Selbyana 7: 318-327

CLASSIFICATION OF THE TRIBE DIURIDEAE (ORCHIDACEAE) I. SUBTRIBE PRASOPHYLLINAE SCHLECHTER

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ABSTRACT

The general limits of the tribe Diurideae are discussed. It is considered that the tribe should be removed from the subfamily Orchidoideae. This paper is restricted to discussions on comparative studies of species in the subtribe Prasophyllinae. Vegetative and floral morphology, frequency of flowering, general distribution, preferred pollinators, and fungal associations are examined. I conclude that there is one genus, *Prasophyllum*, in the subtribe and that *Genoplesium* and *Microtis* should be removed from the Prasophyllinae.

INTRODUCTION

This is the first of a series of papers dealing with the systematics of the tribe Diurideae. It is my intention to review the status of each subtribe in the Diurideae and to present a final paper which will incorporate my data into a new system of classification for the tribe.

The terrestrial Diurideae (sensu Dressler, 1981) include the Australasian subtribes Acianthinae, Caladeniinae, Diuridinae, Prasophyllinae, Pterostylidinae, and the South American-New Caledonian subtribe Chloraeinae. Most Diurideae have root-stem tuberoids except for the Chloraeinae and saprophytic species such as *Genoplesium baueri* R. Br., *Townsonia viridis* (J. D. Hook.) Schltr., *Prasophyllum flavum* R. Br., and *Arthrochilus huntianus* (S. Muell.) Blaxell, which have fleshy, brittle root systems with little or no tuberoid present.

The Diurideae present special problems for the systematist because of different column structures within the tribe. Diuridinae *(Diuris, Orthoceras)* and Prasophyllinae *(Prasophyllum)* have a spiranthoid-type of anther that is inserted near the base of the stigma. Caladeniinae, Acianthinae, Chloraeinae, and Pterostylidinae have an anther that is inserted above or near the apex of the stigma and is similar to the Neottioid-type of anther (Burns-Balogh and Funk, in prep.) In most species of these subtribes the hingelike anther cap appears as a cape enshrouding the pollinarium. The pollinia are composed of monads and tetrads in all but Prasophyllinae which has massulae and rarely tetrads. The viscidium is apical in *Prasophyllum*, *Diuris*, and *Orthoceras*, and basal in *Genoplesium*, *Microtis*, other Diuridinae, Caladeniinae, Acianthinae, Chloraeinae, and Pterostylidinae. In the majority of the Diurideae the rostellum is extremely reduced leaving a notchlike area at the apex of the stigma when the pollinarium is removed. The staminodes are well developed and in some cases quite complex.

Dressler (1981) places the Diurideae with the Neottieae, Diseae, and Orchideae in subfamily Orchidoideae because of the root-stem tuberoids that are found in most genera of all but the Chloraeinae and Neottieae, the usually erect anthers, habit of growth, and the lack of stomatal subsidiary cells. Most other classifications, e.g. Garay (1972), separate the Diseae and Orchideae from the Neottieae and Diurideae, but Dressler believes that the sec-

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tile pollinia, root-stem tuberoids, and basitonic attachment of the pollinia to the viscidium point to a phyletic relationship. He also cites a putative hybrid between *Epipactis* (Neottieae) and *Gymnadenia* (Orchideae) as further indication of a closer relationship than has been thought in the past.

The development of the anther is one of the few consistent characters in the Orchidaceae. In the Orchideae and Diseae the anther connective separates the two thecae and the filament is either absent or completely fused to the column apex. The anther in the Orchidoideae s.s. may be either erect (Orchideae), at right angles to the column axis (Diseae), or reversed in position with the stigma by bending backwards (Satyrieae, sensu Brieger, 1970 - ongoing). In the Diurideae and Neottieae the anther connective does not separate the thecae and is simply an extension of the filament that supports the anther. The pollinia of the Orchidoideae s.s., like the Prasophyllinae, are massulate, but they have an additional character, i.e., the elastoviscin caudicle. In the subtribe Prasophyllinae a rostellar stipe or hamulus has independently evolved, and this is similar to that which occurs in the Tropidiinae (Spiranthoideae). There are a few other characters present in the Orchidoideae s.s. that isolate them from the Neottieae and Diurideae, i.e., the lack of a foot layer in the pollen wall (Burns-Balogh, 1983) intectate exines on the pollen grain, a bursicle, a straplike rostellum, and a totally fused column structure.

Because of the above character disagreements I am removing the Diurideae and Neottieae from the Orchidoideae. The major reason for differing with Dressler (1981) is that I believe column structures that are consistent should take precedence over vegetative structures which are not consistent in classification. On the basis of wider observations and numerical analysis, Lavarack (1976) has also found very little evidence of a relationship between the Orchideae and Diurideae.

MATERIALS AND METHODS

All plant and pollen material, unless otherwise stated, was collected by Mark Clements in the field in Australia or from cultivated plants which are part of the extensive collection of Australian indigenous orchids at the National Botanic Gardens, Canberra, Australia. Fresh or preserved flowers were examined in order to observe the diagnostic structures of the column and perianth. Pollinaria were extracted from fresh flowers by Clements and Peter Berhardt and sent to me to be processed for SEM (Scanning Electron Microscope) observation. Pollinaria were air-dried, placed on an SEM specimen stub, gold-coated, and observed with a Cambridge Mark IIA Stereoscan microscope. All identifications and references to environmental factors were furnished by Mark Clements.

SUBTRIBE PRASOPHYLLINAE: GENERAL CONSIDERATIONS

In the past the Prasophyllinae have been treated as consisting of three genera: *Prasophyllum*, including *Corunastylis (= P. apostasopodes)*, *Microtis*, including *Goadbyella (= M. alba)*, and *Genoplesium* (Dressler, 1981), all three of which were described by Robert Brown (1810).

In Brown (1810), *Prasophyllum* comprised 12 species in two groups, one with an awned anther apex and the other with a mucronate or rostrate apex. There were five species in *Microtis* and one in *Genoplesium*.

Lindley (1830-1840) placed *Microtis* in the subtribe Euarethuseae, tribe Arethuseae while *Prasophyllum* was included in the subtribe Diuridae, tribe Neottieae. He also separated *Prasophyllum* into two sections but these were based on lateral sepal shape.

Nicholls (1969) stated that Bentham divided *Prasophyllum* into three sections: i. *Euprasophyllum* with a sessile labellum; ii. *Podochilus* with a clawed labellum; and iii. *Genoplesium* with a clawed and articulate labellum. Rupp (1943) broadened the concept of section *Euprasophyllum* to include *Podochilus* and recognized the section *Genoplesium*. Later (1949) he recognized *Genoplesium* as a distinct and monotypic genus and suggested the name *Micranthum* as a new sectional name for *Prasophyllum* species formerly included with *Genoplesium*. Since that time *Prasophyllum* has been recognized as having two sections, viz. *Prasophyllum* and sect. *Micranthum*.

In 1926 Schlechter grouped *Microtis* and *Prasophyllum* in his latest concept of the Prasophyllinae. Unfortunately the subtribe was not well defined and because of this there are problems with its circumscription today.

In studying the column morphology of this subtribe I have found two distinct types which are referable to those found in the Spiranthoideae (sensu Dressler, 1981) and the Neottioideae (sensu Burns-Balogh and Funk, in prep.). *Prasophyllum* sect. *Prasophyllum* has the typical spiranthoid type of anther while *Prasophyllum* sect. *Micranthum*, *Genoplesium* and *Microtis* have the typical neottioid type of anther. It is a perplexing situation because one can either separate these groups into two different subfamilies or maintain the genus *Prasophyllum* based on floral morphology. It may be that the two sections of *Prasophyllum* represent two distinct subfamilies which have converged in appearance, a situation not uncommon in the Orchidaceae.

Probably the closest ally is *Epiblema*, subtribe Diuridinae, which shares with the Prasophyllinae a short column, prominent staminodia, and usually narrow, onionlike leaves. Barthlott (in Dressler, 1981) reports a similar seed structure in these two subtribes.

PRASOPHYLLUM

Prasophyllum is composed of approximately 90 species that occur in Australia (including Tasmania), New Zealand, and New Caledonia. Section *Prasophyllum* may be recognized by the spiranthoid type of column, the robust plants, moderate size flowers, and a labellum which is sessile on the column base or attached by a short, rigid claw that is never articulate or movable. Section *Micranthum* is recognized by the neottioid type of column, the short and slender plants, small flowers, and a labellum that is articulate or movable on the conspicuous claw attached to the column base. Both sections have nonresupinate flowers, and there are an approximately equal number of species in each section.

Most species in section *Prasophyllum* occur in southwest Australia, South Australia, Victoria, and Tasmania. A few occur in southern New South Wales and probably only two or three in southeastern Queensland. Two species reach New Zealand. Section *Micranthum* reaches its greatest diversity around Sydney, New South Wales, where there are approximately 20 species. One species reaches northeastern Australia and New Caledonia, and there are two species in New Zealand. In Western Australia there is only a single species.

While the majority of species are autotrophic, *Prasophyllum flavum* (sect. *Prasophyllum*) is semi-saprophytic.

The column in section *Prasophyllum* is very short. The single functional anther is free, there are two stigmatic lobes, and the rostellum varies from long and bladelike to short with a notched rostellum remnant. The stylar canal entrance is located between the apex of the stigmatic surface and the base of the rostellum or within the stigmatic area. The pollinarium is massulate, rarely granulate and possesses a stipe which is connected to the four pollinia by an elastic (?) connection. There appear to be only two pollinia but two in each set are united on the surface facing the rostellum, so there are actually four pollinia. The exine has either a thick broken reticulum or it may be heavily clavate. The staminodes are well developed and consist of two lobes fused at the base, one invariably larger than the other. The larger lobe is about equal to the length of the portion of the column on which the stigmatic surface is situated. All staminodes have entire margins, even in species where other floral segments may be crenulate or fimbriate, e.g., *Prasophyllum fimbria* H. G. Rchb. and *P. hians* H. G. Rchb. The pollinaria are generally exposed at maturity when the anther shrinks back against the dorsal sepal surface, also a characteristic of Spiranthoideae.

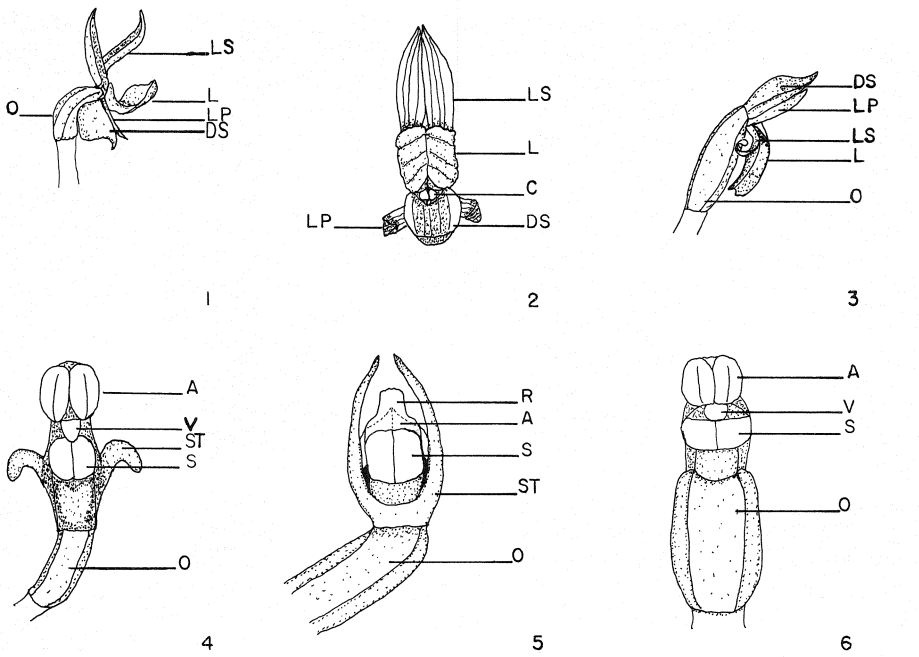
The labellum in section *Prasophyllum* is mostly highly colored and generally has a prominent callus ridge(s). A nectarlike substance is excreted from or near the base of the labellum and can be seen as droplets in most species. Other floral segments are often dull brown or green in color and contrast strongly with the colorful labellum. The labellum (or median petal) is sessile in all species. The general orientation of the column is such that the pollinator (usually a species of wasp), in order to get the nectarlike substance, must approach the flower with its head facing downward. The head then touches the viscidium during the collection of nectar. The callus plates and connate lateral sepals appear to be structures that the pollinator can grasp in order to collect the nectar. The petals are stiff and held more or less parallel to each other and at each side of the column in a number of species. They prevent the pollinator from collecting the nectar when approaching the flower from the side. Most flowers are highly perfumed. *Prasophyllum* sect. *Prasophyllum* is pollinated by wasps (Hymenoptera) and beetles (Coleoptera). Clements has observed a large wasp trying to remove the eight to ten pollinaria stuck to its compound eyes and head. Large flies were also seen with *Prasophyllum* pollinaria attached to the eyes.

Species of *Prasophyllum* show a fair degree of variability in floral morphology between populations. I suggest the very nature of the method used to attract a vector, i.e., scent and color, then reward, does not require stringency of flower shape that other species exhibit, e.g., those that mimic insects. This may be the reason many species are taxonomically difficult to define.

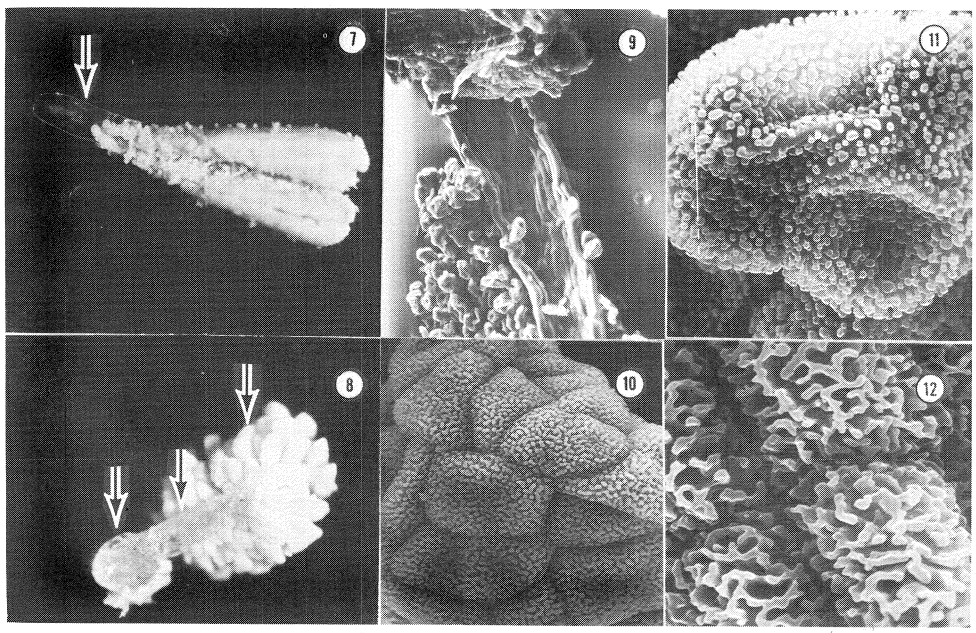
Column and pollinarium morphology of this section are illustrated in figures 2, 5, 7, 9-11.

GENOPLESIUM

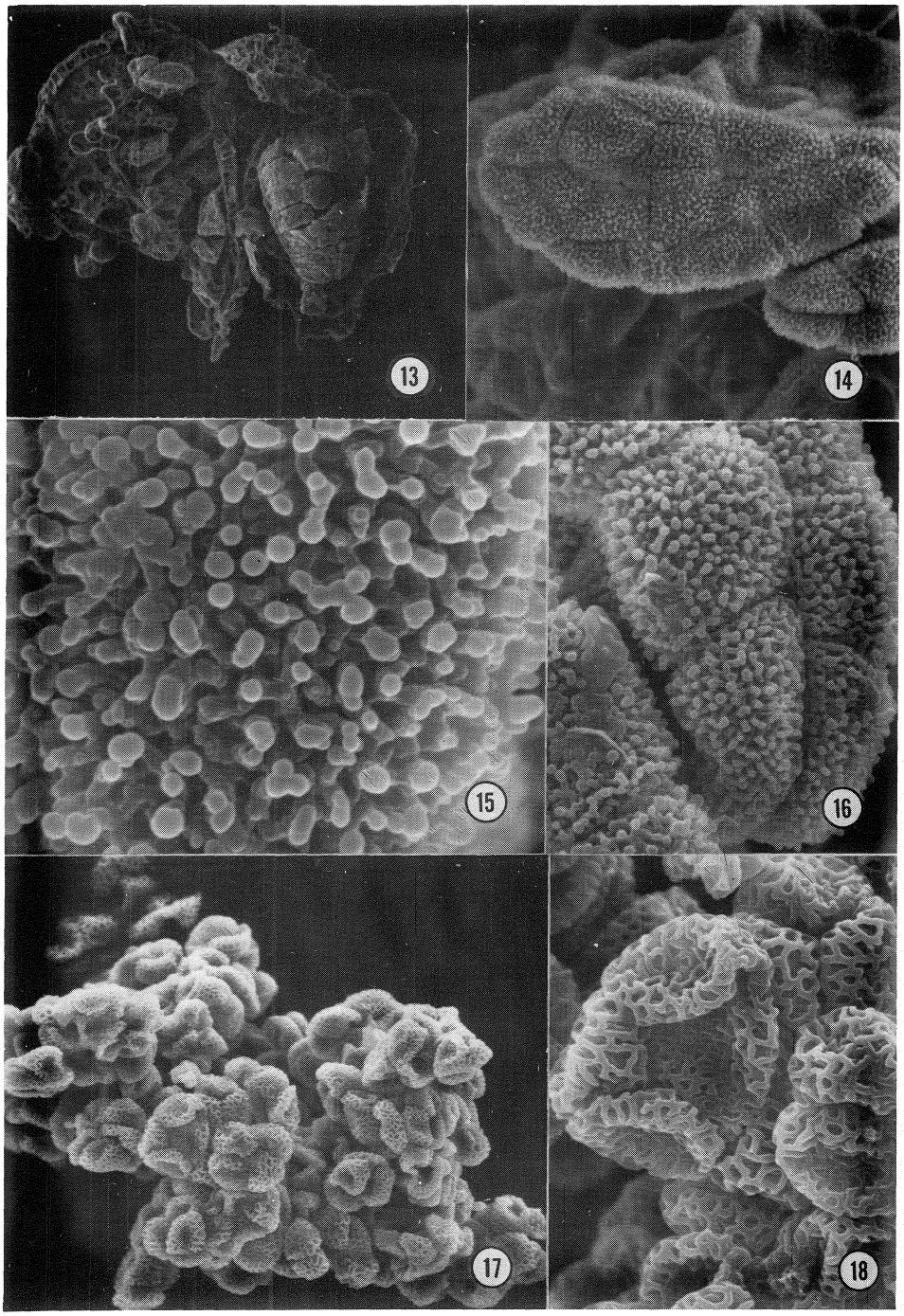
Genoplesium, including *Prasophyllum* sect. *Micranthum* has a column structure differing from *Prasophyllum* sect. *Prasophyllum* in the following ways. The staminodes are usually two-, or rarely, three-lobed with the margins of one or both lobes being entire or ciliate, sometimes extremely so, e.g., *Prasophyllum fimbriatum*. Staminodes are, in most cases, longer than the remainder of the column. The stigmatic surface is small, disclike, and positioned toward the apex of the column. However, the stigma may be elongate and



Figures 1-6. Floral and column morphology of Prasophyllinae and *Microtis*. LS - lateral sepal, LP - lateral petal, O - ovary, DS - dorsal sepal, C - column, A - anther, V - viscidium, S - stigma, ST - staminode. Figs 1, 4. *Genoplesium baueri*. Figures 2, 5. *Prasophyllum* sect. *Prasophyllum*. Figures 3, 6. *Microtis* sp.



Figures 7-12. Pollinarium morphology of *Prasophyllum*. Figure 7. *Prasophyllum* sp. (Clements 2111). Granulate-type. Arrow-viscidium. ×15. Figure 8. *P. morrisii*. (Clement 2316). Top arrow-viscidium, middle arrow-stipe, bottom arrow-massulae. ×35. Figure 9. *Prasophyllum* sp. (Clements 2111). SEM view of stipe. ×100. Figure 10. *P. alpinum*. (Clements 1275). Massula. × 650. Figure 11. *Prasophyllum* sp. (Clements 2111). Tetrad showing clavae. ×1416. Figure 12. *P. fimbriatum*. (Clements 2301). Tetrad showing broken reticulum. ×3245.



Figures 13-18. Pollinarium morphology of *Genoplesium* and *Microtis.* Figure 13. *Genoplesium baueri.* (Clements 2305). Massulae positioned within anther. ×67. Figure 14. *Genoplesium baueri.* (Clements 2305). Massula ×580. Figure 15. *Genoplesium baueri.* (Clements 2305). Exine showing clavae. ×4450. Figure 16. *Genoplesium baueri.* (Clements 2305) Tetrad. ×1425, Figure 17. *Microtis parviflora.* (Bernhardt s.n.). Granulate pollen mass. ×450. Figure 18. *Microtis parviflora.* Tetrads showing reticulate exine. ×1335.

teardrop-shaped in other species, e.g., *P. fimbriatum.* The viscidium is a round, sticky disc and the pollinarium is positioned directly behind or sometimes above the apex of the column. The labellum is attached near the base of the column by a thin but strong claw. The labella of all but one species are mobile, hang loosely, and vibrate with air movements. The labellum margin may be entire, slightly ciliate, or almost tomentose. There are two slightly raised callus plates on most labella but these are somewhat variable in character and appear to be of major significance in only a small number of species. For example, in *Prasophyllum filiforme* Fitzg. the general labellum margin is green while the callus plates are rich red-maroon. This is probably important in attracting pollinators. Most flowers are dark in color, sometimes with a distinctive scent similar to fermenting vegetation. All species are pollinated by small flies (family Chloropidae). The pollinarium attachment is usually on the dorsal thorax.

The structural features of the flower of *Genoplesium* s.s. are, in almost every respect, identical to those of *Prasophyllum* sect. *Micranthum.* However, *Genoplesium* differs in that the staminodes are curved and rarely two-lobed, and the floral bract varies from erose to entire. *Genoplesium* s.s. is also saprophytic, and previous classifications have used the above features (staminodes and floral bract) to delimit it as a monotypic genus. However, in my opinion, the differences are specific rather than generic. Rupp (1949) used the saprophytic habit as a generic character but there are other genera such as *Dipodium* which has four species in Australia, two of which are saprophytic (Warcup, 1981b).

Column and pollinarium morphology of this genus and section are illustrated in figures 1, 4, 8, 12-16.

MICROTIS

Microtis is composed of seven or eight species which occur in New Zealand, New Caledonia, Indonesia, the Philippines, Taiwan, and China. Some of them superficially resemble *Habenaria.*

The floral structure of *Microtis* is quite distinct from that of other Prasophyllinae. The staminodes are simple, single-lobed structures that clasp the apex of the column. The stigma is small and disclike near the apex of the column. It is slightly concave in shape. The viscidium is very small and invariably breaks down leaving the pollinia to fall onto the surface of the stigma so that most species are self-pollinating. The anther loosely covers the pollinia and is positioned at the apex of the column. The rostellum is extremely reduced and is totally removed with the viscidium. The anther is also slightly longer than the stigmatic surface. Unlike *Prasophyllum* and *Genoplesium* the flowers are resupinate. The stylar canal entrance is centrally located on the stigmatic surface. The pollinarium has a reduced stipe, is granulate, composed of tetrads, unlike *Prasophyllum* or *Genoplesium,* and the pollen exine is reticulate. The labellum is sessile in all species with or without callus plates. Occasionally a sweet scent can be detected. Although pollination has been reported by Bates (1981) to be carried out by wasps, Clements has observed only ants as pollen vectors.

Column and pollinarium morphology are illustrated in figures 3, 6, 17-18.

Symbiotic germination trials by Warcup (1981a) and Clements (unpublished) show that while *Prasophyllum* sect. *Prasophyllum* seed will germinate when inoculated with the fungus *Ceratobasidium cornigerum,* species belong-

ing to *Prasophyllum* sect. *Micranthum* or *Genoplesium* do not readily respond to this fungus nor do seeds of *Microtis*. The latter respond to *Sebacina vermifera* or *Tulasnella calospora*.

GENERIC DESCRIPTIONS

Prasophyllum R. Br.
Type: *Prasophyllum australe* R. Br.
Synonym: *Prasophyllum* sect. *Prasophyllum*

Terrestrial herbs with a single erect leaf, 5-200 cm tall, 0.1-2 cm wide with a small green sheath at the base; usually green, sometimes red-purple to almost black. Root-stem tuberoids globular, 2-50 mm diameter (*P. flavum* is semi-saprophytic with a short, brittle rhizome and vestigial tuberoid.) A new tuberoid replaces old ones toward the end of the growing season, sometimes produced at the ends of underground stolons resulting in colonies of the same clone being formed. Simple roots on underground stem above the tuberoids. Inflorescence a spike, originating in the center of the leaf base. Flower stalk growing up inside the leaf-tube before breaking out through a longitudinal slit. Flowers one to several hundred per spike; usually highly colored, often with a sweet scent. The inflorescence may or may not exceed the length of the leaf.

Most species are spring flowering (August to November) but *P. striatum* flowers in autumn and *P. parviflorum* flowers throughout the winter. Several montane species found in swamps flower during summer. The majority of species flower profusely the season after bush fires have swept through the habitat. Most species are thought to be pollinated by species of wasps, and a few are self-pollinating.

Genoplesium R. Br.
Type: *Genoplesium baueri* R. Br.
Synonym: *Prasophyllum* sect. *Micranthum*

Small terrestrial herbs with a single tubular erect leaf 10-30 cm tall, 0.1-0.3 cm wide, gradually tapering to a blunt point in non-flowering plants. In flowering plants, leaf with a free apex, jointed at the base, 7-15 mm long, 1-3 mm wide and subtending the base of the inflorescence. Root-stem tuberoids globular or dichotomously branched at the base, 20-30 mm diameter, replaced each year. Roots few, simple, originating from just above the tuberoid, one growing toward ground surface, usually appressed to the stem and containing the mycorrhizae. Inflorescence a spike, originating from the base of the leaf, growing up inside the center of the leaf-tube. Inflorescence always exceeding the leaf length. Flowers 1-50; labellum mobile, green, brown, red, yellow, often with citrus or fermenting, fruitlike scent.

Most species flower in late summer to autumn (January to April). They are thought to be short-lived plants (5-15 years) and favor heathland environments which are sometimes burnt. Some species are thought to be self-pollinated but the majority of species are pollinated by small flies.

Microtis R. Br.
Type: *Microtis rara* R. Br.

Small terrestrial herbs with a single erect leaf 10-50 cm tall, 0.3-1.5 cm wide, gradually tapering to a point; green or yellow. Root-stem tuberoids globular to ovate, 7-15 mm diameter, replaced every year; new tuberoids also

Character	*Prasophyllum*	(Sect.: *Micranthum*	*Genoplesium*)	*Microtis*
Pollinarium	Elongate-massulate	Elongate-massulate	Elongate, massulate	Short, granulate
Exine	Clavate-reticulate	Clavate, reticulate	Clavate	Reticulate
Rostellum shape	Bladelike, elongate	Short, reduced	Short, reduced	Extremely reduced
Flower	Nonresupinate	Nonresupinate	Nonresupinate	Resupinate
Stylar canal entrance	Above or within the stigma	Above or within the stigma	Above or within the stigma	Within stigma
Staminodes	2-lobed, one longer and smooth	2-lobed and ciliate	2-lobed and curved	One lobe, simple closing
Labellum	Sessile	Mobile	Mobile?	Sessile
Pollinator	Wasp, rarely beetle, rarely flies	Flies	Flies?	Wasps, ants
Position of pollinarium	Top of head and compound eyes	Top of thorax	?	Top of head and compound eyes
Plant habit	Slender to robust	Slender	Slender	Slender to robust
Tuberoid	Round	Round, sometimes lobed	Rhizomatous	Round
Roots	Yes	Yes	No	Yes
Reproduction	Seeds, vegetative	Seeds, rarely vegetative	Seeds	Seeds, vegetative
Flowering time	Spring	Summer-Fall	Fall	Spring-Summer
Fire dependency	Many species	No	No	Many species
Fungal association	*Ceratobasidium* spp.	*Ceratobasidium* spp.	Basidia with clamp connections	*Sebacina* sp., & *Tulasuella* sp.

Table 1. Characteristics of subtribe Prasophyllinae and *Microtis*

produced at the end of stolons, clone forming. Roots few (5-8), simple, originating just above the tuberoids. Inflorescence originating from the base of the leaf, growing up through the center of the leaf and breaking out halfway up through a longitudinal slit in the leaf. Inflorescence a spike which may or may not exceed the length of the leaf. Flowers 3-100, resupinate, rigid; labellum sessile, mostly green, yellow, white, or occasionally red, often with a sweet scent.

Most species flower in late spring to early summer (September to December) and may need a fire from the previous summer to initiate flowering. Pollination is carried out by ants or wasps, but most species appear to be capable of self-pollination and some may be cleistogamous.

CONCLUSIONS

In the Prasophyllinae there are two distinct column morphologies, i.e., the spiranthoid type of *Prasophyllum* s.s., and the neottioid type of *Genoplesium* and *Microtis*. Therefore, the evidence suggests that *Genoplesium* and *Microtis* should be removed from the Prasophyllinae. I am not certain at this time which of the other Diurideae (sensu Dressler) are related to these two genera, and for now their status is uncertain.

ACKNOWLEDGMENTS

I thank Mark Clements for supplying specimens used in this study and also for information concerning the ecology of Prasophyllinae. I also thank B. E. S. Gunning, L. A. Garay, R. L. Dressler, and B. Wallace for their advice during preparation of this manuscript and the Smithsonian Institution for providing time on the scanning electron microscope.

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