTTT-TGTCATTTTCAAGAA----------ACGAAGAAGAAAAAAAAAAAAAGATAAAA-------A
A._vernicosum        TTATTTAATTTGAGTTATGAGTCCGAATTATCTCTTTT-TGTCATTTTCAAGAA----------ACGAAGAAGAAAAAAAAA---GATAAAA-----AAA


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Hedyotis_nigricans   AAGATTGAAATCATAGTCGAATTGATTTGATGATTTTACGGATCCTTTTGTCATTTATTTTGTCATTTATTAGAATTTTCATTTTATACATAGATAAAAA
Houstonia_longifolia AAGATTGAAATCATA---------------------------------------TATTTTGTCATTTATTAGAATTTTCATTTTATACATAGATAAAAA
A._aristatum         AAGATTGAAATCATA-------------------------------TTGTCATTTATTTTATCATTTATTAGAATTTGAATTGTATACCTAGATCAAAA
A._ciliolatum        AAGATTGAAATCATAGTCTAATTGATTTGATGATTTTATGGATCCTTTTGTCATTTATTTTATCATTTATTAGAATTTTAATTTTATACCTAGATCAAAA
A._lavarum           AAGATTGAAATCATAGTCTAATTGATTTGATGATTTTATGGATCCTTTTGTCATTTATTTTATCATTTATTAGAATTTTCATTTGATACCTAGATCAAAA
A._macbridei         AAGATTGAAATCATAGTCTAATTGATTTGATGATTTTATGGATCCTTTTGTCATTTATT-----------AGAATTTTCATTTTATACCTAGATCAAAA
A._muticum           AAGATTGAAATCATAGTCTAATTGATTTGATGATTTTATGGATCCTTTTGTCATTTATTTTATCATTTATTAGAATTTTAATTTTATACCTAGATCAAAA
A._rivetii           AAGATTAAAATCATAGTCTAATTGATTTGATGATTTTATGGATCCTTTTGTCATTTATTTTATCATTTATTAGAATTTTAATTTTATACCTAGATCAAAA
A._setosum           AAGATTGAAATCATAGTCTAATTGATTTGATGATTTTATGGATCCTTTTGTCATTTATTTTATCATTTATTAGAATTTTCATTTTATACCTAGATCAAAA
A._thymifolium       AAGATTGAAATCATAGTCTAATTGATTTGATGATTTTATGGATCCTTTTGTCATTTATTTTGTCATTTATTAG--------TTTTATACCTAGATAAAAA
A._vernicosum        AAGATTGAAATCATAGTCTAATTGATTTGATGATTTTATGGATCCTTTTGTCATTTATTTTATCATTTATTAGAATTTTAATTTTATACCTAGATCAAAA
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Houstonia_longifolia ----ACTTTGGATCAAATTCTTTT-----TTCTCGAGC??????????????????????????????????AGAGAAACCCTGGAATTAATAAAAAGGG-
A._aristatum         TAAAACTTTGGATCAAATTCTTTT-----TTCTCGAGCCGTACGAGGAGAAAACTTCCTATACGTTTCTAGAGAGAAACCCTGGAATTAATAAAAAGGGG
A._ciliolatum        TAAAACTTTGGATCAAATTCTTTT-----TTCTCGAGCCGTACGAGGGGAAAACTTCCTATACGTTTCTAGAGAGAAACCCTGGAATTAATAAAAAGGGG
A._lavarum           TAAAACTTTGGATCAAATTCTTTT-----TTCTCGAGCCGTACGAGGAGAAAACTTCCTATACGTTTCTAGAGAGAAACCCTGGAATTAATAAAAAGGGG
A._macbridei         TCAAACTTTGGATCCAATTCTTTT-----TTCTCGAGCCGTAHGAGGATCAAACTTCCTATACGTTTCTAGAGAGAAACCCTGGAATTAATAAAAAGGGG
A._muticum           TAAAACTTTGGATCAAATTCTTTT-----TTCTCGAGCCGTACGAGGAGAAAACTTCCTATACGTTTCTAGAGAGAAACCCTGGAATTAATAAAAAGGGG
A._rivetii           TAAAACTTTGGATCAAATTCTTTT-----TTCTCGAGCCGTACGAGGGGAAAACTTCCTATACGTTTCTAGAGAGAAACCCTGGAATTAATAAAAAGGGG
A._setosum           TAAAACTTTGGATCAAATTCTTTT-----TTCTCGAGCCGTACGAGGAGAAAACTTCCTATACGTTTCTAGAGAGAAACCCTGGAATTAATAAAAAGGGG
A._thymifolium       TAAAACTTTGGATCAAATTCGTTT-----TTCTCGAGCCGTACGAGGAGAAAACTTCCTATACGTTTCTAGAGAGAAACCCTGGAATTAATAAAAAGGGG
A._vernicosum        TAAAACTTTGGATCAAATTCTTTT-----TTCTTGAGCCGTACGAGGAGAAAACTTCCTATACGTTTCTAGAGAGAAACCCTGGAATTAATAAAAAGGGG


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Hedyotis_nigricans   CAATCCTGAGCCAAATCCTATTTTCCGAAAAAAAA---?----AGGTTCAGAAAGTGAAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAAC
Houstonia_longifolia CAATCCTGAGCCAAATCCTATTTTCCGAAAACAAA---?----AGGTTCAGAAAGTGAAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAAC
A._aristatum         CAATCCTGAGCCAAATCCTATTTTCCGAAAACAAAAAACCAAACGGTTCAGAAAGTGAAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAAC
A._ciliolatum        CAATCCTGAGCCAAATCCTATTTTCCGAAAACAAAAAACCAAACGGTTCAGAAAGTGAAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAAC
A._lavarum           CAATCCTGAGCCAAATCCTATTTTCCGAAAACAAAAAACCAAACGGTTCAGAAAGTGAAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAAC
A._macbridei         CAATCCTGAGCCAAATCCTATTTTCCGAAAACAAAAAACAAAACGGTTCAGAAAGTGAAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAAC
A._muticum           CAATCCTGAGCCAAATCCTATTTTCCGAAAACAAAAAACTAAACGGTTCAGAAAGTGAAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAAC
A._rivetii           CAATCCTGAGCCAAATCCTATTTTCCGAAAACAAAAAACCAAACGGTTCAGAAAGTGAAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAAC
A._setosum           CAATCCTGAGCCAAATCCTATTTTCCGAAAACAAAAA-ACAAACGGTTCAGAAAGTGAAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAAC
A._thymifolium       CAATCCTGAGCCAAATCCTATTTTCCGAAAACAAT---?----AGGTTCAGAAAGTGAAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAAC
A._vernicosum        CAATCCTGAGCCAAATCCTATTTTCCGAAAACAAAAAACCAAACGGTTCAGAAAGTGAAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAAC
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Houstonia_longifolia AAATGGAGTTGGATGCGTTACTCGATAAGTCTTTCCAGGAAAAAGAAAAAAATTCCTAAAGTAAAAATATTTAAGGATAAAGTGAAGGATAAACATATAT
A._aristatum         AAATGGAGTTGGATGCGTTAGTCGATAAGTCTTTCCAGGAAAAA--------TTCCTAAAGTATAAGTATTTAAGGATAAAGTGAAGGATAAACATATAT
A._ciliolatum        AAATGGAGTTGGATGCGTTAGTCGATAAGTCTTTCCAGGAAAAA--------TTCCTAAAGTATAAGTATTTAAGGATAAAGTGAAGGATAAACATATAT
A._lavarum           AAATGGAGTTGGATGCGTTAGTCGATAAGTCTTTCCAGGAAAAA--------TTCCTAAAGTATAAGTATTTAAGGATAAAGTGAAGGATAAACATATAT
A._macbridei         AAATGGAGTTGGATGCGTTAGTCGATAAGTCTTTCCAGGAAAAA--------TTCCTAAAGTATAAGTAGTTAAGGATAAAGTGAAGGATAAACATATAT
A._muticum           AAATGGAGTTGGATGCGTTAGTCGATAAGTCTTTCCAGGAAAAA--------TTCCTAAAGTATAAGTATTTAAGGATAAAGTGAAGGATAAACATATAT
A._rivetii           AAATGGAGTTGGATGCGTTAGTCGATAAGTCTTTCCAGGAAAAA--------TTCCTAAAGTATAAGTATTTAAGAATAAAGTGAAGGATAAACATATAT
A._setosum           AAATGGAGTTGGATGCGTTAGTGGATAAGTCTTTCCAGGAAAAA--------TTCCTAAAGTATAAGTATTTAAGGATAAAGTGAAGGATAAACATATAT
A._thymifolium       AAATGGAGTTGGATGCGTTAGTCGATAAGTCTTTCCAGGAAAAA--------TTCCTAAAGTATAAGTATTTAAGGATAAAGTGAAAGATAAACATATAT
A._vernicosum        AAATGGAGTTGGATGCGTTAGTCGATAAGTCTTTCCAGGAAAAA--------TTCCTAAAGTATAAGTATTTAAGGATAAAGTGAAGGATAAACATATAT


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Hedyotis_nigricans   ACATAGGTAGTGAATAGTATATTAAATGAGTACTGACAGCCCAAACGAATCTCTATTTTTCTCTATGAAAAAATAAAGAATTGTTGTTAATAGATTCCAT
Houstonia_longifolia ACATAGGTATTGAATAGTATATTAAATGAGTACTGACAGCCCAAACGAATCTTTATTTTTCTCTATGAAAAAATAAAGAATTGTTGTTAATAGATTCCAT
A._aristatum         ACATAGGTAGTGAATAGTATATTAAATGAGTACTGACAGCCCAAACGAATCTCTGTTTT-ATCTATGAAAAAAGAAAGAATTGTTCTTAATAGATTCCAT
A._ciliolatum        ACATAGGTAGTGAATAGTATATTCAATGAGTACTGACAGCCCAAACGAATCCCTGTTTT-ATCTATGAAAAAAGAAAGAATTATTCTTA-----------
A._lavarum           ACATAGGTAGTGAATAGTATATTCAATGAGTACTGACAGCCCAAACGAATCTCTGTTTT-ATCTATGAAAAAAGAAAGAATTGTTCTTAATAGATTCCAT
A._macbridei         ACATAGGTAGTGAATAGTATATTCAATGAGTACTGACAGCCCAAACGAATCTCTGTTTT-ATCTATGAAAAAAGAAAGAATTGTTCTTAATAGATTCCAT
A._muticum           ACATAGGTAGTGAATAGTATATTCAATGAGTACTGACAGCCCAAACGAATCTCTGTTTT-ATCTATGAAAAAAGAAAGAATTGTTCTTAATAGATTCCAT
A._rivetii           ACATAGGTAGTGAATAGTATATTAAATGAGTACTGACAGCCCAAACGAATCYCTGTTTT-ATCTATGAAAAAAGAACGAATTATTCTTAATAGATTCCAT
A._setosum           ACATAGGTAGTGAATAGTATATTAAATGAGTACTGACAGCCCAAACGAATCTCTGTTTT-ATCTATGAAAAAAGAAAGAATTGTTCTTAATAGATTCCAT
A._thymifolium       ACATAGGTAGTGAATAGTATATTAAATGAGTACTGACAGCCCAAAGGAATCTCTGTTTT-ATCTATGAAAAAATAAAGAATTGTTCTTAATAGATTCCAT
A._vernicosum        ACATAGGTAGTGAATAGTATATTAAATGAGTACTGACAGCCCAAACGAATCTCTGTTTT-ATCTATGAAAAAAGAAAGAATTGTTCTTAATAGATTCCAT
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Hedyotis_nigricans   GTTT----------------------AAGAAAGAATCGAATATTCAGTGATCAAATGATTCACTCCATAGTCTGATAGATCTTTTCACGAACTGATTAAT
Houstonia_longifolia GTTT----------------------AAGAAAGAATCGAATATTCAGTGATCAAATGATTCACTCCATAGTCTGATAGATCTTTTCATGAACTGATTAAT
A._aristatum         GTTT----------------------AAGAAAGAATCGAATATTCATTGATCAAATGATTCACTCCATAGTCTGATAGATCTTTTCACGAACTGATTAAT
A._ciliolatum        GTTT----------------------AAGAAAGAATCGAATATTCATTGATCAAATGATTCACTCCATAGTCTGATAGATCTTTTCACGAACTGATTAAT
A._lavarum           GTTT----------------------AAGAAAGAATCGAATATTCATTGATCAAATGATTCACTCCATAGTCTGATAGATCTTTTCACGAACTGATTAAT
A._macbridei         GTTT----------------------AAGAAAGAATCGAATATTCATTGATCAAATGATTCACTCCATAGTCTGATAGATCTTTTCACGAACTGATTAAT
A._muticum           GTTT----------------------AAGAAAGAATCGAATATTCATTGATCAAATGATTCACTCCATAGTCTGATAGATCTTTTCACGAACTGATTAAT
A._rivetii           GTTT----------------------AAGAAAGAATCGAATATTCATTGATCAAATGATTCACTCCATAGTCTGATAGATCTTTTCACGAACTGATTAAT
A._setosum           GTTT----------------------AAGAAAGAATCGAATATTCATTGATCAAATGATTCACTCCATAGTCTGATAGATCTTTTCACGAACTGATTAAT
A._thymifolium       GTTTATAGATTCCATGTTTAAGGTTTAAGAAAGAATCGAATATTCATTGATCAAATGATTCACTCCATAGTCTGATAGATCTTTTCACGAACTGATTAAT
A._vernicosum        GTTT----------------------AAGAAAGAATCGAATATTCATTGATCAAATGATTCACTCCATAGTCTGATAGATCTTTTCACGAACTGATTAAT


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Hedyotis_nigricans   CAGACGAGAATAAAGATAGAGTCCCATTTTACATGTCAATGCCGGCAACAATGAAATTTATAGTTAAGAGGAAA-TCCGTCGACTTTTAAAATCGTGAGG
Houstonia_longifolia CAGACGAGAATAAAGATAGAGTCCCATTTTACATGTCAATGCCGGCAACAATGAAATTTATAGTTAAGAGGAAAATCCGTCGACTTTTAAAATCGTGAGG
A._aristatum         CAGACGAGAATAAAGAGAGAGTCCCATTTTACATGTCAATGCCGGCAACAATGAAATTTATAGTTAAGAGGAAAATCCGTCGACTTTTAAAATCGTGAGG
A._ciliolatum        CAGACGAGAATAAAGATAGAGTCCCATTTTACATGTCAATGCCGGCAACAATGAAATTTATAGTTAAGAGGAAAATCCGTCGACTTTTAAAATCGTGAGG
A._lavarum           CAGACGAGAATAAAGAGAGAGTCCCATTTTACATGTCAATGCCGGCAACAATGAAATTTATAGTTAAGAGGAAAATCCGTCGACTTTTAAAATCGTGAGG
A._macbridei         CACACGAGAATAAAGATAGAGTCCCATTTTACATGTCAATGCCGGCAACAATGAAATTTATAGTTAAGAGGAAAATCCGTCGACTTTTAAAATCGTGAGG
A._muticum           CAGACGAGAATAAAGAGAGAGTCCCATTTTACATGTCAATGCCGGCAACAATGAAAGTTATAGTTAAGAGGAAAATCCGTCGACTTTTAAAATCGTGAGG
A._rivetii           CAGACGAGAATAAAGATAGAGTCCCATTTTACATGTCAATGCCGGCAACAATGAAATTTATAGTTAAGAGGAAAATCCGTCGACTTTTAAAATCGTGAGG
A._setosum           CAGACGAGAATAAAGAGAGAGTCCCATTTTACATGTCAATGCCGGCAACAATGAAATTTATAGTTAAGAGGAAAATCCGTCGACTTTTAAAATCGTGAGG
A._thymifolium       CAGACGAGAATAAAGATAGAGTCCCATTTTACATGTCAATGCCGGCAACAATGAAATTTATAGTTAAGAGGAAAATCCGTCGACTTTTAAAATCGTGAGG
A._vernicosum        CAGACGAGAATAAAGAGAGAGTCCCATTTTACATGTCAATGCCGGCAACAATGAAATTTATAGTTAAGAGGAAAATCCGTCGACTTTTAAAATCGTGAGG
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Hedyotis_nigricans   GTTCAAGTCCCTCTATCCCCAAATAAAATAGACTATTCGATTCCCCAACTATCTATTTACCT--------A-----ATCCCTCTTTTGTTATCGGTTCAA
Houstonia_longifolia GTTCAAGTCCCTCTATCCCCAAATAAAATAGACTATTCGATTCCCCAACTATCTATTTACCT--------AATCCTATCCCTCTTTTGTTATCGGTTCAA
A._aristatum         GTTCAAGTCCCTCTATCCCCAAATAAGATAGACTATTCGATTCCCCAACTATCTTTTTACCTATTT-ATTC-----ATCCCTCTTTTGTTATCGGTTCAA
A._ciliolatum        GTTCAAGTCCCTCTATCCCCAAATAAGATAGACTATTCGATTGCCCAACTATCTTTTTACCTATTT-ATTC-----ATCCCTCTTTTGTTATCGGTTCAA
A._lavarum           GTTCAAGTCCCTCTATCCCCAAATAAGATAGACTATTCGATTCCCCAACTATCTTTTTACCTATTT-ATTC-----ATCCCTCTTTTGTTATCGGTTCAA
A._macbridei         GTTCAAGTCCCTCTATCCCCAAATAAGATAGACTATTCGATTCCCCAACTATCTTTTTACCTATTT-ATTC-----ATCCCTCTTTTGTTATCGGTTCAA
A._muticum           GTTCAAGTCCCTCTATCCCCAAATAAGATAGACTATTCGATTCCCCAACTCTCTTTTTACCTATTT-ATTC-----ATCCCTCTTTTGTTATCGGTTCAA
A._rivetii           GTTCAAGTCCCTCTATCCCCAAATAAGATAGACTATTCGATTGCCCAACTATCTTTTTACCTATTT-ATTC-----ATCCCTCTTTTGTTATCGGTTCAA
A._setosum           GTTCAAGTCCCTCTATCCCCAAATAAGATAGACTATTCGATTCCCCAACTATCTTTTTACCTATTT-ATTC-----ATCCCTCTTTTGTTATCGGTTCAA
A._thymifolium       GTTCAAGTCCCTCTATCCCCAAATAAGATCGACTATTTGATTCCCCAATTATCTTTTTACCT--------A-----ATGCCTCTTTTGTTATCGGTTCAA
A._vernicosum        GTTCAAGTCCCTCTATCCCCAAATAAGATAGACTATTCGATTCCCCAACTCTCTTTTTACCTATTT-ATTC-----ATCCCTCTTTTGTTATCGGTTCAA


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Hedyotis_nigricans   AATGCCTTATCT-ATTCAC-----TATATTCTCTTAGAAGTGGTCGGGGCGGCAATGTCCTTTTCTTATCATATCTGTATCATATACATA----ATGTAT
Houstonia_longifolia AATGCCTTATCC-ATTCAC-----TATATTCTCTTAGAAGTGGTCGGGGCGGTAATGTCCTTTTCTTATCATATCTGTATCATATACATA----ATGTAT
A._aristatum         AATGCCTTATAC-ATTCAC-----TATATTCTCTTAGAAGTGGTCGGGGCGACAATGTCCTTTTCTTATCACATCTGTATCATATACATACAAACTGTAT
A._ciliolatum        AATGCTTTATAC-ATTCAC-----TATATTCTCTTAGAAGTGGTCGGGGCGACAATGTCCTTTTCTTATCACATCTGTATCATATACATACAAACTGTAT
A._lavarum           AATGCCTTATAC-ATTCAC-----TATATTCTCTTAGAAGTGGTCGGGGCGACAATGTCCTTTTCTTATCACATCTGTATCATATACATACAAACTGTAT
A._macbridei         AATGCCTTATAC-ATTCAC-----TATATTCTCTTAGAAGTGGTCGGGGCGACAATGTCCTTTTCTTATCACATCTGTATCATATACATACAAACTGTAT
A._muticum           AATGCCTTATAC-ATTCACTATATTATATTCTCTTAGAAGTGGTCGGGGCGACAATGTCCTTTTCTTATCACATCTGTATCATATACATACAAACTGTAT
A._rivetii           AATGCTTTATAC-ATTCAC-----TATATTCTCTTAGAAGTGGTCGGGGCGACAATGTCCTTTTCTTATCACATCTGTATCATATACATACAAACTGTAT
A._setosum           AATGCCTTATCC-ATTCAC-----TATATTCTCTTAGAAGTCGTCGGGGCGACAATGTCCTTTTCTTATCACATCTGTATCATATACATACAAACTGTAT
A._thymifolium       AATGCCTT----------C-----TATATTCTCTTAGAAGTGGTGGGGGCGACAATGTCCTTTTCTTCTCACATCTGTATCATATACATACAAACTGTAT
A._vernicosum        AATGCCTTATAC-ATTCAC-----TATATTCTCTTAGAAGTGGTCGGGGCGACAATGTCCTTTTCTTATCACATCTGTATCATATACATACAAACTGTAT
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Hedyotis_nigricans   ATGTTCAAATGTATATGTTTGAGCAAGAAATCCCCATTTGAATGATTTACAATCAATCGAATTACCCCTAACCTAAAAAA-ACTTGGGAATTCCCTCTTT
Houstonia_longifolia ATGTTCAAATGGATATGTTTGAGCAAGAAATCCCCATTTGAATGATTTATAATCAATCGAATTACTCCTAACCTAAAAAAACCTTGGGAATTCCCTTTTT
A._aristatum         ATGTTCAAATGTATATGTTTGAGCAAGAAATCCCAATTTGAATGATTTACAATCAATCGAATTACTCCTAACCTAAAAAAGCCTTGGGAATTCCCTTTTT
A._ciliolatum        ATGTTCAAATGTATATGTTTGAGCAAGAAATCCCAATTTGAATGATTTACAATCAATCGAATTACTCCTAACCTAAAAAAGCCTTGGGAATTCCCTTTTT
A._lavarum           ATGTTCAAATGTATATGTTTGAGCAAGAAATCCCAATTTGAATGATTTACAATCAATCGAATTACTCCTAACCTAAAAAAGCCTTGGGAATTCCCTTTTT
A._macbridei         ATGTTCAAATGTATATGTTTGAGCAAGAAATCCCAATTTGAATGATTTACAATCAATCGAATTACTCCTAACCTAAAAAAGCCTTGGGAATTCCCTTTTT
A._muticum           ATGTTCAAATGTATATGTTTGAGCAAGAAATCCCAATTTGAATGATTTACAATCAATCGAATTACTCCTAACCTAAAAAAGCCTTGGGAATTCCCTTTTT
A._rivetii           ATGTTCAA????????????????????????????????????????????????????????????????????????????????????????
A._setosum           ATGTTCAAATGTATATGTTTGAGCAAGAAATCCCAATTTGAATGATTTACAATCAATCGAATTACTCCTAACCTAAAAAAGCCTTGGGAATTCCCTTTTT
A._thymifolium       ATGTTCAAATGTATATGTTTGAGCAAGAAATCCCCATTTGAATGATTTACAATCAGTCGAATTACTCCTAACCTCAAAAAACCTTGGGAATTCCCTTTTT
A._vernicosum        ATGTTCAAATGTATATGTTTGAGCAAGAAATCCCAATTTGAATGATTTACAATCAATCGAATTACTCCTAACCTAAAAAAGCCTTGGGAATTCCCTTTTT

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Hedyotis_nigricans   AAGTTTTAATATTACTAATTGTTGGAATTGGTAACCTTCTTTT-----AAATTGACACAGTACCCATTTTCGAGTAAAATAAGAATGC
Houstonia_longifolia AAGTTTTAATATTACTAATTGTTGGAATTGGTAACCTTCTTTT-----AAATTGACACAGTACCCATTTTCGAGTAAAATAAGAATGC
A._aristatum         AAGTTTTAATA---ATAATTGTTGGAATTGGCAACCTTCTTTT-----AAATTGACACAGTACCCATTTTCGAGTAAAATGAGAATGC
A._ciliolatum        AAGTTTTAATA---AGAATTGTTGGAATTGGCAACCTTCTTTTAGTTTCAATTGACACAGTACCCATTTTCGAGTAAAATGAGAATGC
A._lavarum           AAGTTT--------?-----GTTGGAATTGGCAACCTTCTTTT-----AAATTGACACAGTACCCATTTTCGAGTAAAATGAGAATGC
A._macbridei         AAGTTTTAATA---AGAATTGTTGGAATTGGCAACCTTCTTTT-----AAATTGACACAGTACCCATTTTCGAGTAAAATGAGAATGC
A._muticum           AAGTTTTAATA---ATAATTGTTGGAATTGGCAACCTTCTTTT-----AAATTGACACAGTACCCATTTTCGAGTAAAATGAGAATGC
A._rivetii           ????????????????????????????????????????????????????????????????????????????????????
A._setosum           TAGTTTTAATA---ATAATTGTTGGAATTGGCAACCTTCTTTT-----AAATTGACACAGTACCCATTTTCGAGTAAAATGAGAATGC
A._thymifolium       AAGTTTTAATA---ATAATTGTTGGAATTGGCAACCTTCTTTT-----AAATTGACACAGTAACCATTTTTGAGTAAAATGAGAATGC
A._vernicosum        AAGTTTTAATA---ATAATTGTTGGAATTGGCAACCTTCTTTT-----AAATTGACACAGTACCCATTTTCGAGTAAAATGAGAATGC
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Erklärung

Hiermit erkläre ich, dass ich die Arbeit selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.

Ferner erkläre ich, dass ich anderweitig mit oder ohne Erfolg nicht versucht habe, diese Dissertation einzureichen. Ich habe keine gleichartige Doktorprüfung an einer anderen Hochschule endgültig nicht bestanden.

Bayreuth, den 10. Oktober 2005