# THE ORIGIN AND PHYLOGENETIC POSITION OF *CAREX* SECTION *PHYLLOSTACHYS* IN THE GENUS *CAREX* (CYPERACEAE).

by

Julian Richard Starr

Department of Botany

A thesis submitted as part of the requirements for a Degree of Master of Science at the University of Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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# TABLE OF CONTENTS

## ABSTRACT

*Carex* section *Phyllostachys* is a small, highly reduced section of eight species confined to North America, north of Mexico. Although the section is easily circumscribed, its unusual appearance has led to wide speculation concerning its origin and phylogenetic position in *Carex*. In order to gain a better understanding of phylogeny in this complex genus, anatomical, micromorphological, macromorphological, and molecular DNA characters were used to clarify the phylogenetic position of sect. *Phyllostachys* in *Carex*, and to determine the relationships of its species. The taxonomic utility of anatomical, micromorphological, and molecular characters, for species and sectional circumscriptions, was also assessed.

Phylogenetic reconstructions inferred from sequences of the ITS region of nrDNA indicate that all the subgenera in *Carex*, except for subg. *Vignea*, are artificial. Two main groups are identified: (1) a "compound clade", comprised of subg. *Indocarex*, *Primocarex*, and a portion of subg. *Carex*, and (2) a "reduced clade", consisting of sections *Phyllostachys* (subg. *Carex*), *Filifoliae*, and *Firmiculmes* (subg. *Primocarex*). The most basal groups in the "compound clade" are composed of southeast Asian species from subg. *Indocarex*, supporting theories that the group originated in this region and is primitive within a wider subg. *Carex/Indocarex* lineage. All sections surveyed in this analysis were monophyletic and firmly supported. Difficult circumscriptions, such as the separation of sections *Laxiflorae* and *Careyanae*, were strongly upheld. Although the ITS region was a powerful tool for defining sectional limits and for estimating relationships between the sections of *Carex*, it was not variable enough to fully resolve relationships below the sectional level.

A cladistic analysis using anatomical, morphological, and ITS sequence data suggests that section *Phyllostachys* can be divided into two distinct clades: (1) a "wide-scaled" clade consisting of *Carex backii*, *C. saximontana*, and *C. latebracteata*; and (2) a "narrow-scaled" clade consisting of *C. willdenowii*, *C. superata*, *C. basiantha*, *C.*

*juniperorum*, and *C. jamesii*. Trends in character evolution and flower number appear to be related to breeding systems although this is not supported by previous studies utilizing isozymes. Correlations between phytogeography, glacial movements, and phylogeny suggest that speciation in the section may have been influenced by the events of the Pleistocene. Estimates of divergence times based on the mutation rate of the ITS region in the genus *Dendroseris*, indicate that most of the speciation in the section has occurred within the last 1.7 my. The most basal species in sect. *Phyllostachys* is *C. latebracteata*, a narrow endemic of the Ouachita Mountains of Arkansas and Oklahoma - a known glacial refugium. Similar evolutionary patterns within sections *Phyllostachys*, *Griseae*, and *Laxiflorae* may point to the recent diversification of all three of these taxa under the influence of the Pleistocene. Tree topology challenges the hypothesis that chromosome evolution in *Carex* is only unidirectional and ascending.

Anatomical, micromorphological, and molecular DNA characters support the separation of the close species pair of *C. backii* and *C. saximontana*, and the recognition of three species in the *Carex willdenowii* complex. Species-specific characters also distinguish *C. latebracteata*, *C. juniperorum*, and *C. jamesii*. The low levels of infraspecific variation and species-specific autapomorphies in ITS sequences indicate that this region could be used effectively to help clarify taxonomic problems in critical groups. While groups inferred from micromorphological characters are congruent with those expected from morphological and molecular evidence, anatomical characters conflict with these data sets due to high levels of homoplasy.

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# LIST OF TABLES

## TABLE

# LIST OF FIGURES

FIGURE

# CHAPTER 1

## INTRODUCTION

Comprising nearly 2000 species or almost half of the Cyperaceae L., the genus *Carex* L. represents one of the largest, most cosmopolitan, and ecologically significant genera of flowering plants (Reznicek, 1990). Remarkably, despite its global distribution and ecological importance, evolutionary trends and relationships within this genus have remained almost completely unknown (Reznicek, 1990; Naczi, 1992).

Systematic inquiry into the evolution of *Carex* has always been hindered by the nature of anatomical and morphological characters in the genus. Floral reduction (Smith and Faulkner, 1976), uniform vegetative morphology and anatomy (Metcalf, 1971; Standley, 1990a), and the repeated occurrence of parallelisms and reversals (Reznicek, 1990; Naczi, 1992) has obscured phylogenetic trends and has confused relationships. Most infrageneric groups, especially sections, are largely phenetic and are based on either a single trait or only a few macromorphological characters (Naczi 1992). Extreme reduction and an almost complete lack of knowledge of the ontogeny of *Carex* (Alexeev, 1988), further raises homology problems that complicate phylogenetic reconstruction at all levels. To make matters worse, hybridization and introgression are common in certain sections (Faulkner, 1972; Cayouette and Morisset, 1985; Kukkonen and Toivonen, 1988). All these pitfalls have led to the recognition of many artificial taxa which continue to hinder phylogenetic research in *Carex* by obscuring trends and confusing similarities. This helps explain why only three previous phylogenetic reconstructions have been attempted in the genus (Crins and Ball, 1988; Crins, 1990, Naczi, 1992).

One solution to the problem of poor circumscription is to focus evolutionary studies on a number of small subgroups in the genus that appear to be distinct and well circumscribed (Crins 1990). I chose to explore phylogeny in *Carex* by focusing on the evolution and systematic position of a small (i.e., 8 species), well defined group in the genus, *Carex* section *Phyllostachys* (J. Carey) L.H. Bailey. A number of reasons favour

this approach. First, the small size of the section allows for both interspecific and intraspecific variation to be assessed for a wide variety of different character types. Second, its clear delimitation from other sections and recent resolution of a number of taxonomic problems (Naczi, Reznicek, and Ford, 1997; Ford et al., 1997c; chapters 2 and 4) allows for an interpretation of results that will not be confounded by a poorly resolved taxonomy. Third, the evolutionally reduced and unusual inflorescence morphology of the section has led to wide speculation concerning its origin and systematic position within the genus. By using section *Phyllostachys* as a model, I hope to explore the usefulness of traditional and molecular DNA characters for circumscribing sections and for resolving inter- and intrasectional relationships in the genus.

The main purpose of this thesis is to explore the problems encountered in phylogenetic reconstructions in *Carex*, and to provide guidance to future investigators on the usefulness of both traditional (i.e., anatomy, morphology, and micromorphology) and molecular DNA characters for making taxonomic decisions and inferring phylogenies in *Carex*.

This thesis has been divided into three separate studies (i.e., chapters 2-4) that help to clarify the evolutionary position of section *Phyllostachys* within *Carex*, and to reconstruct the phylogenetic history of its species. Each chapter is preceded by an introduction that presents the problem in greater detail and provides a fuller statement of its objectives. The first study (chapter 2) used anatomical and micromorphological data to clarify species limits within the section, and to investigate the advantages and drawbacks of these characters in future phylogenetic studies in the genus. Conflicts between morphological groups and groups inferred from anatomy and micromorphology have led several authors to conclude that anatomical (Standley, 1987, 1990a) and micromorphological (Rettig, 1986; Waterway, 1990a) characters exhibit high levels of homoplasy and may not be reliable indicators of evolutionary relationships. However, these studies have involved either paraphyletic assemblages (Standley, 1987) or large

groups whose taxonomy is still highly confused (Standley, 1990a; Waterway, 1990a). This makes it difficult to determine whether incongruities between morphological groups and those based on anatomy and micromorphology are due to an unresolved taxonomy or homoplasy in characters. The small size of *Carex* section *Phyllostachys*, and its clear demarcation from other Carices, offers a unique opportunity to evaluate the potential and problems of these characters within the context of a putatively monophyletic group. By comparison to trends in the morphology of sect. *Phyllostachys* and to the results of other studies, this chapter investigates how these characters can be used in future phylogenetic studies (see chapter 4).

In the second study (chapter 3), sequences from the internal transcribed spacer region of nuclear ribosomal DNA (i.e., the ITS region) were used to clarify the phylogenetic position of section *Phyllostachys* in the genus *Carex*. Because of morphological and floral reduction, and a generally uniform vegetative morphology and anatomy, few traditional characters are available for resolving phylogenetic relationships in *Carex* (Reznicek, 1990). The relationships of the most evolutionally reduced sections in the genus, like section *Phyllostachys*, have been particularly challenging. Because of their unconventional morphology, these reduced sections have become intimately involved in the controversies surrounding the phylogeny and circumscription of the genus, and its subgeneric taxa. Section *Phyllostachys* alone has been allied with sections representative of three of the four subgenera recognized by Kükenthal (1909). Molecular DNA characters offer an obvious source of variation whose use has yet to be explored in *Carex*. The advantages pertaining to these characters in homology assessment, homoplasy, the scoring of character states, and finally even character numbers (Sytsma, 1990), all suggest that DNA characters could clarify the phylogenetic position of section *Phyllostachys* in *Carex*, where traditional characters have failed.

In the third and final study (chapter 4), anatomical, micromorphological, and molecular characters have been combined with a morphological data set to produce a

robust phylogenetic hypothesis for interpreting phylogeny within section *Phyllostachys*. The phylogenetic hypothesis was used to trace evolutionary trends in the section, and to interpret branching patterns within the context of historical and biological processes. The utility of molecular characters for uncovering subtle taxonomic variation within section *Phyllostachys* was also assessed by comparison to anatomical, morphological, and micromorphological results. The theory that chromosome evolution within *Carex* is unidirectional and ascending (Davies, 1956; Whitkus, 1987) was tested by superimposing chromosome counts upon the phylogeny of the section.

# CHAPTER 2

## SYSTEMATIC IMPLICATIONS OF ANATOMICAL AND MICROMORPHOLOGICAL VARIATION IN *CAREX* SECTION *PHYLLOSTACHYS* (CYPERACEAE)

*Carex* L. section *Phyllostachys* (J. Carey) L.H. Bailey is a small section of eight species that is found throughout temperate North America, but it is most diverse in the southern and eastern United States. Morphologically, the section is easily distinguished from other Carices: its foliaceous pistillate scales, few-flowered androgynous spikes, and apically winged culms (Catling, Reznicek, and Crins, 1993) make sect. *Phyllostachys* one of the most distinctive groups in the genus.

Despite the well marked nature of the section, the characters used to define species are relatively few, resulting in some debate over specific limits. Since Willdenow (1805) described *C. willdenowii*, the taxonomy of this distinctive sedge from the eastern deciduous forest has been largely without controversy. Recent studies of morphology (Naczi, Reznicek, and Ford, 1997) and allozyme divergence (Ford et al., 1997c) within *C. willdenowii* s.l. have suggested, however, that this taxon is a complex of three species (i.e., *C. willdenowii* var. *willdenowii*, *C. willdenowii* var. *megarrhyncha* F. J. Hermann = *C. superata* Naczi, Reznicek, and Ford, and *C. basiantha* Steud.). The morphological distinctions between these three species are few, which explains why most previous authors had treated *C. basiantha* as a synonym of *C. willdenowii* (Kükenthal, 1909; Mackenzie, 1935), and why Hermann (1954) had felt *C. superata* was best treated as a variety of *C. willdenowii* (i.e., *C. willdenowii* var. *megarrhyncha* F. J. Hermann).

The close species pair of *C. saximontana* Mackenzie and *C. backii* Boott is another example of taxonomic ambiguity in the section. Subtle distinctions in the length of the anthers and perigynium beak are the characters most often used to segregate these two taxa (Catling, Reznicek, and Crins, 1993). These seemingly minor differences have

convinced several authors that *C. saximontana* should be treated as a variety of *C. backii* (e.g., Hudson, 1977; Scoggan, 1978; Boivin, 1992), or that the two taxa should be merged (e.g., Moss, 1983; Gleason and Cronquist, 1991).

The last three taxa in the section (*C. jamesii* Schw., *C. juniperorum* Catling, Reznicek, and Crins, and *C. latebracteata* Waterfall), do not present any immediate taxonomic problems, however, it should be noted that a considerable amount of genetic and morphological variability has been observed in *C. jamesii* (Ford et al., 1997b; Ford et al., unpublished data).

Anatomical and micromorphological characters are particularly useful in situations where species limits are not clearly distinguished by morphological characters (Le Cohu, 1970; Shepherd, 1976; Wujek and Menapace, 1986). They have also been used widely to assess phenetic relationships in *Carex* (Standley, 1987, 1990a; Rettig, 1990; Menapace and Wujek, 1987; Menapace, Wujek, and Reznicek, 1986), although it is unclear whether they are appropriate for phylogenetic studies. For example, conflicts with morphological data sets have led several authors to conclude that anatomical (Standley, 1987, 1990a) and micromorphological (Rettig, 1986; Waterway, 1990a) characters exhibit high levels of homoplasy and may not be reliable indicators of evolutionary relationships. However, several of these studies have either worked on only a small portion of a much larger group (Standley, 1987) or upon large groups whose taxonomy is still highly confused (Waterway, 1990a). This makes it difficult to determine whether incongruities with morphology are due to the inaccuracies of traditional classifications or the unreliability of characters (Standley, 1990a; Waterway, 1990a). The small size of *Carex* section *Phyllostachys* and its clear demarcation from other Carices offers an unique opportunity to evaluate these characters within the context of a putatively monophyletic section.

This chapter presents a comparative study of the anatomy of the leaves and culms of all the species presently recognized in *Carex* section *Phyllostachys*. Variation in the

micromorphology of the silica bodies present in the epidermal layers of the achenes was also explored. The purpose of this study is to clarify species limits within section *Phyllostachys*, and to investigate the problems and potential of anatomical and micromorphological characters to future phylogenetic studies in *Carex*.

## 2.1 MATERIALS AND METHODS

### *Anatomical Studies*

Anatomical studies were based upon leaf and culm samples taken from herbarium and live specimens representative of all species in sections *Phyllostachys*. For live specimens, the 3rd or 4th leaf from the base of the pseudoculm was removed, along with any culms, and placed in FAA. Dry material was boiled in water for five minutes and then placed in FAA (Radford et al., 1974). Segments 4-5 cm long were removed from the median portion of preserved leaves for both epidermal and cross-sectional study. Culm segments 2-4 cm long were removed from just below the swelling point of the apical portion of the stem. This was necessary due to the particularly short culms of some species.

Leaves and culms were hand sectioned with a razor blade. Sections were dehydrated in an ethanol series and stained while being heated for 3.5 mins with 2% Toluidine Blue "O" in 100% ethanol. Sections were made permanent by passing them through a 100% ETOH-Histoclear® dehydration series and mounting them in Permount. To increase sample size, cross-sections of live material mounted in water were also made. Leaf epidermal surfaces were prepared by placing FAA preserved material into a glass petri dish containing household bleach and scraping the undesired tissue away with a razor blade. The epidermal surfaces were then passed through three 15 minute water baths and stained for 3 mins with 2% safranin dissolved in water. The epidermal surfaces were then dehydrated, cleared, and mounted as above. Drawings of cross-sections were made with the aid of a camera lucida. The terminology used to describe the anatomy of the leaf and culm follows that of Metcalf (1971). Specimens used in leaf and culm studies are listed in TABLES 1-3.

*Scanning Electron Microscopy*

The lowermost mature perigynium was removed from each of two terminal spikes from three to six individual herbarium specimens representative of the geographic range of each species (see TABLE 4 for vouchers). This was done in order to assess variation within, and between, individuals and species. The perigynium surrounding the achene was dissected away and the achenes were acetolyzed in a 1:9, sulfuric acid: acetic anhydride solution (Tallent and Wujek, 1983). This procedure removed the cuticle and most of the outer periclinal walls, and exposed the silica bodies within the epidermal cells. Achenes were shaken vigorously for 5 min and then left from 24-48 hrs in solution. At the end of this period, achenes were shaken for 5 min, removed, and then washed in distilled water by shaking for a further 5 min. Those achenes whose walls were still persistent after undergoing the above procedure, were sonicated in distilled water at maximum probe intensity with a BIOSONIC ® sonicator (Bronwill Scientific, Rochester, NY) for 1.5 min. The achenes were then dried in an oven overnight at 50° C and mounted onto aluminum stubs with conductive carbon paint (SPI® Supplies). Achenes were coated with 100-200 nm of a gold-palladium alloy in an Edwards Sputter Coater S150B and photographed using a Steroscan 120 SEM (Cambridge Instruments; 20 kV accelerating voltage) connected to a Kontron Elektronik IBAS Image Analyzer. Micrographs were taken along the median portion of the achenes and at an angle appropriate for revealing the topography of the silica bodies in the epidermal cells. Micrographs taken from directly overhead failed to reveal all the features characteristic of each silica body. The terminology employed by Schuyler (1971) was used in the description of silica bodies.

## 2.2 RESULTS

### *Silica Body Morphology*

The overall morphology of the silica bodies in section *Phyllostachys* was very consistent. All the members of the section possessed a single acute, conical central body which arose from the middle of either a convex or concave silica platform. The central body was always smooth, and with the exception of one specimen of *C. basiantha*, it was always mucronate. The epidermal cells of all species had linear cell walls, they were angular, commonly 6-sided, and often isodiametric (i.e., hexagonal); however, irregular cells, and cells with 5 or 7 sides were not uncommon. Differences in the persistence of the periclinal and anticlinal walls were noticed during the digestive process, however, these characters were largely ignored since they often appeared to be related to the age of the achenes (cf. Menapace, Wujek, and Reznicek, 1986). No micromorphological variation was observed within individuals.

The most significant differences between species in the section were seen in the shape and relief of the silica platform. Based on these characters three phenetic species pairs could be recognized; (1) *C. backii* and *C. saximontana* (figs. 1a and 1b); (2) *C. juniperorum* and *C. jamesii* (figs. 1c and 1d); and (3) *C. basiantha* and *C. superata* (figs. 2a and 2c). *Carex willdenowii* and *C. latebracteata* had very distinctive silica bodies and were not easily associated with other species.

The closeness observed in the morphology of *C. backii* and *C. saximontana* was mirrored in the micromorphology of their silica bodies (figs. 1a and 1b). Both species uniquely shared concave silica platforms whose margins were distinctly appressed to the platforms of adjacent cells. The differences seen between these two species were minimal and were mostly considered to be differences in degree as opposed to differences in character states. The central body was generally more robust in *C. saximontana* than it was in *C. backii* and the depth of the silica platform in *C. saximontana* tended to vary over its species range. Individual achenes of *C. saximontana* sampled from British

Columbia (Calder 10729 & Savile DAO) possessed considerably deeper silica platforms than those sampled from Minnesota (Moore 19653 & Huff DAO) and North Dakota (Stevens s.n. DAO; fig. 1b). This was in contrast to *C. backii* where specimens examined from the most easterly distribution of this species in New Brunswick (Dore 45.198 & Gorham DAO) showed no significant differences in structure or size from specimens examined from British Columbia (Calder 17022 et al. DAO; fig. 1a).

The second species pair, *C. juniperorum* and *C. jamesii*, was characterized by tall, acute to abruptly acuminate central bodies that were surrounded by a trough created by a thickened ridge at the margin (figs. 1c and 1d). The platforms of these species were tightly appressed and the silica body was often arched (most pronounced in *C. jamesii.*). As with *C. backii* and *C. saximontana*, one species (*C. juniperorum*) was very consistent over its entire range while the other (*C. jamesii*) was variable. Variation in *C. jamesii* was notable in the degree to which the ridge and trough of the silica platform were developed. The ridge could vary from a distinct nodular embankment (fig. 1d) to a low, poorly developed lip (figs. 1e and 1f). Naturally, the degree to which the ridge was developed had a direct effect on the depth of the trough, with the most developed ridges being associated with the deepest troughs. Despite this variability, all specimens possessed an acute central body with a thickened border that formed a trough around the central cone. Differences were of degree, not in character states. The silica bodies of *Carex juniperorum* were representative of a part of the variability seen in the micromorphology of *C. jamesii* (cf. figs. 1c and 1d). The complete range of the variability in *C. jamesii* is given in figs. 1d, 1e, and 1f.

The last recognizable species pair was seen in *C. basiantha* and *C. superata.* Both of these species shared distinctly raised silica platforms with small conical central bodies. Unlike the silica bodies of rest of the section, the silica platforms of these two species were ornamented. This was particularly evident in *C. basiantha* where the surface of the silica platform was clearly raised, and with the exception of the central body, ruminate in

texture (fig. 2a). In contrast, only small, weakly rugulose or bulbate areas were seen at the periphery of the platform of *C. superata*. Although the degree of convexity of the platform in *C. superata* was similar to that in *C. basiantha*, these two species differed in that the main portion of the platform of *C. basiantha* forms a plateau while that of *C. superata* continually rises until it reaches the base of the central body. Both members of this species pair showed some infraspecific variation. Low, poorly developed nodules, such as those found in a single specimen of *C. jamesii*, were observed on one specimen of *Carex superata* from Tennessee (Kral 42454b. MICH; fig. 2d). One specimen of *C. superata* from Mississippi (Naczi 4013 et al. WIN; Appendix 1) had silica bodies with textured platforms like those in *C. basiantha*, but this feature was confined to just a few epidermal cells. A similar level of infraspecific variation was seen in *C. basiantha*. One specimen from South Carolina (Hill 19477 MICH) possessed all the traits characteristic of this species but had rounded as opposed to acute central bodies. The rugulose portion of the central plateau in *Carex basiantha* also showed some variation in the area it covered. Nonetheless, the main characters of these two species were consistent enough that any one specimen observed could be identified and easily distinguished from any other species in the section.

*Carex willdenowii* had very distinctive silica bodies, although similarities with *C. basiantha* and *C. superata* were evident. Like the latter two species, the silica bodies of *C. willdenowii* were distinctly convex, their central bodies were acute, and they were typically mucronate (fig. 2e). However, the silica bodies of *C. willdenowii* were bordered by numerous conical satellite bodies (5 to 10; fig. 2 e). These satellites were similar in morphology to the central body of the silica platform and they were directed away at an angle from the main axis of the cell. The occurrence of these satellites was remarkably consistent over the entire range of the species and it made these silica bodies the most distinctive in the section.

*Carex latebracteata* had a unique silica body morphology and it was not easily aligned with other members of the section (fig. 2f). The silica body in *C. latebracteata* possessed a convex platform with margins that were not appressed to the platforms of adjacent cells. The very large and lightly sloping central body nearly occupied the entire surface of the silica platform. In general, silica bodies in *C. latebracteata* were more regular in size and shape than those in the rest of the section. Unlike other species of section *Phyllostachys*, the outer periclinal walls in *C. latebracteata* were very long and persistent as compared with the cell walls of other species; even after 48 hours in the acetolyzing solution and sonication for three or more minutes, the majority of the individuals sampled still retained their cell walls. The persistence of the cell walls did not appear to be related to the age of the achene. Most of the characters mentioned above were unique to this species and thus it was difficult to place it alongside one of the above mentioned species groups. The lack of any ornamentation at the margin of the platform suggested that this species may best be placed alongside *C. backii* and *C. saximontana*, however, the possession of a convex silica platform suggested that an affiliation with *C. jamesii* and *C. juniperorum*, or the *C. willdenowii* complex may be more appropriate.

***Leaves***

The anatomy of the leaves in *Carex* section *Phyllostachys* was fairly uniform and corresponded to a common pattern seen in *Carex*. The leaf was dorsiventrally arranged, keeled on the abaxial surface, and possessed a single row of vascular bundles. These bundles were positioned closest to the abaxial surface, and were often connected to the epidermal surfaces by sclerenchymatous girders of varying size and shape. The largest vascular bundles were commonly found at the keel and at the two thickest points on either side of the lamina. The median vascular bundle rested up against a single layer of bulliform cells on the adaxial surface, and like the other bundles, it was surrounded by an inner sclerenchymatous and an outer parenchymatous sheath. The vascular bundles were

separated by well-developed air cavities which were square or rectangular in outline, and were formed by the breakdown of large, thin-walled cells at the centre of the chlorenchyma. The adaxial cells of the epidermis were consistently larger than the abaxial cells, and both epidermal surfaces often possessed papillae and/or prickle hairs. Conical silica bodies were commonly present in the epidermal cells overlying the sclerenchyma of the keel, margins, and girders. The number of cells occupied by silica bodies was directly correlated with the degree of sclerification.

Based on foliar anatomy, two broad groups were distinguished in section *Phyllostachys*: The first group, comprised of *C. latebracteata* and *C. saximontana* was distinguished by a lightly revolute or curved lamina, whose thickest point was equal to or less than the thickness of the keel (figs. 3A and 3B). The keel was prominent and acute, with a large, highly sclerified, horizontal to lightly descending abaxial girder that connected the median vascular bundle to the epidermis. Leaf margins were completely sclerified and the bulliform cells were no larger than the largest cells of the epidermis.

All other taxa in the section possessed V-shaped leaves where the thickest portion of the lamina lies lateral to the median vascular bundle (figs. 4 and 5). The keel was rounded or flat, and the leaves were often lightly flanged or occasionally curled on one half of the lamina. A small sclerenchyma strand on the adaxial surface of the margin was also characteristic of these species, although *C. juniperorum* frequently possessed a completely sclerified margin similar to *C. latebracteata* and *C. saximontana*. Two further characters, the presence of minor vascular bundles above the air cavities and the interruption of adaxial girders by parenchymatous cells, were frequent in this group but rare in *C. latebracteata* and *C. saximontana*. Both these characters appeared to be related to the thickness of the leaf.

***Leaf Epidermis***

The epidermal surfaces of all species were characterized by distinct bulliform cells found on the adaxial surface of the keel and by the presence of many large rectangular cells which dominated the intercostal regions of the epidermis (fig. 6). The anticlinal cell walls of all the species examined were highly sinuous, with the apex of the bends being thickened and often nodular in appearance. These characters were particularly pronounced in the cells between and immediately adjacent to the stomata. Within species, some variability was noted in the lengths of these intercostal cells with some being completely square while others were very elongate and rectangular. Only one species, *C. latebracteata*, consistently displayed more square than rectangular cells. No obvious differences in the lengths or widths of the intercostal cells were noted between surfaces.

In all species, 1 - 3 longitudinal rows of small, square cells were found overlying the sclerenchyma of the vascular bundles (fig. 6B). Within these cells, 1-3 (4) (normally 2) conical silica bodies were typically found resting in a single row along the inner periclinal wall. The only exception to this was in *C. willdenowii*, where as many as 6 silica bodies were seen arranged in 2 rows per cell on the abaxial surface. As was seen in epidermal cells of the achene, the silica bodies of this species often had satellites.

Square cells containing silica bodies were also observed near the margin of the leaf. On the adaxial surface of all species, these cells were usually found in three discontinuous rows (five in *C. latebracteata*) and often contained fewer silica bodies (1 or 2) than cells found over the vascular bundles.

The stomata were found in numerous longitudinal rows in the intercostal region of the abaxial surfaces of the leaves (hypostomaty; fig. 6B). The stomata in these rows were separated by one to several intercostal cells. This often gave the impression they were scattered randomly across the surface. They were typically paracytic, and with the exception of two species (*C. jamesii* and *C. latebracteata;* orbicular to suborbicular) they

were elliptical in outline. In all of the species examined, the intercostal cells adjacent to the stomata appeared to have grown slightly over the subsidiary cells. In some highly papillate individuals of *C. saximontana* (see below), the papillae tended to arch over the stomata (fig. 6B).

The most distinct differences between taxa were seen in the presence or absence of papillae and prickle hairs at the margin and on the surfaces of the leaf. All prickle hairs (fig. 6A) pointed in a retrorse direction and were generally more consistent in their appearance than were papillae. At the margin, well-developed prickle hairs were seen in varying density in all species, except for *C. latebracteata*. The margin of this species was characterized by a tightly crenulate border of low papillae. Three species, *C. jamesii*, *C. basiantha*, and *C. superata* displayed spines at the margin but no papillae.

On the surfaces of the leaf, papillae and prickle hairs were also variable in their consistency and numbers. When they were present, they were best developed on and close to the bulliform cells at the keel, and around the small square-like epidermal cells overlying the sclerenchyma strands (fig. 6A). Three taxa (*C. saximontana*, *C. latebracteata*, *C. willdenowii*) consistently displayed papillae on the adaxial surfaces of their leaves. Papillae were also seen adaxially in *C. juniperorum* and *C. superata*, but not in all individuals. On the abaxial surface, papillae were seen in all samples of *C. willdenowii*, *C. saximontana*, and *C. juniperorum*. Three species, *C. willdenowii*, *C. juniperorum* and *C. basiantha* possessed prickle hairs on their adaxial surfaces. *Carex backii* and *Carex jamesii* were the only two species in the section to show neither papillae, nor prickle hairs on their surfaces.

### *Culm Anatomy*

Culms of all species contained 4 to 10 major, and 1 to 8 minor vascular bundles (figs. 7-10). These were arranged alternately by size and in a circular or triangular fashion within a thin peripheral, chlorenchymatous zone adjacent to the epidermis. With the

exception of *C. latebracteata*, where minor bundles were completely contained within the chlorenchyma tissue (fig. 7), the xylem pole of both major and minor bundles rested against a spongy translucent ground tissue at the centre. Alternating between major and minor bundles were air chambers of various sizes and shapes. In *C. superata*, these chambers were confined to the chlorenchyma of the culm angles due to the highly sclerified nature of its culm (fig. 7)

The culms in sect. *Phyllostachys* were all triangular in shape except for those of *C. juniperorum*. The culm in this species was commonly asymmetrical, and possessed from three to five angles or wings (fig. 8A and 8B). Like *C. basiantha*, it was also unusual in that sclerification of the wings was typically weak or absent; the rest of the section possessed wings that were strongly sclerified. Culm sides in the section varied from convex to concave.

The culms of *C. saximontana*, *C. backii*, and *C. juniperorum* were distinguished by the possession of epidermal papillae (figs. 8 and 9). In *C. backii* and *C. juniperorum*, these papillae were confined to the apices of the wings, however, in *C. saximontana* they covered the entire surface. The papillae on either side of the stomata of *C. saximontana* tended to arch over the guard cells to form an epistomatal cavity as seen in their leaves. As seen in the leaves of the section, the epidermal cells overlying the sclerenchyma of girders and wings frequently possessed small conical silica bodies.

## 2.3 Discussion

### *Taxonomic Trends*

The anatomical and micromorphological data, along with known morphological differences supports the recognition of eight species in *Carex* section *Phyllostachys*. In particular, strong evidence is provided for the separation of the critical species pair of *C. backii* and *C. saximontana*, and for the recognition of three species within the *C. willdenowii* complex.

Leaf and culm anatomy provide strong evidence for the separation of *C. saximontana* from *C. backii*. The completely sclerified margins, thin revolute leaf type, and distribution of papillae on both surfaces of the leaf and along the entire surface of the culm, are all characters that distinguish *C. saximontana* from *C. backii*. In fact, leaf characteristics such as completely sclerified margins, glaucous induments, papillate adaxial surfaces, and revolute leaf shapes, suggest that *C. saximontana* is more closely related to *C. latebracteata* than it is to *C. backii*.

Anatomical and micromorphological evidence also strongly supports the evidence presented by Naczi, Reznicek, and Ford (1997) and Ford et al. (1997c) that *C. willdenowii* s.l. is a complex of three distinct species (i.e., *C. willdenowii* s.s., *C. basiantha*, and *C. superata*). This complex clearly illustrates how useful epidermal (e.g., Le Cohu, 1970) and micromorphological characters (Wujek and Menapace, 1986) can be for clarifying species limits within critical groups. The three taxa recognized by Naczi, Reznicek, and Ford (1997) differ markedly in the distribution of papillae and prickle hairs on their epidermal surfaces. *Carex willdenowii* s.s. has papillae on both its surfaces and prickle hairs on the adaxial surface; *C. basiantha* has only prickle hairs on its adaxial surface; and *C. superata* does not possess prickle hairs and only rarely has papillae on its adaxial surface. The micromorphology of the silica bodies in achene epidermal cells also strongly supports the recognition of three species. The silica platform of *C. basiantha* is roughened while that of *C. superata* is smooth, and neither of these two taxa possesses

the distinctive satellite bodies of *C. willdenowii* s.s. Culm characters also support the recognition of three species in the *C. willdenowii* complex. The culms of *C. basiantha* are generally weakly sclerified, whereas those of *C. superata* are highly sclerified. This may explain why the long culms of *C. basiantha* tend to be lax and droop, while the short culms of *C. superata* tend to be stiff and erect (see Naczi, Reznicek, and Ford, 1997).

The other three species in the section (*C. juniperorum*, *C. jamesii*, and *C. latebracteata*) are all morphologically distinct and no taxonomic problems surround their circumscription. However, some important anatomical characters are worth noting in *C. juniperorum* and *C. latebracteata*. The asymmetrical, weakly sclerified, three- to five-winged culm of *C. juniperorum* is highly unusual and possibly unique in *Carex*. The unique shape of this extremely short culm is probably a result of the pressure exerted by its tight investment in the leaf sheaths of the pseudoculm. However, this character is not evident in the short culms of *C. superata* indicating that it may be genetically controlled. The culm of *C. latebracteata* is unique in possessing its minor vascular bundles completely within the chlorenchyma tissue, and in having a tightly crenulate leaf margin comprised of low papillae. In contrast to the above named species, *C. jamesii* possesses a very nondescript leaf and culm, and does not have any anatomical characters that are unique to it alone. It was, however, the only species to show considerable variation in its micromorphology, which is consistent with the high levels of genetic variability observed within the species as a whole (Ford et al., 1997b).

### *Phylogenetic Implications*

Cladistic analysis of morphological and molecular data (chapter 4) suggests that *Carex* section *Phyllostachys* can be divided into two main groups. The wide-scaled group, comprised of *C. backii*, *C. latebracteata*, and *C. saximontana* is characterized by pistillate scales that conceal the perigynia and stigmas that are short and thickened. The narrow-scaled group is distinguished by pistillate scales that are narrower than the

perigynia and by stigmas that are retrorse and filiform. Within the wide-scaled group, *C. backii* and *C. saximontana* appear to form a species pair owing to their erect, thickened stigmas, and highly reduced staminate spikes. In the narrow-scaled group the reddish basal sheaths, truncate staminate scales, and constricted beak bases of *C. juniperorum* and *C. jamesii* suggest that these two species are closely related. Within the *C. willdenowii* complex, *C. basiantha* and *C. superata* appear to form a group based on similar achene widths and lengths, and on similar numbers of perigynia and leaves (Naczi, Reznicek, and Ford, 1997). The micromorphological data are entirely consistent with these hypothetical relationships. All of the species pairs suggested by silica body characters also correspond to obvious morphological groups, and although not entirely clear, a relationship between all three members of the *C. willdenowii* complex appears to be supported by the presence of convex silica platforms in these species.

Although the congruence between micromorphological and morphological data sets suggests that silica body ornamentation is a good indicator of evolutionary relationships in sect. *Phyllostachys*, controversy surrounds the usefulness of micromorphology for inferring phylogenetic relationships in *Carex* as a whole. While many studies have shown that these characters can be used to circumscribe sections (e.g., Walter, 1975; Menapace and Wujek, 1987, Toivonen and Timonen, 1976; Menapace, Wujek, and Reznicek, 1986), and species (Wujek and Menapace, 1986), others have found no differences between closely related taxa (Walter, 1975; Menapace, Wujek, and Reznicek, 1986) and marked intraspecific variation (Rettig, 1990; Salo, Pykälä, and Toivonen, 1994). Several studies have now noted similarities in micromorphology between distantly related sections in *Carex*, and even between *Carex* and other cyperaceous genera (Salo, Pykälä, and Toivonen, 1994; Waterway, 1990a; Rettig, 1986). The silica bodies characteristic of *C. backii* (fig. 1a) are seen in several species of sect. *Hymenochlaenae* (see fig. 4, *C. castanea*; fig. 9, *C. sylvatica*; and fig. 24, *C. gracillima* of Waterway, 1990a), a species from sect. *Pseudo-Cypereae* (see fig. 3, *C. comosa*; Walter,

1975), an Asian member of sect. *Confertiflorae* Franch. (see fig. 8b, *C. dispalata*; Hoshino, 1984), and even in *C. paniculata* L., a species from subgenus *Vignea* (see fig. 5, Le Cohu, 1973). The silica bodies present in *C. latebracteata* are similar in morphology to those of *Scirpus clintonii* (fig. 60; Tucker and Miller, 1990), and the micromorphology of *Eriophorum comosum* (fig. 52; Tucker and Miller, 1990) approaches that of *C. jamesii* and *C. juniperorum* (figs. 1c and 1d). Trends such as those outlined above have prompted some authors to suggest that homoplasy is widespread and that silica body characters should be used with caution when assessing evolutionary relationships (Rettig, 1986; Waterway, 1990a). Most studies, however, have found a correlation between morphological similarity and silica body variation. For example, the marked intraspecific variation and wide overlap in characters witnessed in the micromorphology of the *C. flava* complex (sect. *Ceratocystis* Dumortier) is also seen in its morphology and anatomy (Salo, Pykälä, and Toivonen, 1994). Species grouped on the basis of their micromorphology also tend to correspond to natural groupings on the basis of morphology (e.g., Walter, 1975; Menapace, Wujek, and Reznicek, 1986), or other types of data such as flavonoids (Toivonen and Timonen, 1976). Within section *Phyllostachys*, all three species pairs identified by micromorphological characters, and the placement of *C. willdenowii* alongside *C. basiantha* and *C. superata*, are consistent with the types of relationships that would be expected on the basis of comparative morphology (see Waterfall, 1954; Catling, Reznicek, and Crins, 1993; Naczi, Reznicek, and Ford, 1997). This suggest that micromorphological characters are good indicators of relationship, but only in tight monophyletic groups composed of closely related species (Standley, 1990a).

Despite the usefulness of micromorphology in elucidating close species relationships, the following problems exist when using silica body characters in cladistic analyses: (1) character states are often subtle and difficult to categorize; (2) the homology of structures is unknown, and (3) the presence of similar silica bodies in distantly related taxa does suggests that character state changes may be limited and that homoplasy could

pose a problems for polarizing character states. Despite these limitations, micromorphological characters can be used for circumscribing species in critical groups (e.g., *C. willdenowii* complex, see above), and for helping to resolve sectional placements. Given the difficulties of using micromorphological characters in cladistic analyses, however, they are best used as external data sets for assessing the robustness of phylogenies produced using more conserved characters.

Although micromorphological characters suggest groups that are consistent with those expected from comparative morphology, groupings based on anatomical data sets (i.e., leaf, culm, and leaf epidermis characters) conflict with each other and with those groups inferred from morphology and micromorphology. This would appear to indicate that homoplasy is high in anatomical characters; a finding which is consistent with the results of other studies (e.g., Akiyama, 1942; Standley, 1987, 1990a). The anatomical differences between the species of sect. *Phyllostachys* are few and are mostly confined to the presence and absence of prickle hairs and papillae. If we consider papillae and prickle hairs to be derived characters as suggested by Standley (1990a), the disparate groups we obtain clearly illustrate the homoplastic nature of anatomical characters. The presence of papillae on the adaxial surface of the leaves of *C. saximontana*, *C. latebracteata*, and *C. willdenowii* might suggest that these three taxa should form a group. However, only *C. saximontana* and *C. willdenowii* have papillae on both leaf surfaces, and *C. willdenowii* shares prickle hairs on its adaxial surface with two completely different taxa, *C. juniperorum* and *C. basiantha*. The presence of papillae on the culms of *C. saximontana*, *C. backii*, and *C. juniperorum*, suggests yet another group, as do other characters, such as the size of the first cell layer of the chlorenchyma (*C. saximontana*, *C. backii*, *C. latebracteata*). Qualitative leaf characters such as the wide, glaucous, revolute leaf with completely sclerified margins of *C. latebracteata* and *C. saximontana* would suggest that these two species are more closely related to each other than to any other species in sect. *Phyllostachys*. However, morphological characters

(Mackenzie, 1906), ITS sequence data (chapters 3 and 4), and isozyme analyses (Ford et al., 1997a; Ford et al., 1997b) strongly suggest that *C. saximontana* is closest to *C. backii*.

Although anatomical characters have been of enormous value in distinguishing morphologically difficult species pairs (e.g., *C. rostrata* and *C. utriculata*; Le Cohu, 1970; *C. panicea* and *C. vaginata*, Shepherd, 1976; *C. rotundata* and *C. membranacea*, Ford and Ball, 1992), distinct qualitative characters are few and they tend to exhibit highly levels of homoplasy (Standley, 1990a). This makes anatomical characters difficult to use in phylogenetic studies and questions their ability to accurately reflect phylogenetic relationships. Shepherd (1976) and Standley (1990a) have both found that although some relationship between anatomical characters and present morphological classifications appears to exist, when anatomical characters are used alone, they produce groups that are clearly unnatural. Some of these incongruities between morphological and anatomical trends can be explained by circumscriptional problems; but if this were the only reason, we would not expect to see the same types of inconsistencies in a small clearly marked section like the *Phyllostachys*, as we see in a large poorly defined group like section *Phacocystis* (Standley, 1987, 1990a). My data suggest that anatomical characters are effective indicators of species limits, but owing to a lack of qualitative characters and high levels of homoplasy, their role in future phylogenetic studies in *Carex* is limited.

## 2.4 CONCLUSIONS

Anatomical and micromorphological characters support the recognition of eight species within *Carex* section *Phyllostachys*. Anatomical characters clearly separate the close species pair of *C. saximontana* and *C. backii*. Micromorphological and anatomical characters support the recognition of three species within the *C. willdenowii* complex (see Naczi, Reznicek, and Ford, 1997; Ford et al., 1997c). Three species pairs have been identified by micromorphological characters: *C. backii* and *C. saximontana*, *C. juniperorum* and *C. jamesii*, and *C. basiantha* and *C. superata*. The phylogenetic affinities of *C. latebracteata* and *C. willdenowii* are unclear, although *C. willdenowii* appears to be affiliated with *C. basiantha* and *C. superata* due to its prominently convex silica platforms. These hypothetical relationships are consistent with those that would be expected from morphological comparisons.

Micromorphological characters are generally more conserved than anatomical characters and are probably good indicators of evolutionary relationship between closely related species in section *Phyllostachys*. Problems with homology assessment, character state scoring, and polarity decisions suggest, however, that these characters should be used as external data sets for assessing the strength of phylogenies constructed using other characters.

Relationships inferred using anatomical characters conflict significantly with morphological and micromorphological data sets, suggesting that the contribution of these characters for phylogenetic reconstruction in *Carex* may be limited. Determining whether observed incongruities between morphological classifications, and anatomical and micromorphological data sets are due to homoplasy in characters or to poor circumscription is difficult to determine. Phylogenetic studies employing numerous different independent data sets are needed to answer this question, and to determine which characters will be most rewarding to future phylogenetic studies of the genus.

TABLE 1. Collection data for achenes sampled in *Carex* section *Phyllostachys*. Vouchers are deposited at KNK and WIN except where noted. Herbarium acronyms follow Holmgren & al. (1990).

***Carex backii* Boott**

CANADA. BRITISH COLOMBIA.: McLeese Lake 22 miles NNW of Williams Lake, 4 June 1956, Calder 17022 et al. (DAO).—NEW BRUNSWICK. Albert Co.: Pleasant Vale, 19 June 1945, Dore 45.198 & Gorham. (DAO).—U.S.A. WISCONSIN. La Cross Co.: Washington Twp., wooded slope bordering a branch of Coon Creek in Bohemian Valley, 19 June 1959, Hartley 1964. (DAO).

***Carex basiantha* Steudel**

U.S.A. ALABAMA. Butler Co.: ca. 0.5 mi N of Oaky Streak, 23 May 1994, Naczi 3991 & Ford.—ARKANSAS. Scott Co.: ca. 2 mi N of Y City, 20 May 1994, Naczi 3938 & Ford.—FLORIDA. Gadsden Co.: Flat Creek Boat Landing, 1 June 1988, Bryson 7893 & Gholson. (MICH).—LOUISIANA. West Feliciana Parish: .2 mi S of jct. of rtes. 61 & 10, 23 May 1994, Naczi 3987 & Ford. (WIN).—OKLAHOMA. McCurtain Co.: ca. 4 mi N of Idabel, 21 May 1994, Naczi 3954 & Ford.—SOUTH CAROLINA. Berkeley Co.: Rte. 52, 1.2 mi. N of Goose Creek, 18 May 1988, Hill 19477. (MICH).—TENNESSEE. Scott Co.: ca. 10 air mi. W of Oneida at rte. 297 crossing of Big South Fork of Cumberland River, 22 June 1993, Naczi 3201 & Reznicek. (WIN).

***Carex jamesii* Schweinitz**

CANADA.—ONTARIO. Kent Co.: Orford twp., Clear Creek, 22 May 1991, Oldham #12554. (MICH).—U.S.A. ALABAMA. Madison Co.: E of Huntsville on Monte Sano Mt., 10 May 1985, Bryson 3874 et al. (MICH).—INDIANA. Martin Co.: ca. 4 miles SE of Loogootee, 21 May 1913, Deam No.12,858. (MICH).—IOWA. Mills Co.: Wilson Timber, 29 May 1992, Wilson 5175. (MICH).—KENTUCKY. Mason Co.: ca. 2 air mi. W of Dover along S side of route 8, 29 May 1994, Naczi 4028 & Flynn. (WIN). —VIRGINIA. Lunenburg Co.: 1/2 mile N of St. Rt. 612, 3 June 1986, Wieboldt 5982. (MICH).—WEST VIRGINIA. Fayette Co.: New River Gorge, about 8 miles NE of Beckley, 20 May 1985, Wieboldt 5523. (MICH).

TABLE 1. Continued.

***Carex juniperorum* Catling, Reznicek, & Crins**

CANADA. ONTARIO. Hastings Co.: Tyendinga Twp., 5.5 Km NE of Shannonville, 11 June 1991, Catling 9102. (MICH).—U.S.A. KENTUCKY. Lewis Co.: Hughes Knob, 3/4 mi. N of East Fork Church, 7 May 1991, Reznicek 8754 et al. (MICH).—OHIO. Adams Co.: Bush Creek Twp., E of Tulip Rd. 3/5 mi. S of Lynx, 6 May 1991, Reznicek 8748 et al. (MICH).—OHIO. Adams Co.: Tiffin twp., W of state Rd. 41, 1.5 mi. N of West Union on S side Adams Lake, 6 May 1991, Reznicek 8742 et al. (MICH).

***Carex latebracteata* Waterfall**

U.S.A. ARKANSAS. Howard Co.: ca. 4 mi. NW of Athens, Ouachita National Forest, 21 May 1994, Naczi 3952 & Ford. (WIN).—ARKANSAS. Polk Co.: ca. 8 mi. E of Vandervoort, 20 May 1994, Naczi 3948 & Ford. (WIN).—OKLAHOMA. McCurtain Co.: ca. 10 road mi. N of Broken Bow, Hochatown State Park, 21 May 1994, Naczi 3953 & Ford. (WIN).

***Carex saximontana* Mackenzie**

CANADA.—BRITISH COLOMBIA.: Half mile east of Nickel Plate Mine along road between Hedley and Mt. Apex, 19 July 1953, Calder No.10729 & Savile. (DAO).—U.S.A. MINNESOTA. Chippewa Co.: Lac Qui Parle State Park, 20 June 1947, Moore No. 19653 & Huff. (DAO).—NORTH DAKOTA. : Bismarck, in *Bouteloua gracilis*, above thicket on hillside, 23 June 1946, Stevens s.n. (DAO).

***Carex superata* Naczi, Reznicek, & B. A. Ford**

U.S.A. ALABAMA. Monroe Co.: ca. 3 mi. W of Midway, T9N, R10E, N 1/2 of sect. 30, 23 May 1993, Naczi 3073.—ALABAMA. Butler Co.: ca. 0.5 mi. N of Oaky Streak, T7N, R15E, SE 1/4 sect. 9, 24 May 1993, Naczi 3103.—FLORIDA. Gadsden Co.: ca. 5.0 air mi. S of Chattahoochee, 24 March 1990, Orzell & Bridges 13002 (MICH).—MISSISSIPPI. Tishomingo Co.: ca. 10 mi. N of Iuka, J.P. Coleman State Park, 25 May 1994, Naczi 4013 et al. (WIN).—TENNESEE. Franklin Co.: End of Jackson Co. Ala rt. 56, 2 May 1971, Kral 42454b. (MICH).

TABLE 1. Continued.

***Carex willdenowii* Willdenow**

U.S.A. KENTUCKY. Whitley Co.: ca. 8 mi. E of Williamsburg along S side of rte. 92, 29 May 1993, Naczi 3153. (WIN).—OHIO. Gallia Co.: Wayne National Forest, 10 miles SW of Gallipolis, 25 May 1988, A.A. Reznicek 8161 & S.A. Reznicek. (MICH).—NEW YORK. Genessee Co.: "The Gulf", 6km ENE of Le Roy, 30 May 1991, Reznicek 8777 et al. (MICH).—PENNSYLVANIA. Warren Co.: Allegheny National Forest, ca. 5 mi. E of Warren, 21 June 1985, Rettig 1344. (MICH).—VIRGINIA. Rockingham Co.: George Washington National Forest, jct. of forest Rds. 87 and 232, 15 June 1991, Cusick #29,658. (MICH).—WEST VIRGINIA. Back Mountain in Rd., 2 mi. NE of Wanless, 30 May 1991, Cusick #29,595. (MICH).

TABLE 2. Collection data for populations of *Carex* section Phyllostachys for which leaf cross-sections were examined. Vouchers are deposited at KNK and WIN except where noted. Herbarium acronyms follow Holmgren & al. (1990).

***Carex backii* Boott**

CANADA. MANITOBA. Hwy. 308, 5 km S of Forestry Road 5, 13 June 1994, Ford 94119 et al.; Delta Marsh, University of Manitoba Field Station, 12 July 1994, Ford 94191 & Starr (3 samples).—ONTARIO. Niagara Regional Mun.: North Grimsby Twp., Beamer Conservation Area, 15 June, 1994. Ball (no voucher) (3 samples). Simcoe Co.: Vespra Twp., 5 miles NW of Barrie, 26 July 1981, Reznicek & Reznicek 6364 MICH. Victoria Co.: Carden Twp. Mun., Carden Alvar, rocky woods 5.3 km S of Uphill, UTM 572507 (map 31D/11), 26 May 1994, Oldham #16032 TRTE.

***Carex basiantha* Steudel**

U.S.A. ALABAMA. Butler Co.: ca. 0.5 mi N of Oaky Streak, 23 May 1994, Naczi 3991 & Ford.—ARKANSAS. Scott Co.: ca. 2 mi N of Y City, 20 May 1994, Naczi 3938 & Ford.—LOUISIANA. West Feliciana Parish: along W side of route 61, just S of St. Francis Hotel, 23 May 1994, Naczi 3987 & Ford.—OKLAHOMA. McCurtain Co.: ca. 4 mi N of Idabel, 21 May 1994, Naczi 3954 & Ford.—TEXAS. Jasper Co.: ca. 12 mi W of Jasper, 22 May 1994, Naczi 3965 & Ford (2 samples).

***Carex jamesii* Schweinitz**

CANADA. ONTARIO. Essex Co., Anderson Twp., 5 km NE of Amherstburg, 22 May 1994, Ball 940526. Niagara Regional Mun.: Louth Twp., Twenty Mile Creek, Jordan, 13 June 1979, Ball 79039 PWB in TRTE. Essex Co.: Pelee Island, 12 June 1994, Oldham (no number) TRTE (2 samples).Waterloo Co.: Wilmot Twp., 8 km W of New Dundee on the Nith River, 3 June 1982 Ball 82074 PWB in TRTE.—U.S.A. ARKANSAS. Franklin Co.: ca. 1 mi N of Cecil, Citadel Bluff Army Corps of Engineers Park, 19 May 1994, Naczi 3923 & Ford. Newton Co.: ca. 3 mi NE of Boxley, Lost Valley Recreation Area of Buffalo National River, 19 May 1994, Naczi 3917 & Ford. Scott Co.: ca. 2 mi N of Y City, W of route 71 and S of Fourche La Fave River, 20 May 1994, Naczi 3939 & Ford.—INDIANA. Grant Co.: Taylor University Arboretum, SW edge of Upland, 17 May 1994, Rothrock 3255; Stellers Road, 1.3 miles N of Matthews, 17 May 1994, Rothrock 3254.—KENTUCKY. Campbell Co.: Highland Heights, 10 May 1994, Naczi 3826. Mason Co.: ca. 2 air mi W of Dover, along S side of route 8, 29 May 1994, Naczi 4028 & Flynn.

Table 2. Continued.

U.S.A.—MISSISSIPPI. DeSoto Co.: ca. 2 mi N of Walls, along E side of route 61, 25 May 1994, Naczi 4026 et al..—VIRGINIA. Bath Co.: ca. 0.4 mi S of Healing Springs, along W side of route 220, 23 June 1994, Naczi 4482 & Thieret.

***Carex juniperorum* Catling, Reznicek, & Crins**
U.S.A. KENTUCKY. Bath Co.: ca. 5 air mi ESE of Owingsville, 16 May 1994, Naczi 3890 (2 samples). Lewis Co.: ca. 3.5 air mi ESE of Trinity, 5 May 1994, Naczi 3808 et al. (2 samples).—OHIO. Adams Co.: ca. 3 air mi NE of Peebles, 16 May 1994, Naczi 3878.

***Carex latebracteata* Waterfall**
U.S.A. ARKANSAS. Howard Co.: ca. 4 mi NW of Athens, 21 May 1994, Naczi 3952 & Ford. Polk Co.: ca. 8 mi E of Vandervoort, 20 May 1994, Naczi 3948 & Ford (2 samples).—OKLAHOMA. McCurtain Co.: ca. 10 road mi N of Broken Bow, 21 May 1994, Naczi 3953 & Ford.

***Carex saximontana* Mackenzie**
CANADA. MANITOBA. Treesbank, 300 m E of Prov. Rd. 530 where road crosses the Assiniboine River, 9 June 1995, Ford 9501 & Starr.—SASKATCHEWAN. Cypress Hills, ca. 6 km NW of Eastend, 11 June 1995, Ford 9526 & Starr; Besant Campground and Recreation Area, ca. 30 km W of Moose Jaw, 14 June 1995, Ford 9547 & Starr.—U.S.A. UTAH. Utah Co.: Timpanogos National Monument parking area. Along highway 92 E of Alpine, 10 August 1993, Naczi 3372 & Thieret.

***Carex superata* Naczi, Reznicek, & B. A. Ford**
U.S.A. ALABAMA. Butler Co.: ca. 0.5 mi N of Oaky Streak, 23 May 1994, Naczi 3990 & Ford; Greenville, 3 mi N of center of town along route 263, 24 May 1994, Naczi 3993 & Ford.—MISSISSIPPI. Tishomingo Co.: ca. 10 mi N of Iuka, J. P. Coleman State Park, 25 May 1994, Naczi 4013 et al.

TABLE 2. Continued.

***Carex willdenowii* Willdenow**

U.S.A. ARKANSAS. Garland Co.: ca. 17 air mi N of Hot Springs, Iron Springs Recreation Area of Ouachita National Forest, 19 May 1994, Naczi 3924 & Ford.—KENTUCKY. Franklin Co.: ca. 6.5 air mi NW of Frankfort, 11 May 1994, Naczi 3835 & Borne.—OHIO. Pike Co.: 1.5 road mi W of Buchanan, 16 May 1994, Naczi 3887.—PENNSYLVANIA. Bradford Co.: ca. 8 mi SW of Towanda, along W side of Preacher Brook Road, 17 June 1994, Naczi 4287 & Thieret.

TABLE 3. Collection data for populations of *Carex* sect. *Phyllostachys* for which leaf epidermal surfaces were surveyed. Vouchers are deposited at KNK and WIN unless otherwise noted. Herbarium acronyms follow Holmgren & al. (1990).

***Carex backii* Boott**

CANADA. MANITOBA. Delta Marsh, University of Manitoba Field Station, 12 July 1994, Ford 94191 & Starr.—ONTARIO. Niagara Regional Mun.: North Grimsby Twp., Beamer Conservation Area, 15 June, 1994. Ball (no voucher). Victoria Co.: Carden Twp. Mun., Carden Alvar, rocky woods 5.3 km S of Uphill, UTM 572507 (map 31D/11), 26 May 1994, Oldham #16032 TRTE.

***Carex basiantha* Steudel**

U.S.A. ALABAMA. Butler Co.: ca. 0.5 mi N of Oaky Streak, 23 May 1994, Naczi 3991 & Ford.—OKLAHOMA. McCurtain Co.: ca. 4 mi N of Idabel, 21 May 1994, Naczi 3954 & Ford.—TEXAS. Jasper Co.: ca. 12 mi W of Jasper, 22 May 1994, Naczi 3965 & Ford.

***Carex jamesii* Schweinitz**

CANADA. ONTARIO. Essex Co.: Pelee Island, 12 June 1994, Oldham (no number) TRTE. Niagara Regional Mun.: Louth Twp., Twenty Mile Creek, Jordan, 13 June 1979, Ball 79039 PWB in TRTE. —U.S.A. ARKANSAS. Franklin Co.: ca. 1 mi N of Cecil, Citadel Bluff Army Corps of Engineers Park, 19 May 1994, Naczi 3923 & Ford.

***Carex juniperorum* Catling, Reznicek, & Crins**

U.S.A. KENTUCKY. Bath Co.: ca. 5 air mi ESE of Owingsville, 16 May 1994, Naczi 3890. Lewis Co.: ca. 3.5 air mi ESE of Trinity, 5 May 1994, Naczi 3808 et al.—OHIO. Adams Co.: ca. 3 air mi NE of Peebles, 16 May 1994, Naczi 3878.

***Carex latebracteata* Waterfall**

U.S.A. ARKANSAS. Howard Co.: ca. 4 mi NW of Athens, 21 May 1994, Naczi 3952 & Ford. Polk Co.: ca. 8 mi E of Vandervoort, 20 May 1994, Naczi 3948 & Ford.—OKLAHOMA. McCurtain Co.: ca. 10 road mi N of Broken Bow, 21 May 1994, Naczi 3953 & Ford.

TABLE 3. Continued.

***Carex saximontana* Mackenzie**
CANADA. MANITOBA. Manitoba Wildlife Management Area, W side of Prov. Rd. 346 where road crosses Souris River, 9 June 1995, Ford 9507 & Starr.—SASKATCHEWAN. Besant Campground and Recreation Area, ca. 30 km W of Moose Jaw, 14 June 1995, Ford 9547 & Starr. U.S.A. UTAH. Utah Co.: Timpanogos National Monument parking area. Along highway 92 E of Alpine, 10 August 1993, Naczi 3372 & Thieret.

***Carex superata* Naczi, Reznicek, & B.A. Ford**
U.S.A. ALABAMA. Butler Co.: ca. 0.5 mi N of Oaky Streak, 23 May 1994, Naczi 3990 & Ford; Greenville, 3 mi N of center of town along route 263, 24 May 1994, Naczi 3993 & Ford.—MISSISSIPPI. Tishomingo Co.: ca. 10 mi N of Iuka, J. P. Coleman State Park, 25 May 1994, Naczi 4013 et al.

***Carex willdenowii* Willdenow**
U.S.A. ARKANSAS. Garland Co.: ca. 17 air mi N of Hot Springs, Iron Springs Recreation Area of Ouachita National Forest, 19 May 1994, Naczi 3924 & Ford.—OHIO. Pike Co.: 1.5 road mi W of Buchanan, 16 May 1994, Naczi 3887.—PENNSYLVANIA. Bradford Co.: ca. 8 mi SW of Towanda, along W side of Preacher Brook Road, 17 June 1994, Naczi 4287 & Thieret.

TABLE 4. Collection data for populations of *Carex* sect. *Phyllostachys* for which culm cross-sections were examined. Vouchers are deposited at KNK and WIN unless otherwise noted. Herbarium acronyms follow Holmgren & al. (1990).

***Carex backii* Boott**

CANADA. MANITOBA. Delta Marsh, University of Manitoba Field Station, 12 July 1994, Ford 94191 & Starr.—ONTARIO. Niagara Regional Mun.: North Grimsby Twp., Beamer Conservation Area, 15 June, 1994. Ball (no voucher). Simcoe Co.: Vespra Twp., 5 miles NW of Barrie, 26 July 1981, Reznicek & Reznicek 6364 MICH.

***Carex basiantha* Steudel**

U.S.A. ARKANSAS. Scott Co.: ca. 2 mi N of Y City, 20 May 1994, Naczi 3938 & Ford.—OKLAHOMA. McCurtain Co.: ca. 4 mi N of Idabel, 21 May 1994, Naczi 3954 & Ford.—TEXAS. Jasper Co.: ca. 12 mi W of Jasper, 22 May 1994, Naczi 3965 & Ford (2 individuals sampled).

***Carex jamesii* Schweinitz**

CANADA. ONTARIO. Essex Co.: Pelee Island, 12 June 1994, Oldham (no number) TRTE. Niagara Regional Mun.: Louth Twp., Twenty Mile Creek, Jordan, 13 June 1979, Ball 79039 PWB in TRTE. —U.S.A. ARKANSAS. Scott Co.: ca. 2 mi N of Y City, W of route 71 and S of Fourche La Fave River, 20 May 1994, Naczi 3939 & Ford.

***Carex juniperorum* Catling, Reznicek, & Crins**

CANADA. ONTARIO. Nastings Co.: Tyendinaga Twp. 5.5 km NE of Shannonville, 11 June 1991, P.M. Catling 9102. (MICH).—U.S.A. KENTUCKY. Bath Co.: ca. 5 air mi. ESE of Owingsville, 16 May 1994, Naczi 3890. Lewis Co.: Hymes Knob, 1 1/4 mi. E of Trinity School, 7 May 1991, A. A. Reznicek 8756, A.W. Cusick & Reznicek. (MICH).—OHIO. Adams Co.: ca. 3 air mi. NE of Peebles, 16 May 1994, Naczi 3878.

***Carex latebracteata* Waterfall**

U.S.A. ARKANSAS. Howard Co.: ca. 4 mi NW of Athens, 21 May 1994, Naczi 3952 & Ford. Polk Co.: ca. 8 mi E of Vandervoort, 20 May 1994, Naczi 3948 & Ford.—OKLAHOMA. McCurtain Co.: ca. 10 road mi N of Broken Bow, 21 May 1994, Naczi 3953 & Ford.

TABLE 4. Continued.

***Carex saximontana* Mackenzie**

CANADA. MANITOBA. Manitoba Wildlife Management Area, W side of Prov. Rd. 346 where road crosses Souris River, 9 June 1995, Ford 9507 & Starr.—SASKATCHEWAN. Besant Campground and Recreation Area, ca. 30 km W of Moose Jaw, 14 June 1995, Ford 9547 & Starr. U.S.A. UTAH. Utah Co.: Timpanogos National Monument parking area. Along highway 92 E of Alpine, 10 August 1993, Naczi 3372 & Thieret.

***Carex superata* Naczi, Reznicek, & B.A. Ford**

U.S.A. ALABAMA. Butler Co.: ca. 0.5 mi N of Oaky Streak, 23 May 1994, Naczi 3990 & Ford; Greenville, 3 mi N of center of town along route 263, 24 May 1994, Naczi 3993 & Ford.—MISSISSIPPI. Tishomingo Co.: ca. 10 mi N of Iuka, J. P. Coleman State Park, 25 May 1994, Naczi 4013 et al.

***Carex willdenowii* Willdenow**

U.S.A. ARKANSAS. Garland Co.: ca. 17 air mi N of Hot Springs, Iron Springs Recreation Area of Ouachita National Forest, 19 May 1994, Naczi 3924 & Ford.—OHIO. Pike Co.: 1.5 road mi W of Buchanan, 16 May 1994, Naczi 3887.—PENNSYLVANIA. Bradford Co.: ca. 8 mi SW of Towanda, along W side of Preacher Brook Road, 17 June 1994, Naczi 4287 & Thieret.


fig. 1. Scanning electron micrographs of silica deposits in achene epidermal cells of *Carex* spp. **a**. *Carex backii* (Calder 17022 et al., DAO). **b**. *Carex saximontana* (Stevens (s.n.), DAO). **c**. *Carex juniperorum* (Reznicek 8742 et al., MICH). **d**. *Carex jamesii*, Virginia (Wieboldt 5982, MICH). **e**. *Carex jamesii*, Indiana (Deam No. 12, 858, MICH). **f**. *Carex jamesii*, Iowa (Wilson 5175, MICH).


fig. 2. Scanning electron micrographs of silica deposits in achene epidermal cells of *Carex* spp. **a**. *Carex basiantha*, Louisiana (Naczi 3987 & Ford, WIN). **b**. *Carex basiantha*, Arkansas (Naczi 3938, WIN). **c**. *Carex superata*, Alabama (Naczi 3103, WIN). **d**. *Carex superata*, Tennessee (Kral 42454b, MICH). **e**. *Carex willdenowii* (Cusick #29, 595, MICH). **f**. *Carex latebracteata* (Naczi 3953 & Ford, WIN).

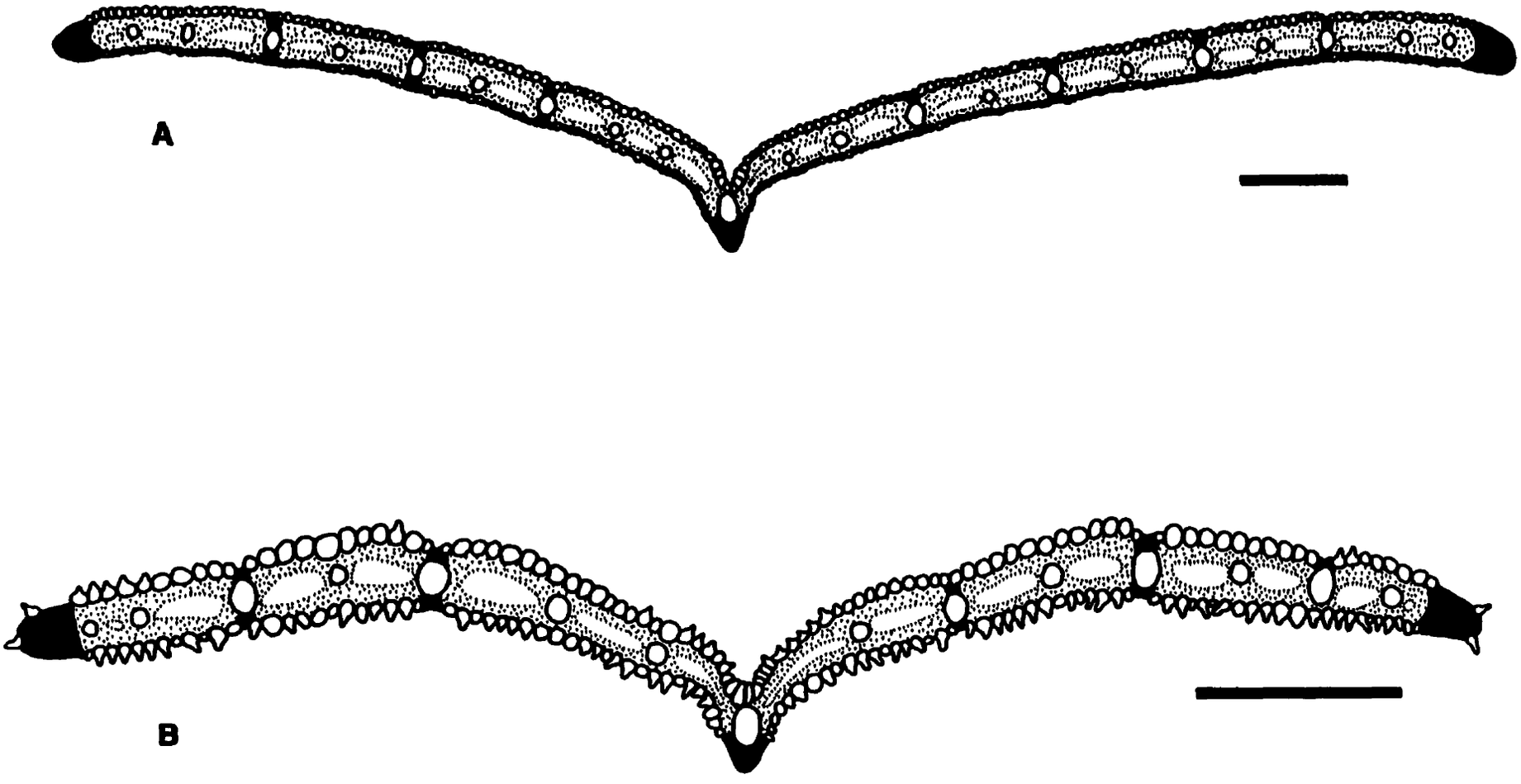


Fig. 3. Cross sections of leaves. **A.** *Carex latebracteata* (Naczi 3948 & Ford, WIN). **B.** *Carex saximontana* (Ford 9507 & Starr, WIN). Scale bars to the right of figures represent 0.5 mm. Darkened areas = sclerenchyma, stippled areas = chlorenchyma, stipple bordered ellipses = air cavities, continuous circles between upper and lower surfaces = vascular bundles.

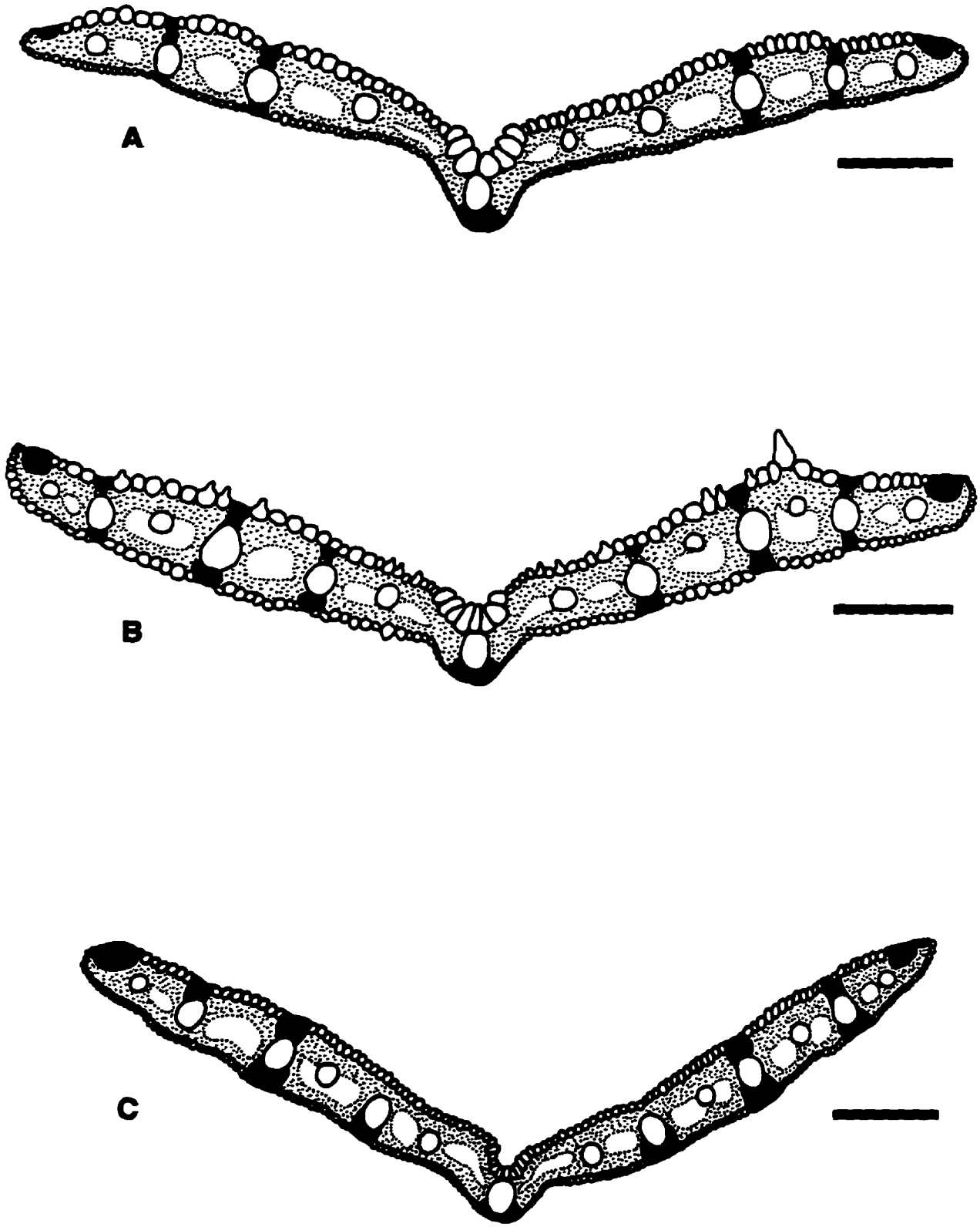


fig. 4. Cross sections of leaves. **A.** *Carex jamesii* (Naczi 3923 & Ford, WIN). **B.** *Carex juniperorum* (Naczi 3878, WIN). **C.** *Carex backii* (Ford 94119 et al., WIN). Scale bars to the right of figures represent 0.25 mm.

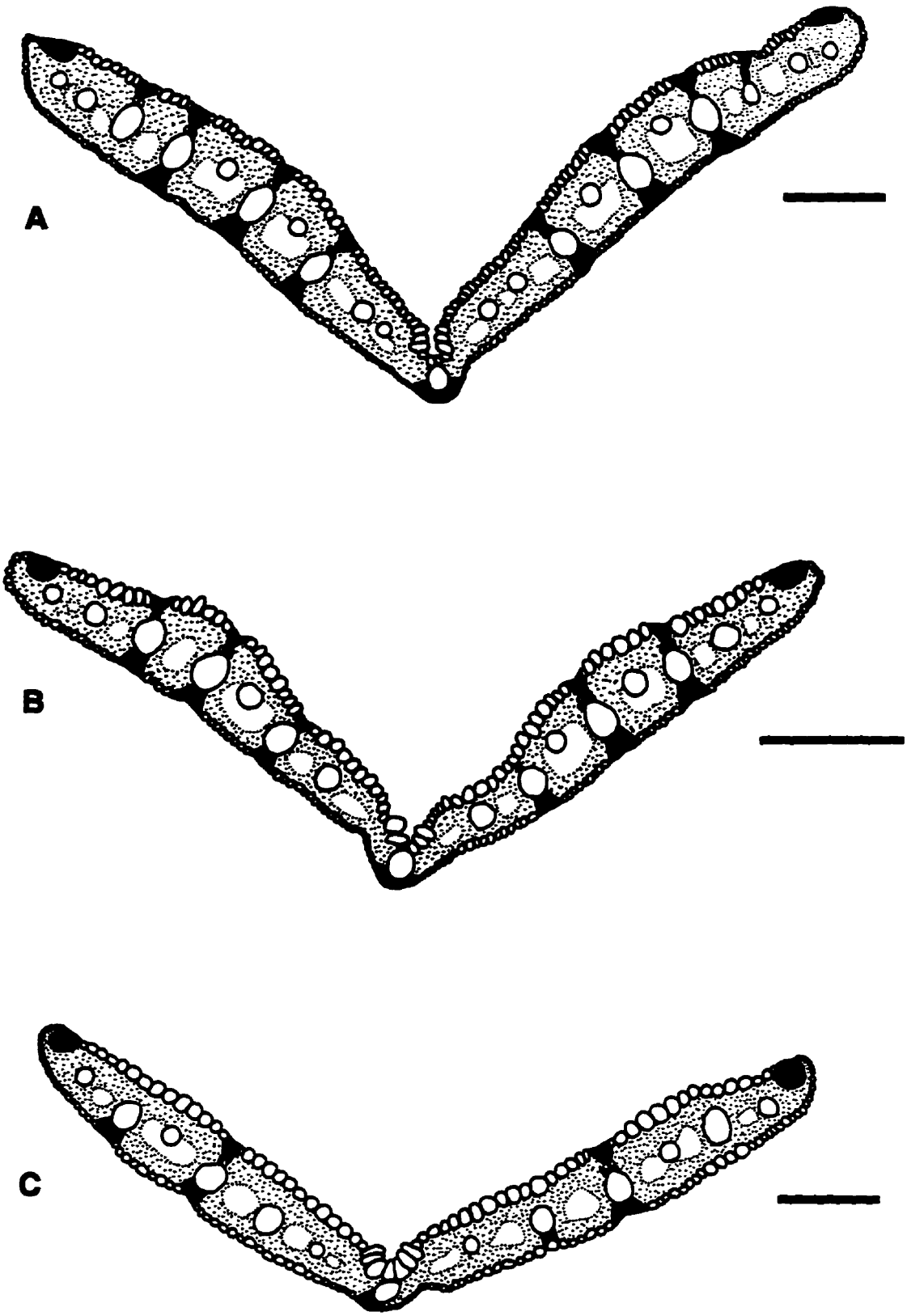


Fig. 5. Cross sections of leaves. **A.** *Carex superata* (Naczi 3993 & Ford, WIN). **B.** *Carex basiantha* (Naczi 3954 & Ford, WIN). **C.** *Carex willdenowii* (Naczi 3924 & Ford, WIN). Scale bars to the right of figures represent 0.25 mm.

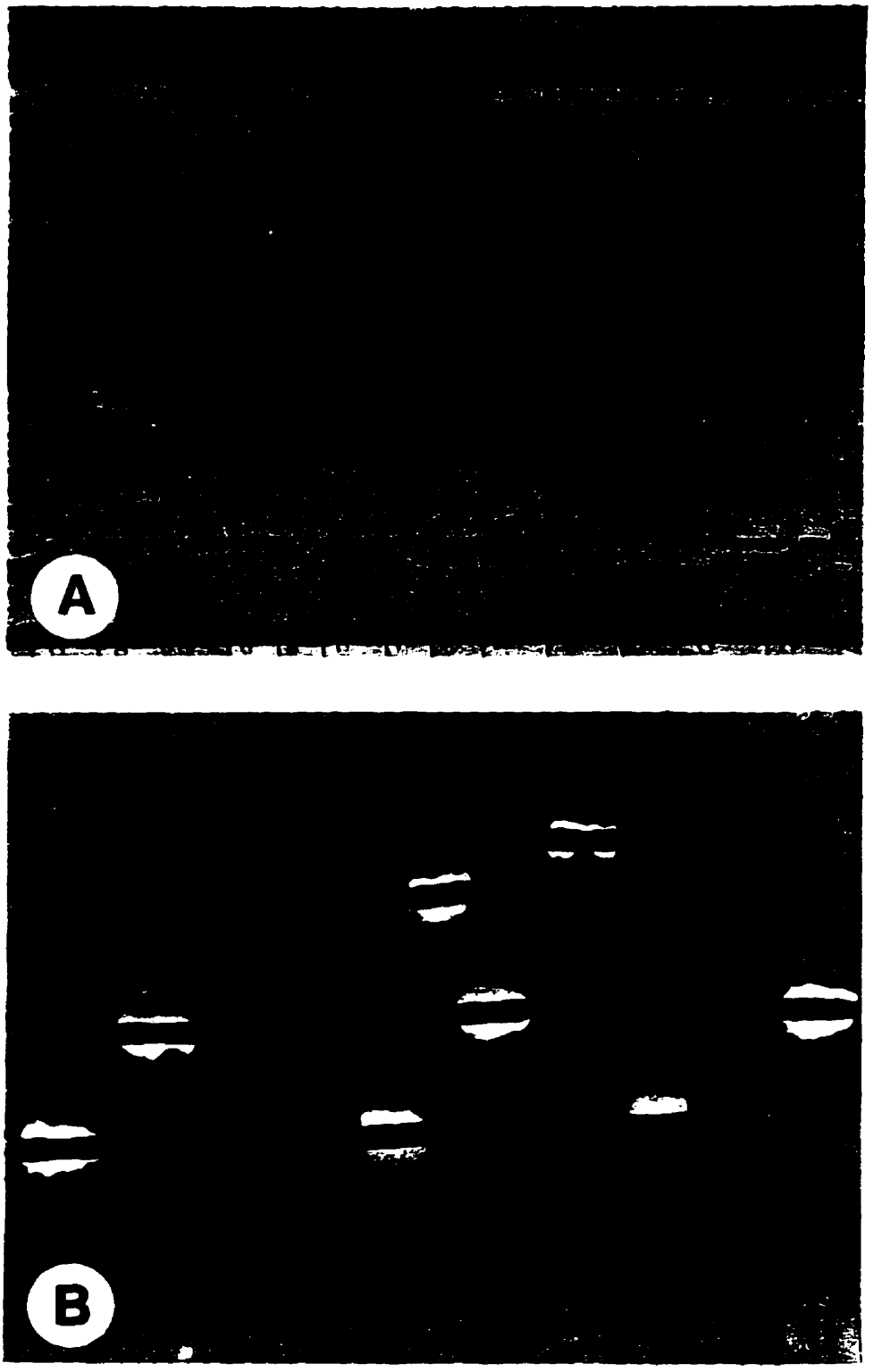


fig. 6. Epidermal surfaces of leaves. **A**. *Carex willdenowii*, adaxial surface (Naczi 4287, WIN). **B**. *Carex saximontana*, abaxial surface (Ford 9507 & Starr, WIN).

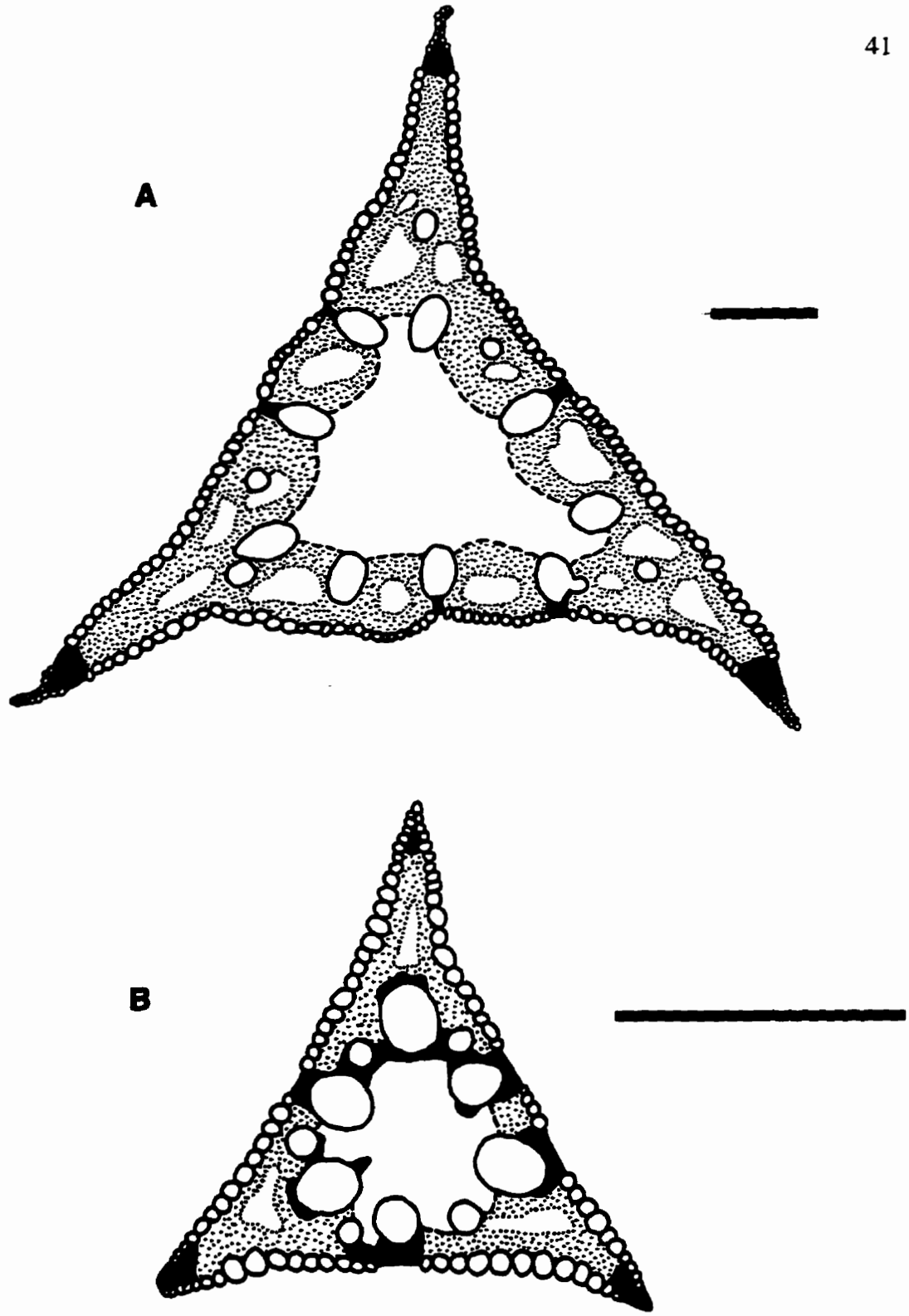


Fig. 7. Cross sections of culms. **A.** *Carex latebracteata*(Naczi 3952 & Ford, WIN). **B.** *Carex superata* (Naczi 3993 & Ford). Scale bars to the right of figures represent 0.25 mm. Darkened areas = sclerenchyma, stippled areas = chlorenchyma, stipple bordered ellipses = air cavities, continuous circles between upper and lower surfaces = vascular bundles. The dashed line separates chlorenchyma from the spongy central ground tissue.

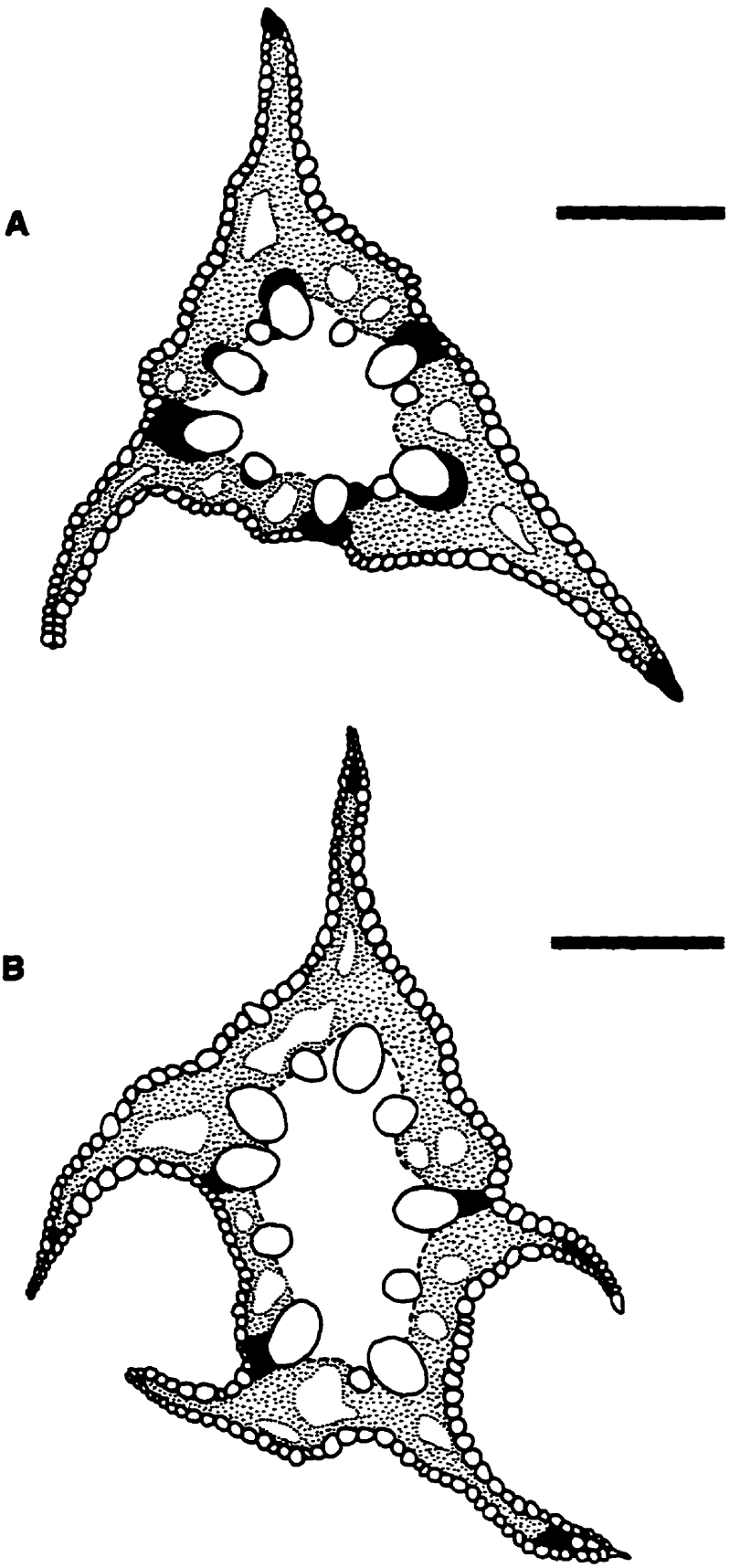


Fig. 8. Cross sections of culms. **A**. *Carex juniperorum*, Ontario (Catling 9102, WIN). **B**. *Carex juniperorum*, Kentucky (Naczi 3890, WIN). Scale bars to the right of figures represent 0.25 mm.

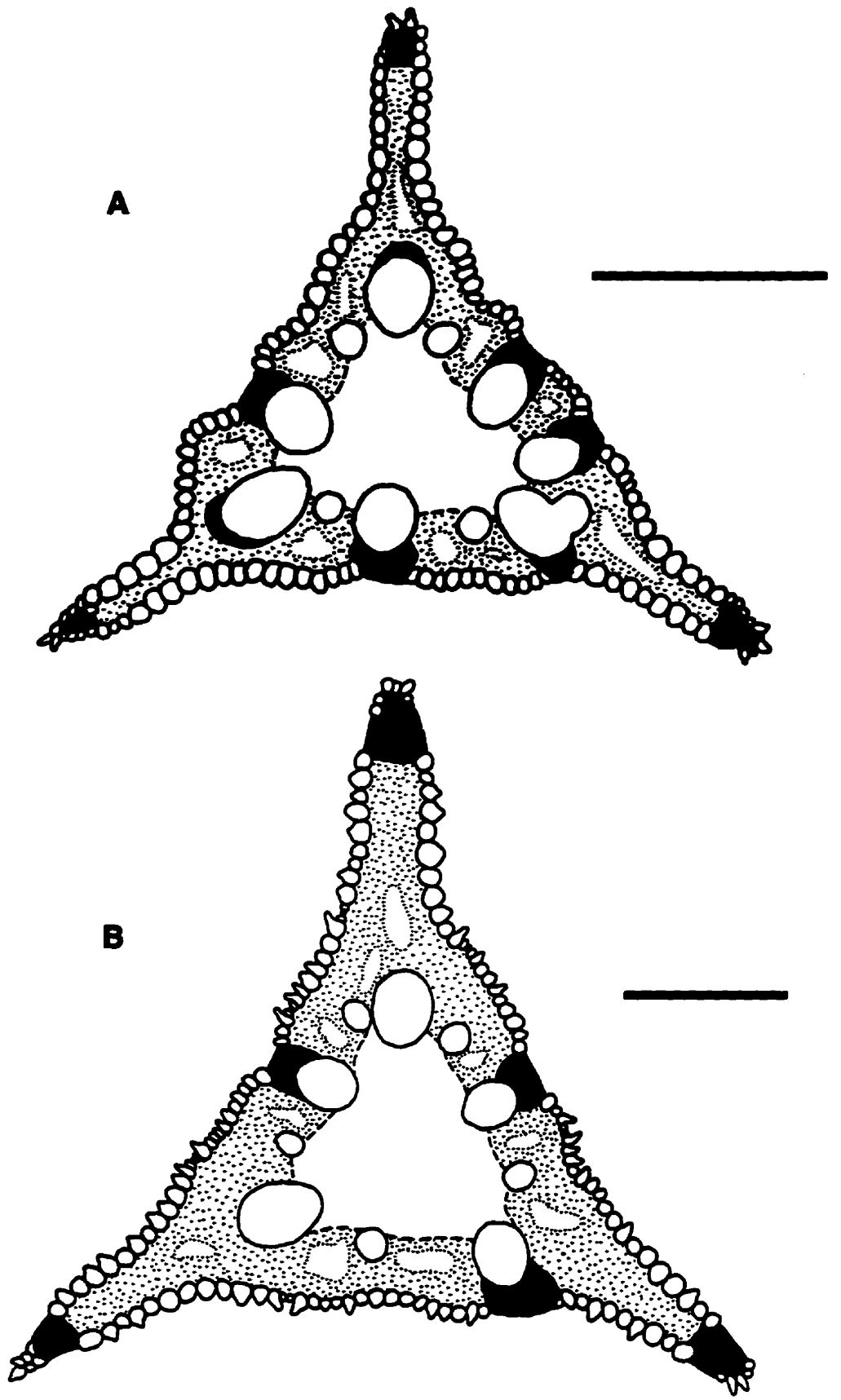


Fig. 9. Cross sections of culms. **A.** *Carex backii* (Ball (s.n.), WIN). **B.** *Carex saximontana* (Ford 9547 & Starr, WIN). Scale bars to the right of figures represent 0.25 mm.

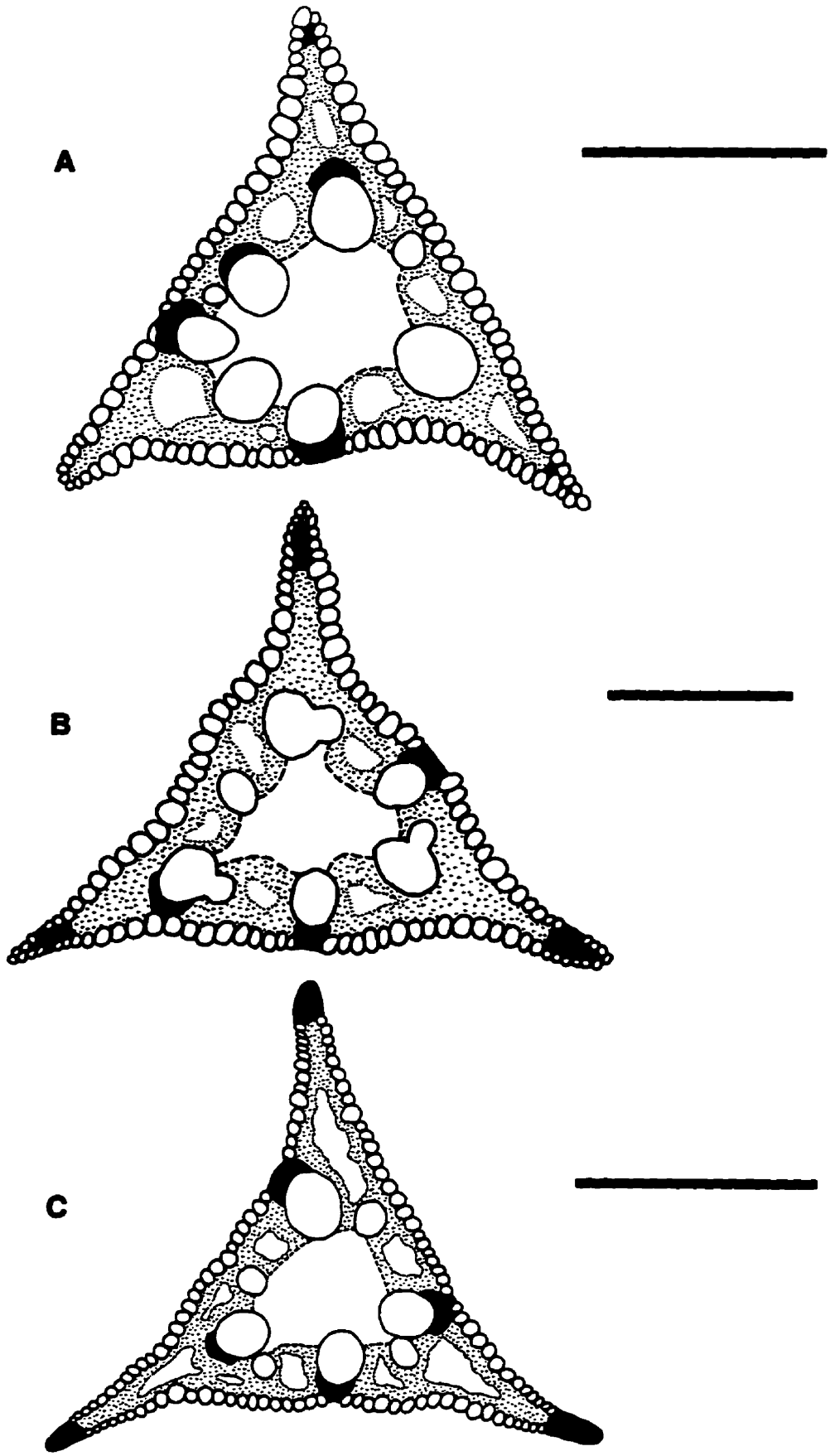


Fig. 10. Cross sections of culms. **A.** *Carex basiantha* (Naczi 3965 & Ford, WIN). **B.** *Carex willdenowii* (Naczi 3924 & Ford, WIN). **C.** *Carex jamesii* (Naczi 3939 & Ford, WIN). Scale bars to the right of figures represent 0.25 mm.

# CHAPTER 3

## PHYLOGENY AND SECTIONAL DELIMITATION IN *CAREX* (CYPERACEAE) USING SEQUENCES FROM THE INTERNAL TRANSCRIBED SPACERS OF NRDNA

Comprising nearly 2000 species or almost half of the Cyperaceae L., the genus *Carex* L. represents one of the largest of all flowering plant genera (Reznicek, 1990). Although the genus is cosmopolitan in distribution, it is primarily a north temperate genus, with centres of diversity in North America and eastern Asia (Ball, 1990; Reznicek, 1990; Naczi, 1992). The habitats in which *Carex* can be found are as diverse as the genus, ranging from the dry open savannas and rain-forests of the subtropical and tropical zones, to the wet-meadows, deciduous forests, and tundra of the temperate and Arctic regions of the earth. The genus, and its tribe the *Cariceae* Kunth ex Dumort., are clearly distinguished from the rest of the Cyperaceae by the possession of imperfect flowers that lack a perianth and by the possession of a perigynium, a sac-like structure of prophyllar origin that surrounds the naked gynoecium (Blaser, 1944). The perigynium forms the most important taxonomic character in *Carex*, and it is primarily through the subtle differences in its shape, size, texture, and nervation, that the enormous number of species in the genus are distinguished (Nelmes, 1951).

Despite the global distribution and ecological importance of this genus, evolutionary trends and relationships within *Carex* have remained almost completely unknown (Reznicek, 1990). This lack of evolutionary understanding is primarily attributable to the nature of morphological and anatomical characters in *Carex*. Floral reduction (Smith and Faulkner, 1976), uniform vegetative morphology and anatomy (Metcalf, 1971; Standley, 1987; etc.), and the repeated occurrence of parallelisms and reversals (Reznicek, 1990; Naczi, 1992; chapter 4) have obscured phylogenetic trends and have led to the recognition of many artificial taxa. The extreme reduction in the

genus and the almost complete lack of knowledge of the ontogeny of *Carex* (Alexeev, 1988), further raises homology problems that complicate phylogenetic reconstruction at all levels. Several recent revisionary studies have clarified some sectional limits (e.g., sect. *Stellulatae* Kunth, Reznicek, 1980; sect. *Hymenochlaenae* (Drej.) L. H. Bailey, Reznicek, 1986; sect. *Ceratocystis* Dumort, Crins and Ball, 1988; sect. *Griseae* (L. H. Bailey) Kükenth., Naczi, 1992), and a number of phylogenetic studies based on morphology and anatomy have been attempted (Naczi, 1992, Crins and Ball, 1988; Crins, 1990; Starr and Ford, 1995), however, the same problem remains; a lack of reliable characters with which to strongly delimit taxa and infer relationships.

Because of the reduced nature of the genus, the search for new characters has been a high priority for caricologists. In the early 1970's, scanning electron microscopy (SEM) of leaf epidermal surfaces (Le Cohu, 1972), and most importantly of the silica bodies in the epidermal cells of the achenes (Le Cohu, 1973; Walter, 1975), provided a new source of characters. Although silica bodies proved useful for circumscribing some species (e.g., Toivonen and Timonen, 1976; Wujek and Menapace, 1986; chapter 2) and sections (e.g., Walter, 1975; Tallent and Wujek, 1983; Menapace and Wujek, 1987), it was soon clear that parallelism would restrict their use in phylogenetic analyses (Rettig, 1986; Waterway, 1990a; chapter 2).

Polycentric chromosomes and the ability of chromosomal fragments to behave independently during meiosis (i.e., agmatoploidy; see Grant, 1981), led many to believe (e.g., Heilborn, 1924; Davies, 1956; Whitkus, 1987) that Carices with lower chromosome numbers were more primitive than those with higher counts. The first phylogenetic tree produced for the genus (Heilborn, 1924) was constructed under this principle, and it has been used by subsequent authors as a means to polarize phylogenetic trees in the absence of outgroups (Crins and Ball, 1988; Crins, 1990), or to hypothesize primitive groups in the genus (Savile and Calder, 1953; Ball, 1990). Unfortunately several problems such as aneuploid series within species (e.g., Wahl, 1940; Faulkner, 1972; Naczi 1992), low

chromosome counts in presumably advanced sections (e.g., section *Acrocystis*, n=15-18; Reznicek, 1990), correlations between counts and habitat types (Bell, 1982; Crins and Ball, 1988; Whitkus, 1988; Naczi, 1992; Hoshino and Waterway, 1995), and the belief that chromosome fusion may also occur (Faulkner, 1972; Whitkus, 1988; Reznicek, 1990; chapter 4), suggest that chromosome counts do not provide a reliable means for inferring evolutionary direction (see chapter 4).

Isozyme analysis has also made significant contributions to our understanding of breeding systems, genetic variability in populations, species boundaries, and species relationships in *Carex* (Bruederle and Fairbrothers, 1986; Standley, 1990b; Waterway, 1990b; Bruederle and Jensen, 1991; Ford, Ball, and Ritland, 1991, 1993; Whitkus, 1992; McClintock and Waterway, 1994). However, as with the above mentioned characters, the numerous problems associated with the use of isozyme data in phylogenetic analyses (see Richardson, Baverstock, and Adams, 1986; Crawford, 1990), have limited their contribution to a broader understanding of evolutionary trends and relationships in *Carex*.

Molecular DNA characters offer an obvious source of variation whose use has yet to be explored in *Carex*. The advantages afforded by these characters in homology assessment, homoplasy, the scoring of character states, and finally even character numbers, all suggest that DNA characters could make a substantial contribution to the systematics of this genus.

In this study I investigate the usefulness of DNA characters to the taxonomy and systematics of *Carex* by using sequences from the internal transcribed spacers of nuclear ribosomal DNA (nrDNA). Because of the enormity of the genus, and the numerous taxonomic problems that surround many of its sections, I chose to explore the systematic position and phylogeny of a small (i.e., 8 species), well defined group in the genus, *Carex* section *Phyllostachys* (J. Carey) L.H. Bailey. A number of reasons favour this approach. First, the small size of the section allows for both interspecific and intraspecific variation to be assessed. Second, its clear delimitation from other sections and the recent resolution

of a number of taxonomic problems (Naczi, Reznicek, and Ford, 1997; Ford et al., 1997c; chapters 2 and 3) allows for an interpretation of results that will not be confounded by a poorly resolved taxonomy. Third, the evolutionally reduced and unusual inflorescence morphology of the section has led to wide speculation concerning its origin and systematic position within the genus. By using section *Phyllostachys* as a model, I hope to explore the usefulness of ITS sequences for circumscribing sections and for resolving inter- and intrasectional relationships in the genus. More specific objectives regarding taxonomic questions and phylogenetic relationships to be tested will be presented at the end of the following section.

## 3.1 SYSTEMATIC BACKGROUND

*Carex* sect. *Phyllostachys* has long been recognized as a very distinctive group within the genus. Described as a "singular section" by Bailey (1886), or as "a very strongly marked group" by Mackenzie (1935), sect. *Phyllostachys* has even been accorded the rank of genus by Torrey (*Phyllostachys* Torrey; 1836). *Carex* sect. *Phyllostachys* does indeed possess several striking diagnostic characters which impart a unique appearance to the group. The large foliaceous pistillate scales, highly reduced and congested androgynous spikes (3 - 61 flowers; Crins, in press), and apically winged culms and rachis (Catling, Reznicek, and Crins, 1993), make this one of the few sections in *Carex* whose limits are currently undisputed. However, like many evolutionally reduced sections in the genus, its unusual appearance has led to wide speculation concerning its origin and phylogenetic position in *Carex*.

Because of their unconventional inflorescence morphology, many reduced sections in *Carex* have become intimately involved in the controversies surrounding the phylogeny and circumscription of the genus and its subgeneric taxa. Deciding what is, and what is not a *Carex*, is certainly one of the most serious problems in a broader understanding of evolutionary trends in *Carex*, but it is beyond the scope of this study. However, due to the pivotal role of sect. *Phyllostachys* in some of the major evolutionary controversies in the genus, a discussion of the systematic problems surrounding the subgenera is necessary before the diverse hypotheses concerning the origins of the *Phyllostachys* can be entertained. The terminology used to describe the vegetative and reproductive morphology of *Carex* follows Reznicek (1990).

### *Subgeneric Classification*

In the only worldwide monograph of the genus, Kükenthal (1909) divided *Carex* into four subgenera (*Primocarex* Kükenth., *Carex*, *Vignea* (P.Beauv. ex Lestib.f.) Peterm., and *Indocarex* Baillon) which he believed were representative of the major

lineages in the genus. Although there are good reasons for reorganizing Kükenthal's subgenera, his classification has been maintained by many authors (e.g., Raymond, 1959; Chater, 1980; Jermy, Chater, and David, 1982; Kukkonen and Toivonen, 1988), because of its usefulness in organizing gross morphological types and because of a lack of alternative widely accepted systems.

Subgenus *Carex* is the largest of the subgenera with 1400 species, and it is considered by many to be one of the most derived (e.g., Kükenthal, 1909; Nelmes, 1951; Smith and Faulkner, 1976; Reznicek, 1990). Although most of its species are characterized by the possession of cladoprophylls and by inflorescences that are composed of terminally staminate, and laterally pistillate inflorescence units, a great degree of variability is seen in the sexual expression and configuration of these inflorescence units (see Kükenthal, 1909). This has prompted a number of taxonomists to suggest that the subgenus as it is presently circumscribed may be unnatural (Nannfeldt, 1977; Reznicek, 1990), but it is unclear how further subdivision should take place.

Subgenus *Indocarex* is a small, mostly tropical taxon of about 100 species (Ball, 1990; Reznicek, 1990). With few exceptions (Kükenthal, 1909), the members of subgenus *Indocarex* have been considered as the most primitive in the genus as inferred from their phytogeography (Kreczetovicz, 1936; Nelmes, 1951; Ball, 1990), and inflorescence structure (Kreczetovicz, 1936; Smith and Faulkner, 1976). The species in subg. *Indocarex* can be distinguished by uniformly androgynous spikes, similar terminal and lateral inflorescence units, the occurrence of cladoprophylls, and most importantly, by the possession of inflorescence prophylls: unique structures at the base of the lateral inflorescence units that are homologous to perigynia (Blazer, 1944), and similar to them in appearance. However, a fundamental blurring of subgeneric limits appears to occur in Asia, where a number of sections such as the *Decorae* (Kükenth.) Ohwi (Raymond, 1959; Koyama, 1957; Koyama, 1962) and the *Hymenochlaenae* (Holm, 1900; Ohwi, 1936; Koyama, 1962), obscure the boundaries between the Indocarices and subg. *Carex*.

Although many authors have merged these subgenera in the past, most modern authors choose to treat them separately (e.g., Kukkonen and Toivonen, 1988; Reznicek, 1990).

Subgenus *Vignea*, comprising 400 to 500 species, is distinguished by the possession of similar terminal and lateral inflorescence units that are sessile, and by the presence of an abaxial false suture on the perigynium (Reznicek, 1990). The pistillate flowers are normally distigmatic, and cladoprophylls and sheathing bracts are generally absent. Unlike the other three commonly recognized subgenera in *Carex*, almost all authors consider subg. *Vignea* to be natural and separate, whether the evidence is based on morphology (e.g., Bailey, 1886; Kükenthal, 1909; Ohwi, 1936; Nelmes, 1951; Koyama, 1962, etc.), anatomy (Le Cohu, 1967), or smut host-parasite data (Savile and Calder, 1953; Nannfeldt, 1977). Although this subgenus is generally considered to be derived (e.g., Nelmes, 1951, Smith and Faulkner, 1976), Reznicek (1990) has pointed out that the most compound inflorescences in the genus are found within this subgenus, a feature associated with primitive Carices.

Subgenus *Primocarex* is a group of approximately 70 species grouped into 14 sections that is distinguished by a single character: a solitary, terminal spike (Nannfeldt, 1977; Reznicek, 1990). Kükenthal (1909) felt that subg. *Primocarex* was primitive, and had independently given rise to both the vignean and indocaricoid lines during the evolution of the genus. Subgenus *Carex*, which he placed last in his classification, was considered a later development from subgenus *Indocarex*. Kreczetovicz (1936) argued, however, that subgenus *Primocarex* was not primitive nor natural, but rather a polyphyletic group that was the result of the repeated independent reduction of multispicate species from the other three subgenera. Kreczetovicz (1936) described two primary modes of reduction that had given rise to the Primocarices: (1) "digressive reduction", or simple quantitative reduction unaccompanied by noticeable changes in structure or function (this was seen primarily in Primocarices descendant from subg. *Carex* and *Vignea*), and (2) "transmutive reduction", in which a fusion and/or a

transformation of parts (e.g., of a bract into a scale) obscured a recent origin from subg. *Indocarex*. Both types of reduction were thought to be the result of climatic changes that had accompanied recent mountain building and glaciation events. Subsequent investigations of inflorescence development by Smith and Faulkner (1976) have also supported the contention that the group is polyphyletic and advanced, although smut host-parasite relationships have indicated that a portion of the group is primitive and natural (Savile and Calder, 1953).

***Carex sect. Phyllostachys***

Early taxonomic treatments of the *Phyllostachys* focused on an origin for the section from within subgenus *Carex*. Tuckerman (1843) treated *C. backii* Boott, *C. willdenowii* Schkuhr ex Willd., and *C. steudelii* Kunth (= *C. jamesii* Schwein.) as a distinct "grex" (a group of unspecified rank) within section *Legitimae* Koch. (=subg. *Carex* in part). These three species were also recognized by Carey (1848) as a natural group, however, Bailey (1885, 1886) expanded sect. *Phyllostachys* by creating two new subsections: (1) the *Bractoideae* Bailey, which contained the three species recognized by Carey (1848) and Tuckerman (1843), and (2) the *Phyllostachyae* Bailey, a subsection comprised of two species that are currently placed in section *Firmiculmes* Kükenth. (*C. multicaulis* L.H. Bailey and *C. geyeri* Boott). In his treatments, Bailey (1885, 1886) provided a very detailed theory on the origin and relationships of sect. *Phyllostachys*. He postulated that the *Phyllostachys* were an offshoot of sect. *Acrocystis*, and that the section was connected to section *Laxiflorae* Kunth via *C. multicaulis*, a species which he considered to be close to *C. hitchcockiana* Dew. Although *C. hitchcockiana* is now placed in section *Griseae* (L.H. Bailey) Kükenth., it forms part of a much larger clade comprised of sections *Laxiflorae*, *Granulares* (O. Lang) Mackenzie, *Careyanae* Tuckerman ex Kükenth., and *Griseae* (Naczi, 1992) to which Tuckerman (1843) had also felt sect. *Phyllostachys* was close. Although later authors essentially ignored this early

hypothesis, a few of the species in these sections have dilated culm apices; a rare condition in *Carex*, but one that is found throughout sect. *Phyllostachys*.

Holm (1900, 1903) and Kreczetovicz (1936) both considered sect. *Phyllostachys* a transitional group that demonstrated that subgenus *Indocarex* was inseparable from subgenus *Carex*. In a study prompted mainly by his exception to the classification proposed by Bailey (1885, 1886), Holm (1900) argued that sect. *Phyllostachys* was not closely related to either sections *Acrocystis* or *Laxiflorae*, and that anatomically, they were closely related species that were distinct from sect. *Firmiculmes*. In his attempts to clarify its systematics, Holm noted that the peculiar habit of sect. *Phyllostachys*, in which a terminal and several basal androgynous spikes arise from a single pseudoculm, hinted at a relationship to both the "*Carices genuinae*" (i.e., subg. *Primocarex* in part and subg. *Carex*) and section *Vigneastra* Tuckerman (=subg. *Indocarex*). The connection to subg. *Indocarex* was explored by a demonstration of positional homology between a teratological specimen of *C. backii* and a normal multispicate species from subg. *Indocarex*, *C. cladostachya* (= *C. polystachya*). The similarities between the two did not suggest an immediate relationship, but indicated that a wider "*Carices Genuinae*", which included subg. *Indocarex*, should be recognized. Holm argued instead that the species assigned to the *Phyllostachys* were not sufficiently distinct to constitute a section, but were best treated as primitive members of the *Hymenochlaenae*; a many-flowered section in subgenus *Carex* that, like sect. *Phyllostachys*, emulates the indocaricoid condition when the abnormal growth of the rachilla (a reduced secondary axis) within basal perigynia leads to the production of lateral androgynous inflorescence units.

Kreczetovicz (1936) believed that sect. *Phyllostachys* was a highly derived group that had evolved only recently (Pliocene or Pleistocene) from subg. *Indocarex* via transmutive reduction. In sect. *Phyllostachys* this process had proceeded by a reduction of the lateral inflorescence units to a vestigial axis (i.e., a rachilla); by the conversion of the inflorescence prophylls into perigynia, and by the transformation of inflorescence bracts

into leafy pistillate scales. The inflorescence was thus "pseudo-monostachyous", each perigynium representing the remnants of a peduncled lateral inflorescence unit that because of concrescence and a transformation of parts masked its multispicate origin. The strongest evidence for such a transformation was seen in sections *Phyllostachys* (subg. *Carex*) and *Firmiculmes* (subg. *Primocarex*); both groups had pseudo-monostachyous inflorescences, and yet Kükenthal (1909) had placed them in different subgenera. The presence and abnormal growth of the rachilla in these sections was a sign of their recent and incomplete reduction from the Indocarices.

Based on smut host-parasite records, Savile and Calder (1953) proposed a phylogenetic hypothesis for *Carex* that maintained the four traditional subgenera, and created a new subgenus (*Kuekenthalia* Savile and Calder) for those species in subg. *Carex* with persistent styles and bladdery perigynia. Section *Phyllostachys* was placed at the tip of a lineage in subg. *Carex* that included section *Limosae* (Tuckerm.) Christ and the evolutionally reduced section *Scirpinae* Tuckerm. (placed in subg. *Primocarex* by Kükenthal). Although they agreed with Kreczetovicz (1936) that subgenus *Primocarex* sensu Kükenthal was artificial, smut records suggested that nearly half its species were still a natural group, and that the presence of a rachilla, among other morphological characters, clearly demonstrated that the group was primitive. Despite the fact that they considered the simple spikes of their Primocarices as primitive, other reduced groups, like sections *Phyllostachys* and *Scirpinae*, were considered highly evolved because their smuts shared advanced characters with smuts that infected Carices typical of evolutionally advanced subgenera.

Although not all authors explicitly discuss their views regarding evolution, the arrangement of taxa within their monographs presents an implicit phylogenetic hypothesis. The placement of sect. *Phyllostachys* in the works of Mackenzie (1935) and Kükenthal (1909), is here worthy of mention owing to the great influence of these monographs on the present systematics of the genus.

In his treatment of the North American Carices, Mackenzie (1935) placed sect. *Phyllostachys* between the monotypic section *Polytrichoideae* (Tuckerm.) Mackenzie and the small, western North American section *Filifoliae* Tuckerman. As an hypothesis of relationship this treatment is unique, but it cannot be considered implausible. Like many evolutionally reduced groups in *Carex*, the lack of macromorphological characters in sections *Polytrichoideae*, *Filifoliae*, and *Phyllostachys* means that none of these groups can be unambiguously aligned with any other section. These sections do share a caespitose habit, a lack of inflorescence bracts, and few-flowered androgynous spikes, but this condition is commonly seen among reduced Carices. They differ considerably, however, in the morphology of their perigynia and in their ecological preferences, which are the most likely reasons why they have not been suggested as being related before. Unfortunately, Mackenzie rarely expressed his views on evolution in the genus, so it is difficult to determine whether he truly felt a relationship existed between these three sections or whether he was simply employing a convenient arrangement.

Kükenthal (1909) placed sect. *Phyllostachys* between two old-world sections, the *Rhomboidales* Kükenth. and the *Elatae* Kükenthal. Morphologically these two sections differ markedly from the *Phyllostachys*. They are comprised of many-flowered, multispicate species that possess highly ribbed perigynia, persistent style bases, and sheathing inflorescence bracts. None of these characters is present in sect. *Phyllostachys*.

### *Objectives*

In order to gain a better understanding of the phylogeny and evolutionary position of *Carex* sect. *Phyllostachys*, I conducted a cladistic analysis of sect. *Phyllostachys* and its putatively related sections using sequences from the two internal transcribed spacers (ITS 1 and ITS 2) and portions of the 5.8S gene (i.e., the ITS region) of nrDNA. This locus has recently become the sequence of choice for low level phylogenies in plants (Baldwin et al., 1995). Its high mutational rate, ease in alignment and amplification, and

relatively small size have meant that phylogenetic questions within genera (e.g., *Astragalus*, Wojciechowski et al., 1993; *Epilobium*, Baum, Sytsma, and Hoch, 1994; *Antennaria*, Bayer, Soltis, and Soltis, 1996) and even between closely related species (e.g., *Dendroseris*, Sang et al., 1994; *Senecio*, Bain and Jansen, 1995; *Robinsonia*, Sang et al., 1995) are now routinely addressed by sequence data. These studies suggest that the ITS region is the most appropriate sequence currently available for addressing phylogenetic questions within *Carex*.

The objectives of this study were to: (1) assess the utility of the ITS region for resolving both phylogenetic and taxonomic questions in *Carex*; (2) determine whether *Carex* section *Phyllostachys* is monophyletic, and (3) determine the systematic position of this section within the genus *Carex* by comparing sequence data of section *Phyllostachys* with species representative of putatively related sections.

## 3.2 MATERIALS AND METHODS

### *Choice of Taxa*

Localities and collection numbers of all species and populations sequenced in this study, and the taxonomy of *Carex* employed, are given in TABLES 5 and 6. Taxa were chosen as representatives of specific groups or hypothetical lineages to which *Carex* section *Phyllostachys* has been previously associated (see above). Some of the taxa considered by Mackenzie (1935) and Kükenthal (1909) to be close to sect. *Phyllostachys* could not be included in this study because material was unavailable or because problems were encountered in the amplification or sequencing of their ITS regions.

### *Choice of Outgroup*

Attempts were made to align *Carex* ITS sequences with a member of the Cyperaceae from outside the *Cariceae* (*Cyperus*); however, due to considerable size and base sequence dissimilarity an unambiguous alignment could not be obtained and this taxon was excluded. Efforts were also made to sequence the ITS region of a species from a different genus in the *Cariceae* (*Kobresia simpliciuscula* (Wahlb.) Mackenzie; Keleher & Punter #94/155, WIN) to use as an outgroup. Unfortunately, only partial sequences of ITS 1, ITS 2, and 3' end of the 5.8S gene were attained for this taxon and it was thus excluded from all analyses. Given these difficulties, the two species sequenced from subgenus *Vignea* were chosen as the most appropriate outgroup for polarizing trees. This decision was based on the general agreement that this subgenus is natural (see systematic background), that it is unrelated to sect. *Phyllostachys*, and that the subgenus may be primitive within *Carex* (Reznicek, 1990). Unrooted searches supported the assumption of monophyly for the ingroup; i.e., a single branch partitioned the ingroup from the outgroup (Swofford et al., 1996).

***DNA Isolation and PCR Amplification***

DNAs from all eight of the species presently recognized in section *Phyllostachys*, were extracted from live plants collected in the wild and maintained in a greenhouse (TABLE 5). In order to assess the amount of infraspecific variation that might be expected for the ITS region in *Carex*, two to four individuals from across the range of each of the eight species in section *Phyllostachys* were sequenced. In two cases, individuals from single populations of *C. saximontana* (Ford 9501 & Starr; TABLE 1) and *C. superata* (Naczi 4013 et al.; TABLE 5) were sequenced to determine whether any variation might exist at the populational level. For all other taxa, DNAs were extracted from the live or dried leaf tissues of a single individual.

Total genomic DNA was isolated from 1.0-1.5g of fresh, pressed (within 2 years), or silica dried (Chase and Hillis, 1991) leaf tissue following a modified CTAB method (Doyle and Doyle, 1987) with 1.0% beta-mercaptoethanol in the extraction buffer. Proteins and lipids were removed by adding 250µl of chloroform/isoamyl alcohol (24:1 v/v), inverting the mixture gently for 10 mins, and by centrifugation at maximum speed (14 000 rpm) for a further 10 mins. The supernatant was removed, and the DNA was then reprecipitated with ice-cold 95% ETOH (2-3 hrs in 4° C refrigerator), washed in 70% ETOH (10 min), and re-suspended in 150-300µl of TE buffer.

***PCR Amplification and Sequencing of ITS.***

Double-stranded DNA for the complete ITS region (3' 18S - 5' 26S fragment) was PCR (polymerase chain reaction) amplified from total genomic DNA using the forward primer ITS-L (Hsiao et al., 1995), and the reverse primer ITS-4 (White et al., 1990; see fig. 11). As in grasses (Hsiao et al., 1995), ITS-L provided better amplification than did the forward primer ITS-5 (White et al., 1990). Most successful amplifications involved ITS-L and ITS-4, however, in a few instances this primer pair produced double-banded products. In such cases, a single band was obtained by either increasing the annealing

temperature or by replacing ITS-4 with the reverse primer 307R (D. Nickrent, personal communication to R. J. Bayer). This points to a possible second priming site for ITS-4 in the ITS region of some Carices. Each 100µl reaction mixture contained 10µl of 10X *Taq* reaction buffer (500 mM KCl, 100 mM Tris-HCl (pH 9.0), 1.0% Triton X-100), 6 µl of 25 mM magnesium chloride solution, 65-70µl of MILLI-Q water, 5 µl of each of the primers in 5 pmol concentrations, 2 µl of a 10 mM dNTP solution in equimolar ratio, 1µl of *Taq* DNA polymerase (1 unit), and 1µl-5µl of unquantified template DNA. The PCR samples were heated to 95° C for 3 min prior to the addition of the polymerase in order to denature proteases and nucleases, and to avoid unspecific priming and strand extension during the initial ramping. Double-stranded PCR products were produced on a GeneE® thermal cycler (Techne Cambridge Ltd.) via 30 cycles of DNA denaturation at 95° C for 1 min, primer annealing at 48-55° C for 1 min, and DNA strand extension by *Taq* polymerase at 72° C for 2 min. The PCR was terminated at the end of 30 cycles by a final extension at 72° C for 7 min to allow for the completion of any unfinished strands. Three to five microliters of the double-stranded PCR products were resolved on 1.4% agarose gels using 1 X TBE as the gel buffer. Successful PCR resulted in a single DNA band of = 700 bp in size. In cases where amplification was weak or unsuccessful, the template DNAs were either cleaned using a resin method (Wizard PCR Prep Kit - Promega Corporation, Madison, WI), or subjected to a dilution series. Each procedure, alone or in combination, often resulted in successful PCR amplification. The double stranded DNAs were then purified by differential filtration using ULTRAFREE®-MC filter units (30000 NMWL - Millipore Corporation, Bedford, MA) and diluted with Millipore water based on the strength of the amplification.

Cycle sequencing of the purified amplification product was performed using the dideoxy chain termination method (Sanger, Nicklen, and Coulson, 1977) employed by the *fmol*®*1 Sequencing System (Promega Corporation, Madison, WI). All sequencing primers were 5' end-labeled in a preliminary reaction involving T4 polynucleotide kinase

and [$\gamma$ - $^{32}$P] - dATP (Amersham). Termination products for both ITS 1 and ITS 2 were produced from the double-stranded template DNA with the terminal primers ITS-L and ITS-4, and the internal primers ITS-2 and ITS-3 of White et al. (1990; see fig. 11). However, problems with product secondary structure and the length of gel runs favoured the use of the terminal primers for most sequencing. Fragments were separated electrophoretically on 0.4 mm, denaturing, 6% polyacrylamide gels (8M urea) at 2300v/60W. Gels were fixed in 10% acetic acid for 20 mins, washed in distilled water, and patted dry with paper towels to remove excess fluid. The Gels were then placed in an oven at 65° C for 3 hrs to dry, and exposed to Kodak BIOMAX-100 or BIO-MAX MR film for 24-48 hrs depending on the activity of the gel.

*Sequence Analysis*

The boundaries of the coding (18S, 5.8S, and 26S rDNA) and spacer regions were determined by comparison with the published sequences for rice (Takaiwa, Oono, and Sugiura, 1985). Complete sequences for the entire ITS region (ITS 1, ITS 2, and the 5.8S gene) were not obtained for all taxa, therefore, only ITS 1, ITS 2, and a small variable portion of the 3' end of the 5.8S gene were included in the analysis. DNA sequences were aligned initially using CLUSTAL V (Higgins et al., 1992), then adjusted manually to minimize gap number using SeqApp version 1.8a (Gilbert, 1992) and MacClade 3.0 (Maddison and Maddison, 1992). Regions of ambiguous alignment were excluded from all distance calculations and phylogenetic analyses in order to reduce the chance of systematic error (Swofford et al., 1996). Absolute pairwise distances between sequences were determined in PAUP 4.xx (Swofford, 1997) using the DISTANCE MATRIX option. Gaps were coded as missing for all phylogenetic analyses as recommended by Wojciechowski et al. (1993). Primary sequence lengths and GC contents were determined in Amplify 1.2 (Engels, 1993). These values were manually recalculated for those sequences with ambiguous nucleotide characters (e.g., N, Y, R) which are unacceptable

to the program. Transition/transversion ratios were determined in MacClade 3.0 based on trees produced in PAUP 4.xx.

***Phylogenetic Analysis***

All phylogenetic reconstructions were performed using the computer program PAUP, version 4.xx (Swofford, 1997) run on a Power Macintosh. Heuristic searches were performed on equally weighted characters using Fitch parsimony (1971; all characters unordered) and a SIMPLE stepwise addition of taxa. The "save all minimal trees" (MULPARS), "tree-bisection-reconnection" (TBR), "collapse all zero length branches (COLLAPSE)", and "accelerated transformation" (ACCTRAN) options provided by PAUP were used in searches for optimal trees. Five hundred replicates employing a RANDOM addition sequence were also used in heuristic searches for islands of most parsimonious trees (Maddison, 1991). In order to assess the confidence that could be placed in the monophyly of clades, bootstrap (Felsenstein, 1985) and decay analyses (Bremer, 1988; Donoghue et al., 1992) were performed. Decay indices were estimated using the "converse constraint" method of Baum, Sytsma, and Hoch (1994). In this procedure, multiple heuristic TBR searches using a random addition sequence of 500 replicates were constrained to search for only those trees lacking the hypothesized clade seen in the strict consensus tree of parsimony analyses. A simple subtraction of the shortest trees found in these searches from the most parsimonious tree found in parsimony analyses was equal to the decay index for that clade. Bootstrap values were determined from 500 replicates using heuristic searches and a SIMPLE stepwise addition of taxa. The amount of phylogenetic information in the parsimony analysis was assessed by use of the consistency index (CI; Kluge and Farris, 1969), the retention index (RI; Farris, 1989), and the $g_1$ statistic (Hillis and Huelsenbeck, 1992). Values for the $g_1$ statistic were estimated from the tree-length distribution of 10 000 random trees produced using the RANDOM TREES option in PAUP. This statistic was used to evaluate the

amount of non-random structure in the data set. The number of unambiguous characters state changes along branches was determined in MacClade 3.0. A preliminary analysis was conducted to assess the effect of intraspecific variation in *Carex* section *Phyllostachys* on the topology of trees. The strict consensus and 50% majority rule trees of heuristic searches involving all 42 sequences were compared to those trees produced from searches using only the 1st, 2nd, or 3rd individuals (and combinations thereof) of each of the species sampled more than once (data not shown). No topological differences were observed in any of these analyses, therefore, only the first individual was used in all subsequent analyses (26 sequences in total) in order to reduce computational time. To evaluate the contribution of each of the spacers to the analysis and to determine their level of congruence, both ITS 1 and ITS 2 sequences were analyzed separately. Heuristic tree searches only employed a SIMPLE addition sequence, owing to the length of search times.

The phylogenetic utility of insertions/deletions (indels) was also explored by incorporating indels into the data set as binary characters (bases present = 1/absent = 0). A small portion of the 5.8S gene in which the only variability in this coding region was seen was included in the analysis.

Generalized parsimony (Swofford et al., 1996) using two character transformation weighting schemes that favour transitions over transversions 1.1:1 and 2.2:1 was implemented using the USERTYPE STEPMATRIX option in PAUP. The former weighting scheme was chosen arbitrarily, while the second was derived from the actual number of transitions and transversions observed in the most parsimonious trees. This procedure helps correct for two problems associated with the assumption of equal weighting in normal parsimony: (1) it helps minimize the effect of superimposed changes on the analysis, and (2) it accounts for the common observation that transitions and transversions do not occur at the same rate (e.g., Zurawski and Clegg, 1987). Simulation studies indicate that this type of data transformation significantly improves parsimony's

ability to recover the correct tree with fewer base pairs (Hillis, Huelsenbeck, and Cunningham, 1994).

In addition to parsimony analyses, phylogenetic reconstruction was performed using the minimum evolution and maximum likelihood methods in searches for optimal trees. Unlike parsimony analyses, these methods attempt to estimate the actual amount of evolutionary change and are not as susceptible to the problem of superimposed mutations as parsimony analysis (Swofford et al., 1996). Minimum evolution searches using both a Jukes-Cantor (Jukes and Cantor, 1969) and Log/Det (log-determinant; Lockhart et al., 1994) model of sequence evolution were conducted heuristically utilizing TBR branch swapping and an "as is" addition sequence. These two distance measures complement each other since the simplicity of the Jukes-Cantor model makes it a robust estimator of phylogeny for short sequences (e.g., ITS), while the generality of the Log/Det model reduces the chance that the assumptions of the model will be violated.

Maximum likelihood trees were estimated heuristically using TBR branch swapping and an "as is" addition sequence. A molecular clock was not enforced during these analyses and as a consequence all trees were unrooted. Tree searches were conducted using both a simple Jukes-Cantor model of sequence evolution and a more complex Hasegawa-Kishino-Yano model (HKY85; Hasegawa, Kishino, and Yano, 1985). Parameters for the HKY85 model were estimated from the strict consensus tree of parsimony analysis, and included a correction for rate heterogeneity across sites (i.e., a gamma distribution; Yang, 1993). Simulation studies have demonstrated that a difference in mutational rates between sites can have a serious effect on maximum likelihood estimation as in other tree building methods (Swofford et al., 1996). Functional constraints due to RNA secondary structures and splicing recognition sequences mean that even in non-coding regions, like the ITS region, variable mutation rates across sites should be expected.

## 3.3 RESULTS

***Sequence Analysis***

Aligned sequences for ITS 1, ITS 2, and 17 bp at the 3' end of the 5.8S gene of all 42 individuals sequenced in *Carex* are presented in fig. 12. Sequence for the complete ITS region including both spacers and the entire 5.8S gene of *Carex superata* is given in fig. 13. Summary statistics for all sequences are given in TABLE 7.

The spacer lengths for all Carices examined ranged from 217 bp (*C. peckii* and *C. baccans*) to 223 bp (*C. rugosperma*) for ITS 1 (TABLE 8; fig. 11), and from 211 bp (*C. filifolia*) to 231 bp (*C. pensylvanica* and *C. peckii*) for ITS 2 (TABLE 8; fig. 11). On average, ITS 2 was 3 bp longer than ITS 1. GC contents ranged from 56.4 - 69.5% (mean = 63.4%) for ITS 1, and from 58.8 - 72.7% (mean = 67.8) for ITS 2. The 5.8S subunit in Carices, as determined from three complete sequences (*C. superata*, Naczi 4013 et al., *C. willdenowii*, Naczi 4287 & Thieret, and *C. eleocharis*, Bayer AB-96004 et al.; TABLES 5 and 6), is 166 bp in length. Alignment of the 5.8S gene with sequences from the Cyperaceae (*Kobresia* Willd. and *Cyperus*; sequences from this study), Poaceae (*Oryza sativa*; Takaiwa, Oono, and Sugiura 1985), and two dicot families (i.e., Fabaceae, *Vicia faba*; Tanaka, Dyer, and Brownlee, 1980; and Cucurbitaceae, *Cucumis sativus*; Torres, Ganal, and Hemleben, 1990) identified a three base pair insertion (5'>CAT<3') near the 3' end that was shared by the genus *Carex* and *Kobresia* (fig. 14). With the exception of an autapomorphic mutation in *C. filifolia*, the only variation in 5.8S sequences in *Carex* was a T⇒C transition at the third position of this insertion. In order to align sequences, a nearly equal number of unambiguous insertion/deletion (indel) events had to be inferred for each spacer (ITS 1 = 11; ITS 2 = 10). All of the indels in ITS 1 were 1 bp in length, whereas 5 of the 10 indels in ITS 2 were greater than 1 bp, including a unique 10 bp deletion in the ITS 2 of *C. filifolia*. Nine of the 21 indels in the analysis were autapomorphic, and of the remaining twelve, 7 had a CI=1.0.

The levels of nucleotide divergence in *Carex* ranged from 0.0 to 20.90% in ITS 1 and from 0.0 to 18.72% in ITS 2 (TABLE 7). When both spacer regions were considered, the pairwise sequence divergence between Carices ranged from 0.0 to 19.70%. With the exception of the *Firmiculmes* (7.19%), sequence divergence within sections for which more than one individual was sequenced was low (0.0 - 3.83%; TABLE 9). In contrast, variation between sections was considerably higher, with the lowest values being found in comparisons between sections *Careyanae* and *Acrocystis* (6.41 - 7.13%; TABLE 10), and the highest between sections *Phyllostachys* and *Laxiflorae* (11.08 - 14.22%; TABLE 10). In all cases, the highest divergence values were obtained when members of sections in the "reduced" clade were compared to those in the "compound" clade (see below). Within section *Phyllostachys*, sequence divergence between species was low, ranging from complete identity (e.g., *C. juniperorum* vs. *C. jamesii*, and *C. basiantha* vs. *C. superata*; but see below) to 3.83%. Infraspecific variation was extremely low and never exceeded 1% of the base pairs being compared (TABLE 11). The highest divergence between populations within a species was seen in *C. backii*, where divergence values between the three populations examined ranged from 0.48 - 0.96%. A single point mutation difference was seen in one of the three individuals sampled for both *C. saximontana* and *C. willdenowii*. All other variation within the species of section *Phyllostachys* was due to ambiguous characters that may or may not be true polymorphisms. It should be noted, however, that length variation (± 1 base pair) between tandem repeats within individuals (e.g., *C. juniperorum* and *C. superata*), populations (*C. superata* - 2 bp), and species (*C. basiantha* and *C. superata*) was commonly seen at the 3' poly-A tail of ITS 1 (fig. 12). Because of the volatility of this region, it was excluded from pairwise divergence calculations and phylogenetic analyses.

***Phylogenetic Analysis***

The alignment of ITS spacer sequences resulted in a matrix of 465 characters of which 23 positions in ITS 1, and 6 positions in ITS 2 were deleted due to alignment ambiguities (marked by asterisks in fig. 12). Of the remaining 436 characters, 181 (41.5%) were variable (94 in ITS 1; 87 in ITS 2), and 105 (24.0%) were potentially phylogenetically informative (57 in ITS 1; 48 in ITS 2). Heuristic searches of the reduced 26 taxon data matrix using both a STEPWISE and a RANDOM addition sequence of taxa, produced the same five most parsimonious trees of 341 steps each. All of the most parsimonious trees had a consistency index of 0.66, and a retention index of 0.75. The consistency index for this analysis was considerably higher than the expected value of 0.46 as predicted from the regression analysis of the consistency values from 60 studies by Sanderson and Donoghue (1989). The $g_1$ statistic, as determined from the random distribution of 10 000 trees, for ITS 1, ITS 2, and a combined ITS 1 and ITS 2 data set, was -0.443192, -0.397686, and -0.436309 respectively (TABLE 3). Ignoring invariant sites, the critical values for the $g_1$ statistic of ITS 1 and ITS 2 ($g_1$ = -0.12;100 characters, 25 taxa), and for the combined data set ($g_1$ = -0.09; 250 characters, 25 taxa), were all significant at the $P < 0.01$ level. This suggested that the data had not been randomized with respect to phylogenetic history and were appropriate for phylogenetic analysis (Hillis and Huelsenbeck, 1992; Hillis, Huelsenbeck, and Cunningham, 1994).

The one of five most parsimonious trees topologically identical to the 50% majority rule tree, is presented in fig. 15 along with bootstrap values, decay indices, and unambiguous character changes per branch. The only difference between this tree and the strict consensus of all five trees, was seen in the loss of resolution in section *Phyllostachys* (dotted branches in fig. 15). Two major clades were distinguished in this analysis: (1) a "reduced" clade consisting of sections *Phyllostachys*, *Filifoliae*, and *Firmiculmes*, and (2) a "compound" clade comprising sections *Careyanae*, *Laxiflorae*, *Hymenochlaenae*, *Acrocystis*, *Scirpinae*, *Indicae*, *Cruciatae*, and *Polystachyae*. Other

strongly supported groups in this analysis included a clade basal to the "compound" clade containing two southeast Asian Indocarices, *C. baccans* and *C. cruciata*, and a robust clade consisting of sections *Phyllostachys* and *Filifoliae*. All of the sections in which more than one individual was sequenced were monophyletic, and were strongly supported (bootstrap = 89 - 100%; decay = 6 - 12), with the exception of section *Acrocystis* (bootstrap = 69%; decay = 3). Although the data did not provide firm evidence for the monophyly of section *Acrocystis*, they did support the existence of two strong clades within it; viz. *C. rugosperma* and *C. albicans*, and *C. pensylvanica* and *C. peckii*. Most of the internal branches in the "compound" clade were weak, including the *Careyanae/Laxiflorae* lineage. Within section *Phyllostachys* three species pairs were recognized: *C. backii* and *C. saximontana*, *C. juniperorum* and *C. jamesii*, and *C. basiantha* and *C. superata*. The species pair of *C. juniperorum* and *C. jamesii* formed the best supported clade in the analysis (decay = 2; 86% bootstrap). Little resolution was seen within section *Phyllostachys*, and the separation of the section into "narrow" and "wide" scaled clades (sensu Starr and Ford, 1995) was poorly supported. *C. latebracteata*, a member of the wide-scaled clade, was included in a group that comprised the members of the narrow-scaled clade.

Heuristic searches of a separate ITS 1 data set produced 79 trees, 180 steps long with a CI=0.68 and RI=0.78. The strict consensus of this analysis was entirely compatible with the combined analysis and differed only in the level of resolution achieved. The strict consensus of the 4 trees derived from the analysis of ITS 2 sequences (154 steps; CI=0.67 and RI=0.74), however, was slightly different from the combined analysis in the placement of *C. polystachya* at the base of the "compound clade" instead of the *C. baccans/C. cruciata* clade. Nonetheless, the two analyses were complementary, and the combination of data sets resulted in increased resolution and support for clades that were otherwise absent or weak in either analysis alone (data not shown).

Reanalyzing the data with the inclusion of insertion/deletions (indels) and the single character found at the 3' end of the 5.8S gene had little effect on parsimony analyses. The five trees obtained from these searches were topologically identical to the five found using point mutations alone. The trees from both of these analyses had the same consistency index (CI = 0.66) and retention index (RI = 0.75), and differed only marginally by certain bootstrap and decay values, and by the number of evolutionary steps (cf. figs. 15 and 16).

Searches that used the arbitrary transition/transversion (Ti/Tv) weighting scheme of 1.1:1 produced the same five trees seen in parsimony analyses (data not shown). Topological differences were seen, however, in those analyses that employed the observed Ti/Tv ratio of 2.2:1. A strict consensus of the 8 trees found in these searches is presented in fig. 17. This poorly resolved tree was the only one in this study that placed *C. polystachya* in a basal position within the compound clade, and the only tree to suggest that *C. arctata* and *C. scirpoidea* should form a clade.

Minimum evolution searches using the Jukes-Cantor (JC) and the Log/Det models of sequence evolution each produced a single tree. The least squares lengths for the Jukes-Cantor and Log/Det trees were 0.79 and 0.91, respectively. Both trees accompanied by their bootstrap values are presented in figs. 18 and 19. These two trees were not only very similar to each other but they were also highly congruent with parsimony trees. All of the differences between the two analyses can be isolated to those branches that had extremely short lengths (i.e., < 0.0015). While the topology of the "compound" clade in the JC tree (fig. 18) was identical to that in the parsimony analysis, the Log/Det (fig. 19) analysis showed the *Laxiflorae* to be sister to the *Hymenochlaenae* and not the *Careyanae*. This was the only tree that did not unequivocally place the Indocarices as basal. Most of the differences between the two analyses were seen in section *Phyllostachys*. Although all three of the species pairs identified in the parsimony

analysis of section *Phyllostachys* were also present in both minimum evolution trees, the order of branching within the section was slightly different. A clade comprising *C. juniperorum*, *C. jamesii*, *C. latebracteata*, *C. basiantha* and *C. superata* was present in both trees but either *C. willdenowii* or a clade containing *C. backii* and *C. saximontana* was found in the most basal position.

Searches using the optimality criterion of maximum likelihood and both a JC and HKY85 (with correction for rate heterogeneity) model produced 15 trees each with -Ln likelihoods of 2510.72 and 2339.39 respectively. A single tree for each substitution model was arbitrarily chosen from amongst the 15 best trees and these are presented in figs. 20 and 21. These two trees differed only in the placement of *C. arctata* as sister to the *Laxiflorae* as opposed to being sister to a *Laxiflorae/Careyanae* clade (seen in the Log/Det tree as well). As seen in the minimum evolution JC analysis, both maximum likelihood searches placed *C. willdenowii* basal to the rest of section *Phyllostachys*.

***Summary of Results***

The consensus of parsimony, minimum evolution, and maximum likelihood analyses pointed to the existence of two divergent clades. The first of these large clades, characterized as the "compound" clade, comprised four sections from subgenus *Carex* (i.e., sections *Careyanae*, *Laxiflorae*, *Hymenochlaenae*, and *Acrocystis*) that formed a weak but stable clade in all analyses, a section from subg. *Primocarex* (i.e., the *Scirpinae*), and three sections from subg. *Indocarex*. In all of the analyses, species representative of subgenus *Indocarex* did not form a natural group. With one exception, searches placed either *C. polystachya* (subg. *Indocarex*), or the clade consisting of *C. baccans* and *C. cruciata* (subg. *Indocarex*) in a basal position within the "compound" clade. Two weak, but consistent clades were seen within the "compound" clade: one comprises *Carex scirpoidea* and *C. polystachya*, while the other consists of the species

from sections *Careyanae*, *Laxiflorae*, and *Hymenochlaenae*. In most instances, sections *Careyanae* and *Laxiflorae* formed a clade separate from the *Hymenochlaenae*, however, this topology was not always stable.

The second major clade, characterized as the "reduced" clade, was made up of two sections from subg. *Primocarex* (i.e., sections *Filifoliae* and *Firmiculmes*), and a single section from subg. *Carex* (i.e., section *Phyllostachys*). The relationships between these three taxa were identical in all analyses; the *Firmiculmes* constituted the most basal clade, while the *Filifoliae* and *Phyllostachys* were sisters. Variation in the topology of the "reduced" clade was only seen within section *Phyllostachys*. Three species pairs (*C. backii* and *C. saximontana; C. juniperorum* and *C. jamesii*; *C. basiantha* and *C. superata*) and a clade comprising *C. latebracteata*, *C. juniperorum*, *C. jamesii*, *C. basiantha*, and *C. superata* were always present in analyses, however, their branching order was variable, and the most basal group was either *C. willdenowii*, or a clade comprising *C. backii* and *C. saximontana*.

## 3.4 Discussion

***ITS Sequence Evolution in Carex.***

The sizes of the ITS region (602-617 bp) and spacers (ITS 1 = 217-223 bp; ITS 2 = 211-231 bp) in *Carex* are similar to those reported for a wide variety of flowering plants (reviewed in Baldwin et al., 1995). In general, sequences from the Poaceae showed the greatest similarities to *Carex* in both the size of the ITS region ( Poaceae, 585-603 bp, Hsiao et al., 1995a, 1995b) and its spacers (ITS 1, 214-221 bp; ITS 2, 205-221 bp; Hsiao et al., 1995a, 1995b). However, the range of sequence divergence within *Carex* is generally much higher than seen in the Poaceae, where the levels of variation observed between species of *Carex* would be associated with relatively distant genera (cf. Hsiao et al., 1995a, 1995b). Generally speaking, the range of sequence divergence within *Carex* (0.00 - 19.70%) was much higher than what is normally seen for genera in the ITS region (e.g., *Epilobium* 0.0 - 12.9%, Baum, Sytsma, and Hoch, 1994; *Antennaria* 14%, Bayer, Soltis, and Soltis, 1996; but cf. *Arceuthobium*, Nickrent, Schuette, and Starr, 1994) and was comparable to the values that are commonly observed between genera within families (e.g., *Maloideae* 2.7% - 16.1%, Campbell et al., 1995). The reasons for this are unknown, although the age of the genus and/or poor circumscription (see below) could be involved.

All of the highest pairwise sequence divergence values were seen between *C. filifolia* and the rest of the Carices. The sequence of this species had a number of features that made it distinct. It had the shortest combined ITS 1 and ITS 2 sequence by 7 bp, it possessed a considerably lower GC content (57.5%) than all other Carices (62% - 71.1%), it had a unique 10 bp deletion in ITS 2, and it possessed extremely long branch lengths on all trees. *Carex filifolia* represents one of many reduced species that has blurred the generic limits between *Carex* and the rest of the *Cariceae*. Its completely closed but membranaceous perigynium has suggested to some authors that it is best placed in the genus *Kobresia* (*Kobresia globularis*; Dewey, 1836; Ivanova, 1939),

whereas its well developed rachilla has suggested to others that it is best treated as an *Uncinia* Persoon (*U. breviseta*; Torrey, 1836). Although this analysis strongly suggests that *C. filifolia* (sect. *Filifoliae*), is sister to sect. *Phyllostachys*, and that along with the *Firmiculmes* these sections may represent a much larger "reduced" lineage within *Carex*, the phylogenetic position of this clade, and of *C. filifolia*, will not be truly resolved until a proper taxonomic sampling regime of *Carex* and the other genera in the *Cariceae* has been conducted. The poor support seen in the lower branches of the compound clade is most likely due to poor taxonomic sampling, but it could also be an indication of rapid radiation. Many authors have felt that widespread speciation has taken place as recently as the Pleistocene (Kreczetovicz ,1936; Whitkus, 1981; Kukkonen and Toivonen, 1988; Ball, 1990), and based on phytogeographical evidence, Stebbins (1981) has suggested that most speciation in *Carex* has taken place within the last 15 million years.

Some difficulty was encountered in determining the boundaries of the 5.8S gene in *Carex* since efforts to align sequences with several families, including the Poaceae, suggested that a number of mutations have occurred at both the 5' and 3' ends of this gene. Because there are no other sequences from the Cyperaceae to help with alignment, the tail ends of the 5.8S gene of a species from both the genus *Kobresia* and *Cyperus* were sequenced. The alignment of these sequences revealed a 3 base pair insertion near the 3' end of the gene (fig. 14), that was shared by the genera *Carex* and *Kobresia* (both in the *Cariceae*), but not with the genus *Cyperus* (*Cypereae*). Such size differences in the 5.8S gene within a single family is highly unusual given that this gene is nearly invariant (163-164 bp) in all other flowering plant taxa that have been sequenced to date (Baldwin et al., 1995; but see Ritland and Straus, 1993, and Nickrent, Schuette, and Starr, 1994). To my knowledge, this is the largest insertion known to have occurred within the 5.8S gene of flowering plants, and it may prove to be a useful character for delimiting either the *Cariceae* or a clade within this tribe.

***Subgeneric Circumscriptions in Carex***

In this study, two major clades are identified in *Carex*: (1) an evolutionally "reduced" clade consisting of sections that are traditionally placed in either subg. *Carex* or subg. *Primocarex* (i.e., sections *Phyllostachys*, *Filifoliae*, and *Firmiculmes*), and (2) a "compound" clade comprising sections typically placed in subg. *Carex*, *Primocarex*, and *Indocarex* (i.e., sections *Careyanae*, *Laxiflorae*, *Hymenochlaenae*, *Acrocystis*, *Scirpinae*, *Indicae*, *Cruciatae*, and *Polystachyae*). These two groups suggest that three of the four subgenera recognized by Kükenthal (*Primocarex*, *Carex*, and *Indocarex*) are unnatural, and they shed new light on the historical controversies that have surrounded the circumscriptions of the subgenera and the evolution of the genus.

Subgenus *Carex* with its enormous number of species and high degree of variability in the sexual expression of the spikes is, not surprisingly, polyphyletic in this analysis (Nannfeldt, 1977; Reznicek, 1990). Although the subgenus is unnatural, this is only due to the placement of sect. *Phyllostachys* within the reduced clade. The multispicate "core" sections (i.e., sections *Careyanae*, *Laxiflorae*, *Hymenochlaenae*, and *Acrocystis*) typical of subgenus *Carex*, with terminally staminate and laterally pistillate inflorescence units, still form a weak, albeit consistent clade in my analyses. However, this clade does not appear to represent a completely separate lineage, but seems instead to represent the most derived element of a much wider lineage in *Carex* that includes a portion of subg. *Primocarex*, and has, at its base, sections representative of subg. *Indocarex*.

This "compound" clade is consistent with a wide number of different evolutionary hypotheses and taxonomic classifications of the genus that may seem conflicting, but are generally concordant when examined within the context of the trees produced in this analysis. The derived position of the "core" subg. *Carex* clade and the basal arrangement of subg. *Indocarex* is concordant with the prevailing belief that the clade is advanced and that it is has evolved from the Indocarices by a reduction and specialization of the

inflorescence (e.g., Kükenthal, 1909; Nelmes, 1951; Smith and Faulkner, 1976; Reznicek, 1990). The paraphyly of subgenus *Indocarex* and the inclusion of a section from subg. *Primocarex* is also consistent with the decisions of Ohwi (1936) and Koyama (1962) to recognize only two subgenera, *Eucarex* Coss. et Germ. (=*Carex*) and *Vignea*, the former group comprised of subg. *Primocarex*, in part, and all of subg. *Carex* and *Indocarex*. The position of subg. *Indocarex* at the base of the compound clade is also significant because it is consistent with several hypotheses based on the study of phytogeography (Kreczetovicz, 1936; Nelmes, 1951; Ball, 1990) and inflorescence structure (Smith and Faulkner, 1976), that suggest that the group is primitive and the possible progenitor of a subg. *Carex/Primocarex* line (e.g., Kreczetovicz, 1936; Koyama, 1957, 1962; Smith and Faulkner, 1976).

The other major finding of this study is that subgenus *Primocarex* appears to be polyphyletic. The similarity of their purple basal sheaths and pubescent perigynia, and the frequent occurrence of rudimentary lateral inflorescence units and sterile bracts in section *Scirpinae*, strongly suggested to both Kreczetovicz (1936) and even Kükenthal (1909), that sect. *Acrocystis* and sect. *Scirpinae* were closely related. However, in nearly all my analyses sect. *Scirpinae* formed a weak clade with *C. polystachya*, a Mexican member of subg. *Indocarex*. The clear separation of the *Scirpinae* from the members of the "reduced" clade and its placement next to a member of subg. *Indocarex* is significant in that it confirms the common belief that extreme reduction has occurred along several independent evolutionary lines in *Carex* (Kreczetovicz, 1936; Nelmes, 1952; Smith and Faulkner, 1976). Unfortunately, the ultimate origin of the *Scirpinae* or the reduced clade cannot be determined in this study due to the small taxonomic sample and the lack of taxa from outside the genus to polarize the tree. Whether the origin of these diverse species lies within the genus or whether some of the species have evolved outside the genus as suggested by many authors (e.g., Kreczetovicz, 1936; Ivanova, 1939; Nelmes, 1952), will need to be determined in a wider tribal level study.

***Sectional Delimitation and Taxonomic Utility of the ITS Region in Carex***

One of the main reasons that phylogeny within *Carex* is so poorly known can be traced to the problem of circumscribing infrageneric taxa, particularly sections (Crins, 1990). Extreme floral reduction (Smith and Faulkner, 1976), uniform vegetative morphology and anatomy (Reznicek, 1990; Metcalf, 1971; Standley, 1990a), and repeated events of parallelism and reversal (Reznicek, 1990; Naczi, 1992; chapter 4), have obscured phylogenetic trends and have led to the recognition of many artificial sections. A number of recent studies have clarified the limits of some sections (e.g. *Carex* sect. *Stellulatae*, Reznicek, 1980; sect. *Hymenochlaenae*, Reznicek, 1986; sect. *Ceratocystis*, Crins and Ball, 1988; sect. *Griseae*, Naczi, 1992), and a few of these groups have even been used in cladistic analyses (i.e., sect. *Ceratocystis*, Crins and Ball, 1988; sect. *Griseae*, Naczi, 1992), but the most serious obstacle to a fuller understanding of the evolution of *Carex* remains; a lack of conserved characters that can be easily used to strongly delimit sections and infer their relationships.

The general trend in this study of low infrasectional and high intersectional sequence divergence, coupled with strong statistical support for the monophyly of sections, suggests that the ITS region may provide evolutionally conserved characters appropriate for defining sections. The differences seen between species of sections *Laxiflorae* (*C. laxiflora* and *C. blanda*) and *Careyanae* (*C. plantaginea* and *C. careyana*), are particularly enlightening in this regard. Most modern authors (e.g., Kükenthal, 1909; Mackenzie, 1935; Fernald, 1950) have not recognized section *Careyanae* as being distinct from the *Laxiflorae*. Recent morphological (Bryson, 1980) and biochemical (Manhart, 1986) treatments of the *Laxiflorae* have likewise continued to recognize only a single section, but do concede that the *Laxiflorae* s.l. is composed of two distinctive groups. Naczi (1992) recognized the *Laxiflorae* and *Careyanae* as distinct sections, and demonstrated in a phylogenetic analysis that the *Careyanae* were sister to section *Griseae*, and not to section *Laxiflorae* s.s. The present phylogenetic analysis supports the

conclusions of Naczi (1992) in that it indicates weak support for a *Careyanae/Laxiflorae* clade, but provides strong evidence (DI=10-15; 100% bootstrap) for the recognition of two distinct sections. Sequence divergence within (0.95%, *Laxiflorae*; 2.86%, *Careyanae*; TABLE 9) and between (8.15 - 8.59%; TABLE 10) sections *Laxiflorae* and *Careyanae* further supports such a conclusion. In fact, the levels of divergence observed between sections *Laxiflorae* and *Careyanae* were higher than the levels of divergence that either of these sections displayed with species from section *Acrocystis* (6.41-7.13% *Careyanae*; 6.67-7.40% *Laxiflorae*; TABLE 10). Differences in perigynium characters between the two groups, such as the greater number of nerves in the *Careyanae* (>40; Naczi, 1992), and the possession of acute sides and edges (sect. *Careyanae*) as opposed to obtuse (sect. *Laxiflorae*) (Manhart, 1986; Naczi, 1992), clearly indicate that these are natural groups that should be recognized at the sectional level.

Despite the low levels of sequence divergence observed within sections, the ITS region can in some instances provide conserved characters that are useful for circumscribing critical groups below the sectional level. A good example is seen in the clear separation of *C. albicans* from *C. peckii* (sect. *Acrocystis*). Although these two taxa are distinct at the species level, Gleason (1952) has treated them as varieties of *C. nigro-marginata* Schw. The present analysis, however, places these taxa into two separate and strongly supported clades (DI=3-4; >90% bootstrap), and identifies at least twelve mutational differences in their ITS regions. While the above example may seem trivial, it does suggest that simple sequence comparisons could provide important characters to future taxonomic studies in the genus. Section *Acrocystis*, in particular, has numerous unresolved taxonomic problems (e.g., *C. rugosperma* complex or *C. rossii* complex) where the ITS region could be an effective aid in resolving the complex taxonomy of this group.

***The Phylogenetic Position of Carex section Phyllostachys***

My data show little concordance with former theories on the origin and phylogenetic position of sect. *Phyllostachys* in *Carex*. Bailey's (1885, 1886) very detailed and complex hypotheses of relationship are entirely contradicted by the ITS sequence data. His enlarged *Phyllostachys* that includes two subsections, one containing species here placed in section *Phyllostachys* (subsection *Bractoideae*), and the other comprising species currently placed in section *Firmiculmes* (subsection *Phyllostachyae*), is a polyphyletic group in this analysis. The position of sect. *Phyllostachys* in a clade separate from section *Acrocystis* also contradicts the hypothesis that sect. *Phyllostachys* is an offshoot of this section, as does the potential link to members of a *Laxiflorae*, *Granulares, Careyanae*, and *Griseae* clade to which Tuckerman (1843) had also felt sect. *Phyllostachys* was close.

My ITS sequence data largely support the general conclusion reached by Holm (1900) that Bailey's classification was artificial; however, my data also refute the classification Holm proposed to resolve it. Holm's claim that the *Phyllostachys* were not "sufficiently characteristic" to be considered a section is clearly contradicted by the frequent recognition of its singularity in *Carex* (e.g., Bailey, 1886; Mackenzie, 1935; Catling, Reznicek, and Crins, 1993), and by the robust statistical (bootstrap = 99%; DI = 6) and character (7 synapomorphies) support found for its monophyly in this molecular study. Holm's decision to consider the *Phyllostachys* as primitive within section *Hymenochlaenae* is equally untenable, and is not supported by this study. The basis of this decision is not clearly stated but seems to be inspired by his adherence to Drejer's (1844) concept of the "grex" (equivalent to the section) as composed of "weakened", "central", and "diverging" forms (the *Phyllostachys* being weakened forms, i.e., "*formae hebetatae*"), and by his reliance on the abnormal growth of the rachilla as a conceptional means by which the compound inflorescences of sect. *Hymenochlaenae* may have evolved (see below). It is quite likely that Drejer's (1844) rejection of the genus

*Phyllostachys* Torrey, and his failure to treat the group as a distinct section, provided further impetus to Holm to place them in sect. *Hymenochlaenae*. Although Holm (1900, 1903) successfully rejected several unlikely hypotheses made by previous authors, his final decision to place sect. *Phyllostachys* in *Hymenochlaenae* only confused the systematics of this section even further by creating yet another artificial taxon.

Although this study supports the conclusions reached by Holm (1900, 1903) and Kreczetovicz (1936) for an expanded concept for subg. *Carex* that includes the Indocarices, it does not support the reasoning by which these authors reached these conclusions, or the important role that sect. *Phyllostachys* played in their conception. Both of these authors stressed the importance of the rachilla and its abnormal growth in their determinations of evolution in the genus. Kreczetovicz (1936) considered the rachilla a derived character whose aberrant growth, among other characters, pointed to a recent and direct origin for sections *Firmiculmes* and *Phyllostachys* from subg. *Indocarex* by transmutive reduction. Similarly, Holm (1900, 1903) used a teratological specimen of *C. backii* to argue for an expanded subg. *Carex*, noting that sect. *Phyllostachys* was, "in no wise to be distinguished from the *Vigneastra* (=subg. *Indocarex*)". The present analysis, however, fails to find evidence to suggest that the presence of rachillae, or their abnormal growth, is any indication of phylogenetic relatedness, atavism, or primitiveness. The same type of abnormal growth of the rachilla present in sections *Phyllostachys* and *Firmiculmes*, is also prevalent in groups in the compound clade such as sections *Hymenochlaenae* (Koyama, 1962) and *Acrocystis* (Svenson, 1972). In fact, the presence of rachillae and the abnormal growth of rachillae, occurs sporadically in all four of the subgenera in *Carex* (Snell, 1936; Svensen, 1972; Smith and Faulkner, 1972; Reznicek, 1990), suggesting that these characters do not confer any special insight into the phylogeny of this genus (Reznicek, 1990). Complicated theories to explain the origin and derivation of the evolutionally reduced sections in the genus, such as transmutive reduction, or hypotheses that rely on the presence and proliferation of rachillae appear to

be false (Reznicek, 1990). Section *Phyllostachys* may have had a multispicate origin, however, it does not appear to lie within either subgenus *Indocarex* or *Carex*.

Particularly unreliable, were Savile and Calder's (1953) hypotheses of phylogeny in *Carex*. In their arrangement, sect. *Phyllostachys* is placed in an advanced lineage within subg. *Eucarex* (=*Carex*) that includes sect. *Scirpinae*, while sect. *Firmiculmes* is far removed from the *Phyllostachys* in subg. *Carex*, and sect. *Filifoliae* is distantly situated at the base of subg. *Primocarex*. The ITS data, on the other hand, indicate that sections *Phyllostachys* and *Filifoliae* are sister groups, that they are closely related to sect. *Firmiculmes*, and that the *Scirpinae* are at best a distantly related section in a completely separate lineage. Although their phylogeny was based primarily on smut host-parasite records, morphological and cytological evidence also played a large part in their reconstruction. However, conflicts between these data sets were essentially ignored in favour of preconceived notions of evolution in the genus. For example, the rachilla was considered to be a character of "fundamental importance" that indicated that their Primocarices were "plainly primitive", and yet, they considered evolutionally reduced sections like the *Phyllostachys* that have rachillae (Mackenzie, 1935; Starr, personal observation), and even sections like the *Scirpinae* that they removed from subgenus *Primocarex* and which also has rachillae (Kükenthal, 1909), as highly evolved, because their smuts appeared to be related to smuts that infect multispicate members in subg. *Carex*. Although smut infection records can provide useful data, a lack of correlation between these characters and other conventional characters at higher taxonomic levels most likely confines their utility to the sectional or infrasectional levels (Smith and Faulkner, 1976).

In my examination of the hypotheses of relationship of section *Phyllostachys* to the rest of the Carices, only Mackenzie (1935) has suggested a relationship between sections *Phyllostachys* and *Filifoliae*. Although he places the *Firmiculmes* slightly more distant from the *Phyllostachys*, his classification is the one that agrees best with my

molecular results. Nonetheless, it is similar to all of the above hypotheses in that it positions section *Phyllostachys* firmly within an expanded or reduced concept for subg. *Carex*. Thus my finding that sections *Phyllostachys*, *Filifoliae*, and *Firmiculmes* might form a clade separate from the members of subg. *Carex* is a novel hypothesis.

***Phylogeny in Carex section Phyllostachys***

Although I have succeeded in clarifying the phylogenetic position of section *Phyllostachys* within *Carex*, the ITS region was not variable enough adequately to resolve infrasectional relationships. Owing to a lack of characters, clade composition and branching order was highly variable in my analyses. The only consistent clades were those of the three species pairs previously identified in chapter 2 (i.e., *C. backii*/*C. saximontana*, *C. juniperorum*/*C. jamesii*, and *C. basiantha*/*C. superata*).

The very low levels of sequence divergence observed in sect. *Phyllostachys* is consistent with the hypothesis that its species are very closely related, and recent in origin (Kreczetovicz, 1936; chapter 4). The levels of sequence divergence detected within section *Phyllostachys* are comparable to the levels observed in the genus *Robinsonia* (Sang et al., 1995) and *Dendroseris* (Sang et al., 1994); both of these genera are endemic to the volcanic Juan Fernandez Islands of Chile, where they are thought to have originated shortly after the creation of the archipelago approximately 4 million years ago (Stuessy et al., 1984).

The notable differences in anatomy, micromorphology, and macromorphology within section *Phyllostachys* (Naczi, Reznicek, and Ford, 1997; Catling, Reznicek, and Crins, 1993; chapter 2) suggest that the rate of evolution in these characters is higher than the mutational rate of ITS. Combining all data sets could resolve infrasectional relationships in sect. *Phyllostachys* by taking advantage of the different evolutionary rates of each character type. If the two sections closest to sect. *Phyllostachys* (*Firmiculmes* and *Filifoliae*) are used as the only outgroups in such a combined analysis, it is also possible

that ITS regions that had to be excluded at the subgeneric level because they were too variable, could provide phylogenetically informative characters in an infrasectional study. A combined analysis using anatomy, micromorphology, macromorphology, and ITS sequence data will be used in chapter 4 to resolve phylogenetic relationships within sect. *Phyllostachys*.

## 3.5 CONCLUSIONS

My study provides strong evidence to suggest that subgenus *Carex*, *Indocarex*, and *Primocarex*, as presently circumscribed, are artificial. As many previous authors have believed (e.g., Bailey, 1886; Kreczetovicz, 1936; Ohwi, 1936; Koyama, 1962), subgenus *Carex* and *Indocarex*, along with a portion of subg. *Primocarex* forms a monophyletic group. The most basal species in this clade are Asian Indocarices, supporting phytogeographical and morphological studies (e.g., Kreczetovicz, 1936; Nelmes, 1951; Ball, 1990) that have suggested that this group is primitive. Phylogenetic hypotheses and relationships based on the presence or abnormal growth of the rachilla are not supported in this analysis. Extreme reduction appears to have occurred along several different lineages in *Carex* as was commonly believed, and the data support the existence of an evolutionally "reduced" clade comprising sections *Phyllostachys*, *Filifoliae*, and *Firmiculmes* that is separate from the main body of the *Indocarex/Carex/Primocarex* line. Unfortunately, the ultimate origin of this clade and of the most basal clade in the Carices cannot be determined since trees were not polarized with taxa from outside of the genus. Whether this clade has a vignean origin, or an origin from outside of the genus as suggested by Nelmes (1952) for sections *Filifoliae* (from *Kobresia*) and *Firmiculmes* (from *Uncinia*), can only be resolved by a much wider tribal level study.

One of the most enduring of all problems in the systematics of *Carex* has been the circumscription of sections. Although the ITS region was not variable enough to resolve relationships within sect. *Phyllostachys*, it shows great potential for clarifying sectional

limits and for hypothesizing relationships between sections in *Carex*. The strong support for the monophyly of the morphologically similar sections *Laxiflorae* and *Careyanae*, fully illustrates the potential of the region for sectional delimitation.

Any study that includes only a small portion of a taxon as large as *Carex* must come with a caveat. Four hundred and fifty base pairs are not going to produce a fully resolved *Carex* phylogeny, and 26 taxa from a genus of 2000 species are not going to resolve subgeneric problems that have existed for hundreds of years. Many of the relationships that have been enumerated herein can only be expected to change as a wider taxonomic sample is introduced. However, the great potential of this region, and of DNA characters in general, for helping to resolve many long-standing phylogenetic and taxonomic problems in this genus has been demonstrated. The first step, and the most pressing if we are to understand the evolution of this genus, must be to address the problem of circumscribing *Carex* from its satellite genera in the *Cariceae*. The extreme reduction seen in this genus coupled with an extraordinary range of subtle morphological variation has given rise to the identification of numerous "transitional species" that blur limits of the genera in the tribe (Nelmes 1951; Reznicek, 1990). The circumscription of the genus *Carex* is the central problem to understanding the evolution of the *Cariceae*, and until the question of "what is, and what is not a *Carex*" is answered, the results of any investigations into the phylogeny of the tribe or genus can only be regarded as speculative.

TABLE 5. Collection data for populations of species sampled from *Carex* section *Phyllostachys*. Vouchers are deposited at KNK and WIN unless otherwise noted. Herbarium acronyms follow Holmgren & al. (1990). Individuals sampled from the same population are numbered (1) and (2).

| Subgenus | Species studied | Voucher |
|---|---|---|
| 1. *Carex* | *C. backii* Boott | CANADA. Manitoba: Treesbank, Ford 9502 & Starr. Ontario. Simcoe Co., Reznicek & Reznicek 6364 MICH. North Grimsby Twp., Ball (s.n.). |
| | *C. saximontana* Mackenzie | CANADA. Saskatchewan. Cypress Hills, Ford 9526 & Starr. (1) Manitoba. Treesbank, Ford 9501 & Starr (2) Ford 9501 & Starr |
| | *C. latebracteata* Waterfall | U.S.A. Arkansas. Polk Co., Naczi 3948 & Ford. Oklahoma. McCurtain Co., Naczi 3953 & Ford. |
| | *C. juniperorum* Catling, Reznicek, & Crins | CANADA. Ontario. Hastings Co., Oldam (s.n.) et al. TRTE. U.S.A. Ohio. Adams Co., Naczi 3878. Kentucky. Bath Co., Naczi 3890. |

TABLE 5. Continued.

| Subgenus | Species studied | Voucher |
|---|---|---|
| 1. *Carex* | *C. jamesii* Schweinitz | CANADA. Ontario. Essex Co., Pelee Island, Oldham (s.n.) TRTE. Virginia. Bath Co.; Naczi 4482 & Thieret. |
| | *C. willdenowii* Willdenow | U.S.A. Ohio. Pike Co, Naczi 3887. Pennsylvania. Bradford Co., Naczi 4287 & Thieret. Kentucky. Franklin Co., Naczi 3835 & Borne. |
| | *C. superata* Naczi, Reznicek, & B. A. Ford | (1) U.S.A. Mississippi. Tishomingo Co., Naczi 4013 et al. (2) Naczi 4013 et al. Alabama. Butler Co., Naczi 3990 & Ford. |
| | *C. basiantha* Steudel | U.S.A. Alabama. Butler Co., Naczi 3991 & Ford. Mississippi. Itawamba Co., Naczi 4005 et al. Louisiana. West Feliciana Parish, Naczi 3987 & Ford. |

TABLE 6. Taxonomy and collections of taxa used in this study Vouchers are deposited at KNK and WIN unless otherwise noted. Herbarium acronyms follow Holmgren & al. (1990).

| Subgenus | Section | Species studied and voucher |
|---|---|---|
| 1. *Carex* | *Acrocystis* Dumortier | *C. albicans* Willd. |
| | | Arkansas, Scott Co. |
| | | Ford & Naczi 9440. |
| | | *C. peckii* Howe |
| | | Alberta, Edmonton, Starr 96010. |
| | | *C. pensylvanica* Lam. |
| | | Manitoba, Portage la Prairie, |
| | | Ford et al. 9604. |
| | | *C. rugosperma* Mackenzie |
| | | Manitoba, Whiteshell Provincial |
| | | Park. Ford 94111. |
| | *Careyanae* Tuckerm. | *C. careyana* Torr. |
| | ex Kükenth. | Ontario, Ball (s.n.). |
| | | *C. plantaginea* Lam. |
| | | Peterborough Co. Bakowsky |
| | | #96-174. |
| | *Hymenochlaenae* (Drejer) | *C. arctata* Boott |
| | L.H. Bailey | Manitoba, Whiteshell Provincial |
| | | Park. Ford et al. 9624. |

TABLE 6. Continued

| Subgenus | Section | Species studied and voucher |
|---|---|---|
| | ***Laxiflorae*** Kunth | ***C. laxiflora*** Lam.<br>Arkansas, Ford & Naczi 9443.<br>***C. blanda*** Dewey<br>Ontario, Peterborough Co.<br>Bakowsky #96-176. |
| 2. ***Indocarex*** Baill. | ***Cruciatae*** (C.B. Clark) Nelmes | ***C. cruciata*** Wahlenb.<br>Mulu National Park, Sarawak,<br>Malaysia, Yen (s.n.) '95 WS. |
| | ***Polystachyae*** Tuckerm. | ***C. baccans*** Nees.<br>Wu-lai, Taiwan, Yen (s.n.) '95 WS. |
| | ***Indicae*** Tuckerm. | ***C. polystachya*** Sw.<br>Cayo district, Belize,<br>Jones & Wipff 11275 MICH. |
| 3. ***Primocarex*** Kükenth. | ***Filifoliae*** Tuckerm. | ***C. filifolia*** Nutt.<br>Manitoba, Lauder Sand Hills<br>Punter & Punter (s.n.). |
| | ***Firmiculmes*** Kükenth. | ***C. multicaulis*** L. H. Bailey<br>California, San Diego Co.<br>Ford & Starr 9567. |

TABLE 6. Continued

| Subgenus | Section | Species studied and voucher |
|---|---|---|
| | | *C. geyeri* Boott<br>Montana, Cascade Co.<br>Starr et al. MT96039. |
| | *Scirpinae* Tuckerm. | *C. scirpoidea* Michx.<br>Alberta, Jasper National Park.<br>Bayer AB-96010 et al. |
| 4. *Vignea* (P. Beauv. ex Lestib. f.) Peterm. | *Deweyanae* Tuckerm. | *C. deweyana* Schw.<br>Alberta, Edmonton, Starr 96007. |
| | *Divisae* Christ | *C. eleocharis L. H.* Bailey<br>Bayer AB-96004 et al. Alberta. |

TABLE 7. General sequence statistics for ITS 1 and ITS 2 (alone and combined).

| | ITS 1 | ITS 2 | Combined (ITS 1 + ITS 2) |
|---|---|---|---|
| Length range (bp) | 217 - 223 | 211 - 231 | 431 - 450 |
| Length mean (bp) | 220.27 | 223.50 | 443.77 |
| Aligned length (bp) | 231 | 234 | 465 |
| G+C content range (%) | 56.4 - 69.5 | 58.8 - 72.7 | 57.5 - 71.1 |
| G+C content mean (%) | 63.4 | 67.8 | 65.6 |
| Sequence divergence (%) | 0.00 - 20.90 | 0.00 - 18.72 | 0.00 - 19.70 |
| Number of excluded sites | 23 (10.0%) | 6 (2.6%) | 29 (6.2%) |
| Number of indels | 11 | 10 | 21 |
| Number of variable sites | 94 (45.2%) | 87 (38.2%) | 181 (41.5%) |
| Number of potentially informative sites | 57 (27.4%) | 48 (21.1%) | 105 (24.1%) |
| Number of constant sites | 114 (54.8%) | 141 (61.8%) | 255 (51.6%) |
| Number of autapomorphic sites | 37 (17.8%) | 37 (16.2%) | 74 (17.0%) |
| Transistions (minimum) | 116 | 111 | 227 |
| Transversions (minimum) | 59 | 44 | 103 |
| Transistions/transversions | 1.97 | 2.52 | 2.20 |
| Skewness of tree-length distribution | -0.443192 | -0.397686 | -0.436309 |
| ($g_1$ value for 10 000 random trees) | P<0.1 | P<0.1 | P<0.1 |

TABLE 8. The length and G+C content for ITS1, ITS2, and the combined ITS 1 and ITS 2 spacers of the taxa used in this study.

| Species | ITS1 | | ITS2 | | ITS1 + ITS2 | |
|---|---|---|---|---|---|---|
| | Length (b.p.) | G+C (%) | Length (b.p.) | G+C (%) | Length (b.p.) | G+C (%) |
| *C. backii* | 222 | 61.7 | 222 | 65.8 | 444 | 63.7 |
| *C. saximontana* | 221 | 62.0 | 222 | 65.3 | 443 | 63.7 |
| *C. latebracteata* | 222 | 59.0 | 223 | 65.5 | 445 | 62.2 |
| *C. juniperorum* | 221 | 62.4 | 221 | 67.4 | 442 | 64.9 |
| *C. jamesii* | 221 | 62.4 | 221 | 67.4 | 442 | 64.9 |
| *C. willdenowii* | 222 | 61.7 | 221 | 67.4 | 443 | 64.6 |
| *C. superata* | 222 | 62.2 | 221 | 67.0 | 443 | 64.6 |
| *C. basiantha* | 222 | 62.2 | 221 | 67.0 | 443 | 64.6 |
| *C. filifolia* | 220 | 56.4 | 211 | 58.8 | 431 | 57.5 |
| *C. geyeri* | 220 | 66.8 | 222 | 69.8 | 442 | 68.3 |
| *C. multicaulis* | 220 | 62.3 | 222 | 69.8 | 442 | 66.1 |
| *C. eleocharis* | 222 | 61.0 | 220 | 66.8 | 442 | 63.9 |
| *C. deweyana* | 219 | 66.7 | 219 | 69.4 | 438 | 68.0 |
| *C. rugosperma* | 223 | 64.6 | 227 | 67.4 | 450 | 66.0 |
| *C. albicans* | 221 | 64.7 | 227 | 68.3 | 448 | 66.5 |

TABLE 8. Continued.

| Species | ITS1 | | ITS2 | | ITS1 + ITS2 | |
|---|---|---|---|---|---|---|
| | Length (b.p.) | G+C (%) | Length (b.p.) | G+C (%) | Length (b.p.) | G+C (%) |
| *C. pensylvanica* | 219 | 64.2 | 231 | 68.0 | 450 | 66.1 |
| *C. peckii* | 217 | 64.4 | 231 | 68.0 | 448 | 66.2 |
| *C. plantaginea* | 221 | 67.9 | 225 | 70.2 | 446 | 69.1 |
| *C. careyana* | 220 | 69.5 | 227 | 72.7 | 447 | 71.1 |
| *C. laxiflora* | 219 | 65.8 | 226 | 72.1 | 445 | 69.0 |
| *C. blanda* | 219 | 65.8 | 226 | 71.7 | 445 | 68.8 |
| *C. arctata* | 220 | 66.8 | 222 | 71.2 | 442 | 69.0 |
| *C. scirpoidea* | 219 | 59.4 | 226 | 68.1 | 445 | 64.0 |
| *C. polystachya* | 221 | 62.4 | 227 | 67.4 | 448 | 65.0 |
| *C. baccans* | 217 | 62.2 | 226 | 64.4 | 443 | 63.3 |
| *C. cruciata* | 220 | 64.1 | 227 | 66.5 | 447 | 65.2 |
| *Cyperus* | 181 | 56.4 | 249 | 60.6 | 430 | 58.8 |

TABLE 9. Sequence divergence within sections for which more than one indivdidual was sequenced.

| Section | N | Within sections (%) |
|---|---|---|
| *Phyllostachys* | 24 | 0.00 - 3.83 |
| *Careyanae* | 2 | 2.86 |
| *Laxiflorae* | 2 | 0.95 |
| *Firmiculmes* | 2 | 7.19 |
| *Acrocystis* | 4 | 0.94 - 3.56 |

TABLE 10. Sequence divergence between sections for which more than one individual was sequenced. Percent divergence is given above the diagonal, absolute differences below the diagonal.

| Section | *Phyllostachys* | *Careyanae* | *Laxiflorae* | *Firmiculmes* | *Acrocystis* |
|---|---|---|---|---|---|
| *Phyllostachys* | - | 11.11 - 14.15% | 11.08 - 14.22% | 8.15 - 11.27% | 7.43 - 11.75% |
| *Careyanae* | 46 - 59 | - | 8.15 - 8.59% | 11.03 - 13.53% | 6.41 - 7.13% |
| *Laxiflorae* | 46- 59 | 34 - 36 | - | 10.60 - 12.77% | 6.67 - 7.40% |
| *Firmiculmes* | 34 - 47 | 46 - 56 | 44 - 53 | - | 9.11 - 12.23% |
| *Acrocystis* | 31 - 49 | 27 - 30 | 28 - 31 | 38 - 51 | - |

TABLE 11. Sequence divergence within species of *Carex* sect. *Phyllostachys*

| Species | N | Location of Populations Sampled | Sequence Variation (%) |
|---|---|---|---|
| *C. backii* | 3 | MB(2), ONT | 0.48 - 0.96 |
| *C. saximontana* | 3* | MB(2), SASK | 0.00 - 0.48 |
| *C. latebracteata* | 2 | ARK, OK | 0.00 |
| *C. juniperorum* | 3 | ONT, OH, KY | 0.00 |
| *C. jamesii* | 4 | ONT, VA, ARK, KY | 0.00 - 0.24 |
| *C. willdenowii* | 3 | KY, OH, PA | 0.00 - 0.24 |
| *C. superata* | 3* | AL, MS(2) | 0.00 |
| *C. basiantha* | 3 | AL, LA, MS | 0.00 |

*Two individuals are from the same population

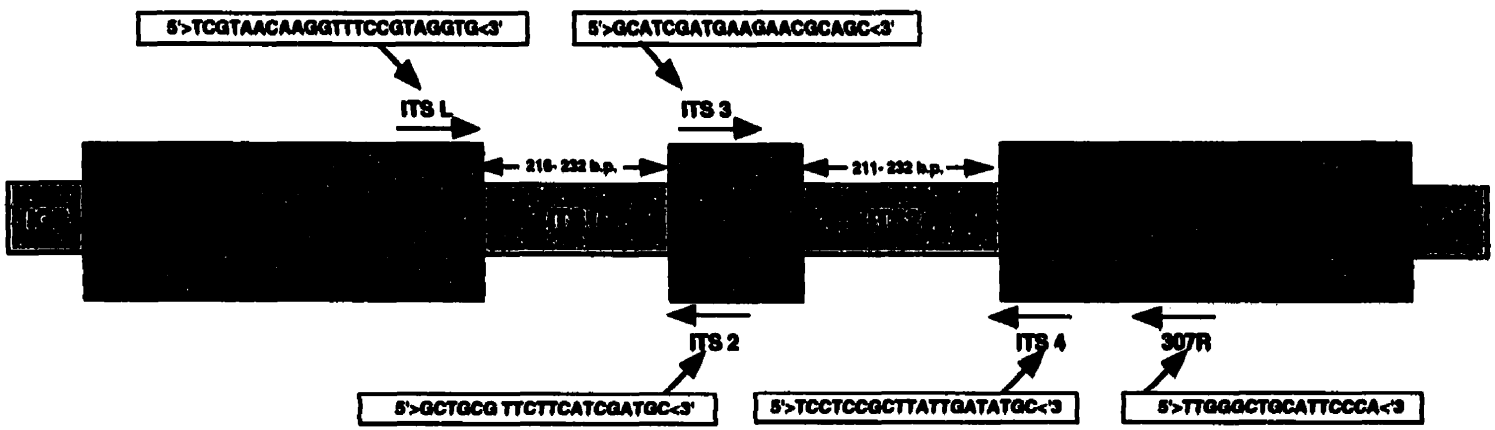


fig. 11. The structure of nrDNA in *Carex* showing positions of the two internal transcribed spacers (ITS 1 and ITS 2) relative to the locations of the 18S, 5.8S, and 26S genes, and the intergenic spacer (IGS) between tandem repeats. The sequences of the primers used in PCR and DNA sequencing and their relative positions in coding sequences is given. Variation in the length of ITS 1 and ITS 2 in the carices sequenced is indicated above the spacer regions.

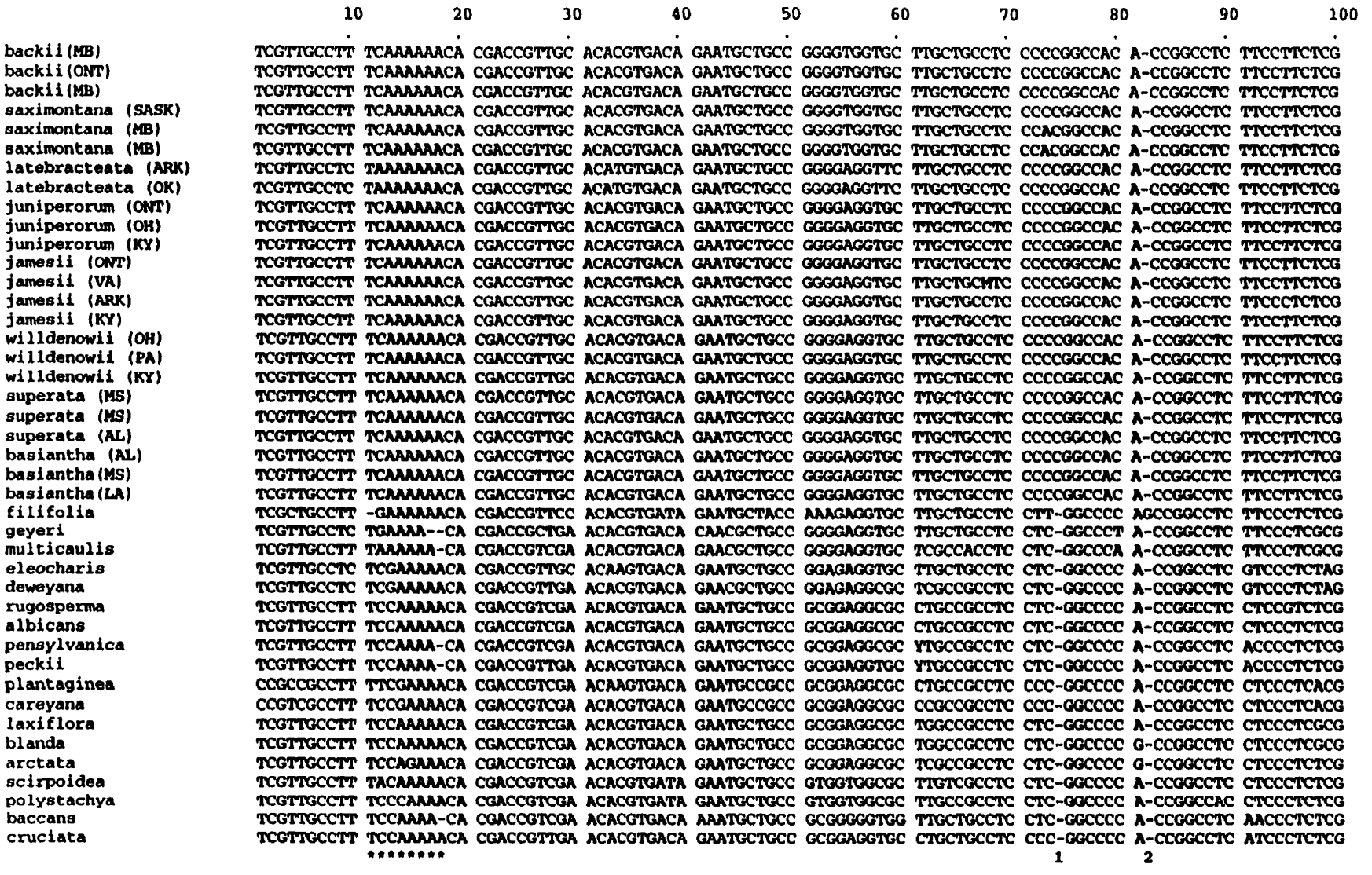


fig. 12. The complete sequence for ITS 1 (positions 1-232), ITS 2 (positions 247-482), and 17 bp at the 3' end of the 5.8S gene (positions 233-247), of all the taxa used in this study. Regions of ambiguous alignment that were not used in the analysis are indicated by asterisks below the sequence. Insertions/deletions (indels) used in the analysis are indicated by number below the sequence. The complete length of the combined ITS 1 and ITS 2 spacers (excluding the 17 bp of the 5.8S gene) for each taxon used in this study is given in brackets at the end of each sequence.

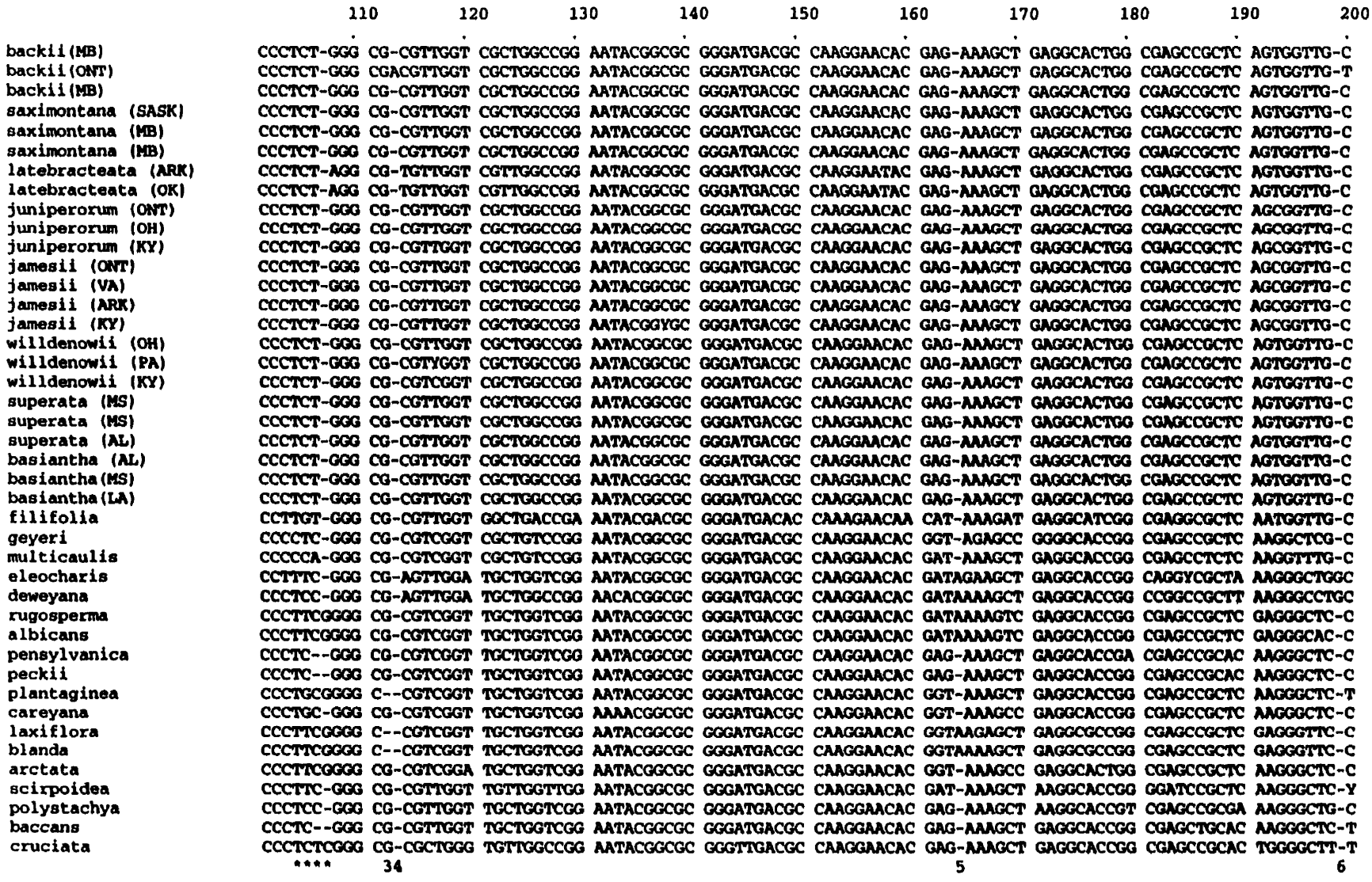


fig. 12. Continued.

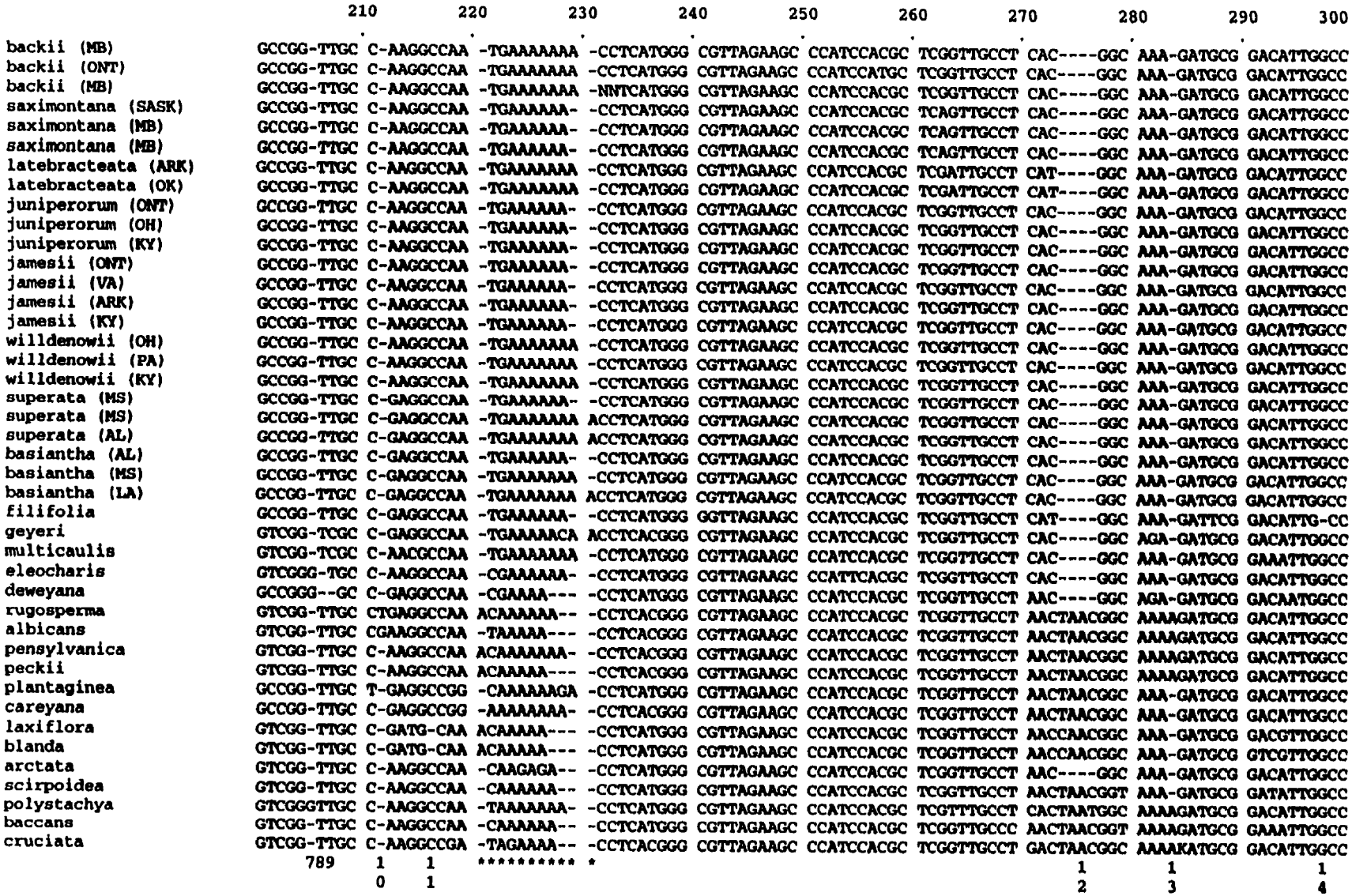


fig. 12. Continued.

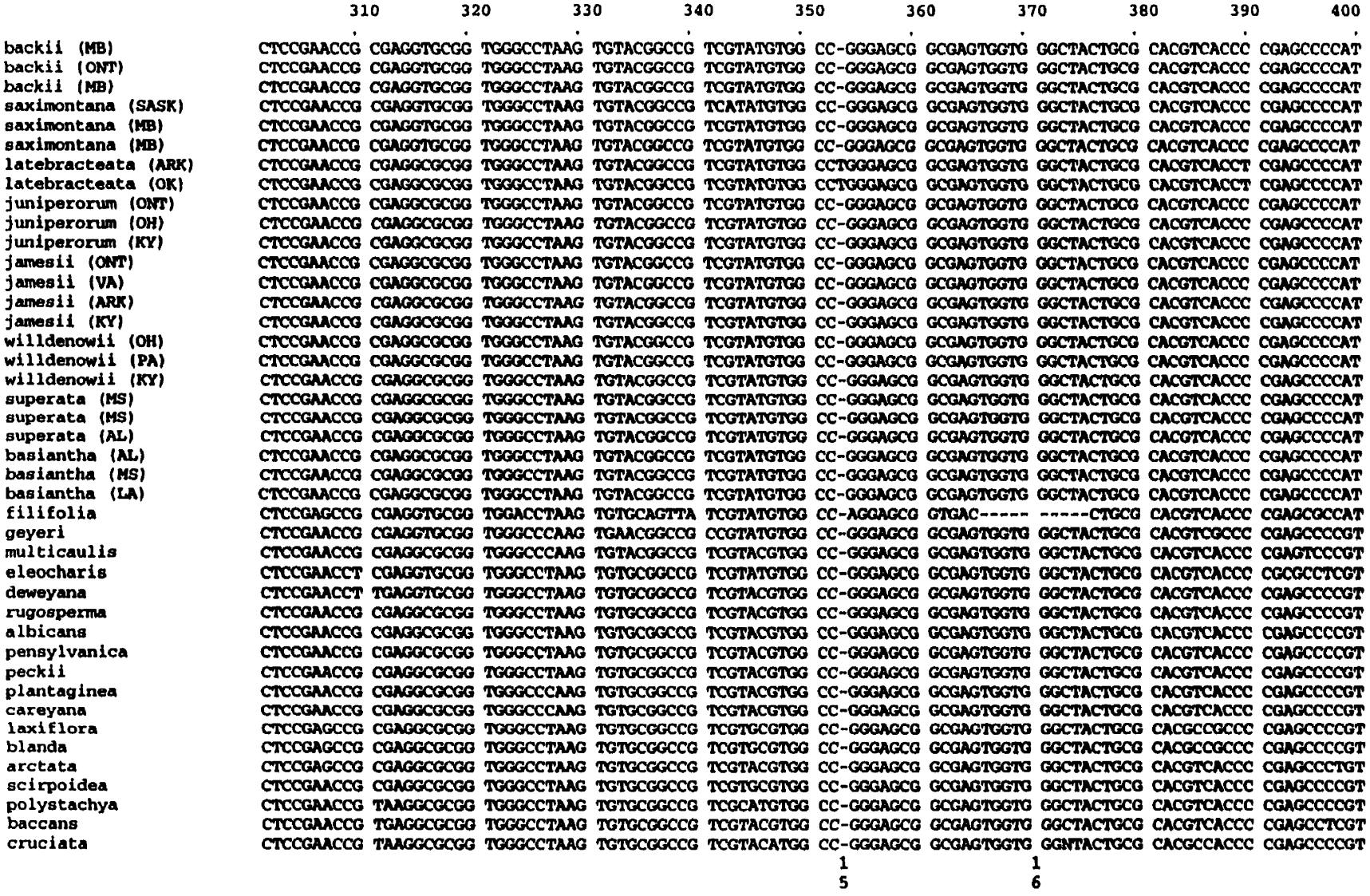


fig. 12. Continued.

```
                          410        420        430        440        450        460        470        480
                           .          .          .          .          .          .          .          .
backii (MB)               AAGGACACAG GGCCTTGTTT GACCCCCTAA CGAGGAGC-- --ATGCTGTC GCGGCTTGTG C--TGTGCGG CACCTTCGGA CC [444]
backii (ONT)              ACAGACACAG GGCCTTGTTT GACCCCCTAA CGAGGAGC-- --ATGCTGTC GCGGCTTGTG C--TGTGCGG CACCTTCGGA CC
backii (MB)               ACAGACACAG GGCCTTGTTT GACCCCCTAA CGAGGAGC-- --ATGCTGTC NCGGCTTGTG C--TGTGCGG CACCTTCGGA CC
saximontana (SASK)        ACCGACACAG GGCCTTGTTT GACCCCCTAA CGAGGAGC-- --ATGCTGTC ACGGCCTGTG C--TGTGCGG CACCTTCGGA CC [443]
saximontana (MB)          ACCGACACAG GGCCTTGTTT GACCCCCTAA CGAGGAGC-- --ATGCTGTC ACGGCCTGTG C--TGTGCGG CACCTTCGGA CC
saximontana (MB)          ACCGACACAG GGCCTTGTTT GACCCCCTAA CGAGGAGC-- --ATGCTGTC ACGGCCTGTG C--TGTGCGG CACCTTCGGA CC
latebracteata (ARK)       ACCGACACAG GGCCTTGTTT GACCCCTGAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC [445]
latebracteata (OK)        ACCGACACAG GGCCTTGTTT GACCCCTGAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC
juniperorum (ONT)         ACCGACACGG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC [442]
juniperorum (OH)          ACCGACACGG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC
juniperorum (KY)          ACCGACACGG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC
jamesii (ONT)             ACCGACACGG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC [442]
jamesii (VA)              ACCGACACGG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC
jamesii (ARK)             ACCGACACGG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC [442]
jamesii (KY)              ACCGACACGG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC
willdenowii (OH)          ACCGACACAG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGCGCGG CACCTTCGGA CC [443]
willdenowii (PA)          ACCGACACAG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGCGCGG CACCTTCGGA CC
willdenowii (KY)          ACCGACACAG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGCGCGG CACCTTCGGA CC
superata (MS)             ACCGACACAG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC [443]
superata (MS)             ACCGACACAG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC
superata (AL)             ACCGACACAG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC
basiantha (AL)            ACCGACACAG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC [443]
basiantha (MS)            ACCGACACAG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC
basiantha (LA)            ACCGACACAG GGCCTTGTTT GACCCC-TAA CGAGGAGC-- --ATGCTGTC GCGGCCTGTG C--TGTGCGG CACCTTCGGA CC
filifolia                 ATCTACATAG TGCCTTGTTT GATCCCCTAA CAATGAGC-- --ATGCTGTC GCGGCTTATG C--TGCGCAG CACCTTCGAA CC [431]
geyeri                    ACGAACACAG GGCCTTGTTC GACCCCCTAA CGAGGAGC-- --ATGCCGTC GCGGCTTGCG C--CGTGCGG CACCTTCGGA CC [442]
multicaulis               ACGCACACAG GGCCTTGTTT GACCCCCTAA CGAGGAGC-- --ATGCCGCC GCGGCTCGCG C--CGCGCGG CACCTTCGGA CC [442]
eleocharis                ACCGACACAG GGCCTTTTTT GACCCC-TAA CGAGGAGC-- --TTGCCGTT GCGGCTTG-G C--TGCGCGG CGCCTTCGGA CC [442]
deweyana                  ACCGACACAG GGCCTCTTAT GACCCC-TCA CGAGGAGC-- --TTGCCGT- -CGGCTCGCG C--CGCGCGG CGCCTTCGGA CC [438]
rugosperma                AATGACGCAG GGCCTTGTTG GAACCCCTAA CGAGGAGC-- --ATGCTGCC GCGGCTTCGG C--TGCGCGG CGTCTTCGGA CC [450]
albicans                  ACCGACGCAG GGCCTTGTTG GAACCCCTAA CGAGGAGC-- --AASCTGCC GCGGCTTCGG C--TGCGCGG CGTCTTCGGA CC [448]
pensylvanica              ACCGACGCAG GGCCTTGTTG GAACCCCTAA CGAGGAGCCA AATCGCTGCC GCGGCTTCGG C--TGCGCGG CGTCTTCGGA CC [450]
peckii                    ACCGACGCAG GGCCTTGTTG GAACCCCTAA CGAGGAGCCA ATTCGCTGCC GCGGCTTCGG C--TGCGCGG CGTCTTCGGA CC [448]
plantaginea               GCCGATGCAG GGCCTTGTTC GACCCC-TAA CGAGGAG--- --GCG-CGCC GCGGCTTCTG CCGTGCGTGG CGCCTTCGGA CC [446]
careyana                  GCCGACGCAG GGCCCCGTTC GACCCC-TAA CGAGGAGC-- --GCGGCGCC GCGGCTTCTG CCGCGCGCGG CGCCTTCGGA CC [447]
laxiflora                 AGCGACGCAG GGCCTTGTTG GAACCCCTAA CGAGGAGC-- --GCGCCGCC GCGGCTTGTG C--TGCCGTG CGCCTTCGGA CC [445]
blanda                    AACGACGCAG GGCCTTGTTG GAACCCCTAA CGAGGAGC-- --GCGCCGCC GCGGCTTGTG C--TGCCGTG CGCCTTCGGA CC [445]
arctata                   ACCGACGCAG GGCCTTGTAC GACCCCCTAA CGAGGAGC-- --ACGTCGCC GCGGCCTGCG C--CGCGCGG CACCTTCGGA CC [442]
scirpoidea                ACCGACGTAG GGCCTTGTAC GACCCCCTAA CGAGGAGC-- --ATGCCGCT GCAGCTTCGG C--CGCACGG CGCCTTCGGA CC [445]
polystachya               ACCGACGCAG GGCCTTCTTT GAACCCCTAA CGAGGAGC-- --ACGCCGCT GCGGCTTCCG C--TGCGCGG CGCCTTCGGA CC [448]
baccans                   ACCGATGCAG GGCCTTGTTT GATCCC-TAA CGAGGAGC-- --ATGTCGTT GCGGCTTNTG C--TGCGCGG TGCCTTCGGA CC [443]
cruciata                  ACCGATGCAG GGCCTTGTTT GAACCCCTAA CGAGGAGC-- --ACGTCGCT GCGGCTTNTG C--TGCGCGG CGCCTTCGGA CC [447]
                                      ******          1  1    1     2            2
                                                      7  8    9     0            1
```

fig. 12. Continued.

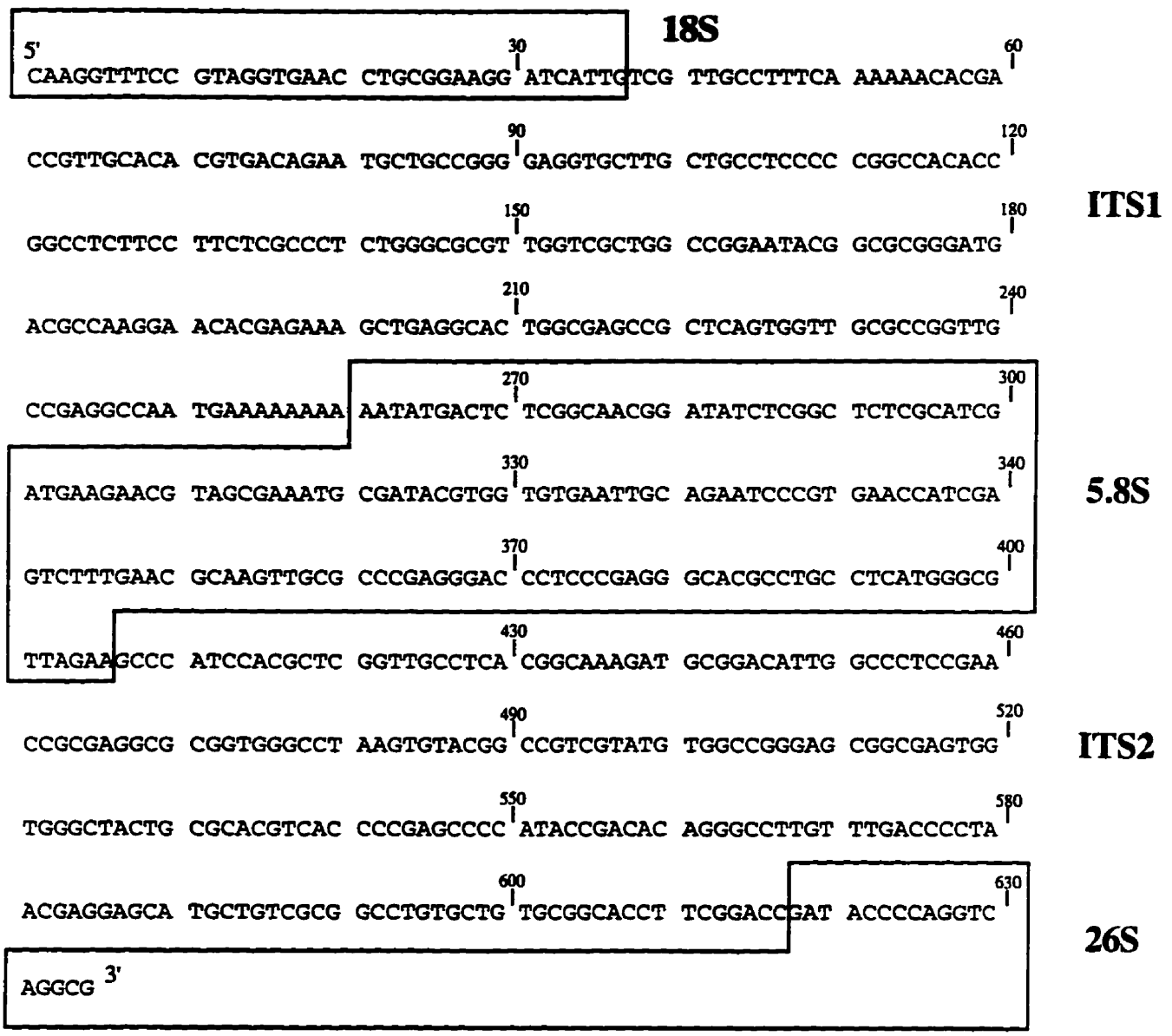


fig. 13. The complete sequence for the ITS region of *Carex superata*, showing the ITS 1 and ITS 2 spacers, the complete 5.8S gene, and portions of the 18S and 26S genes of nrDNA. Coding sequences are boxed.

**3' end of 5.8S rDNA**

```
Oryza sativa      GCCGAGGGCACGCCTGCCT---GGGCGTCACGC
Cucumis sativus   ...........T......---..........
Vicia faba        ...........T......---...T.....AT
Cyperus           C..................---......T.GAA
Kobresia          ..................CAT......T.NAA
Carex backii      ..................CAT......T.GAA
```

ITS2 →

fig. 14. The aligned 3' end of the 5.8S gene for the Cyperaceae (*Cyperus, Kobresia, Carex*; Starr, unpublished), Poaceae (*Oryza sativa*; Takaiwa, Oono, and Sugiura 1985), Fabaceae (*Vicia faba*; Tanaka, Dyer, and Brownlee, 1980), and Cucurbitaceae (*Cucumis sativus*; Torres, Ganal, and Hemleben, 1990). Note the three base pair insertion (5'>CAT<3') shared by *Carex* and *Kobresia*.

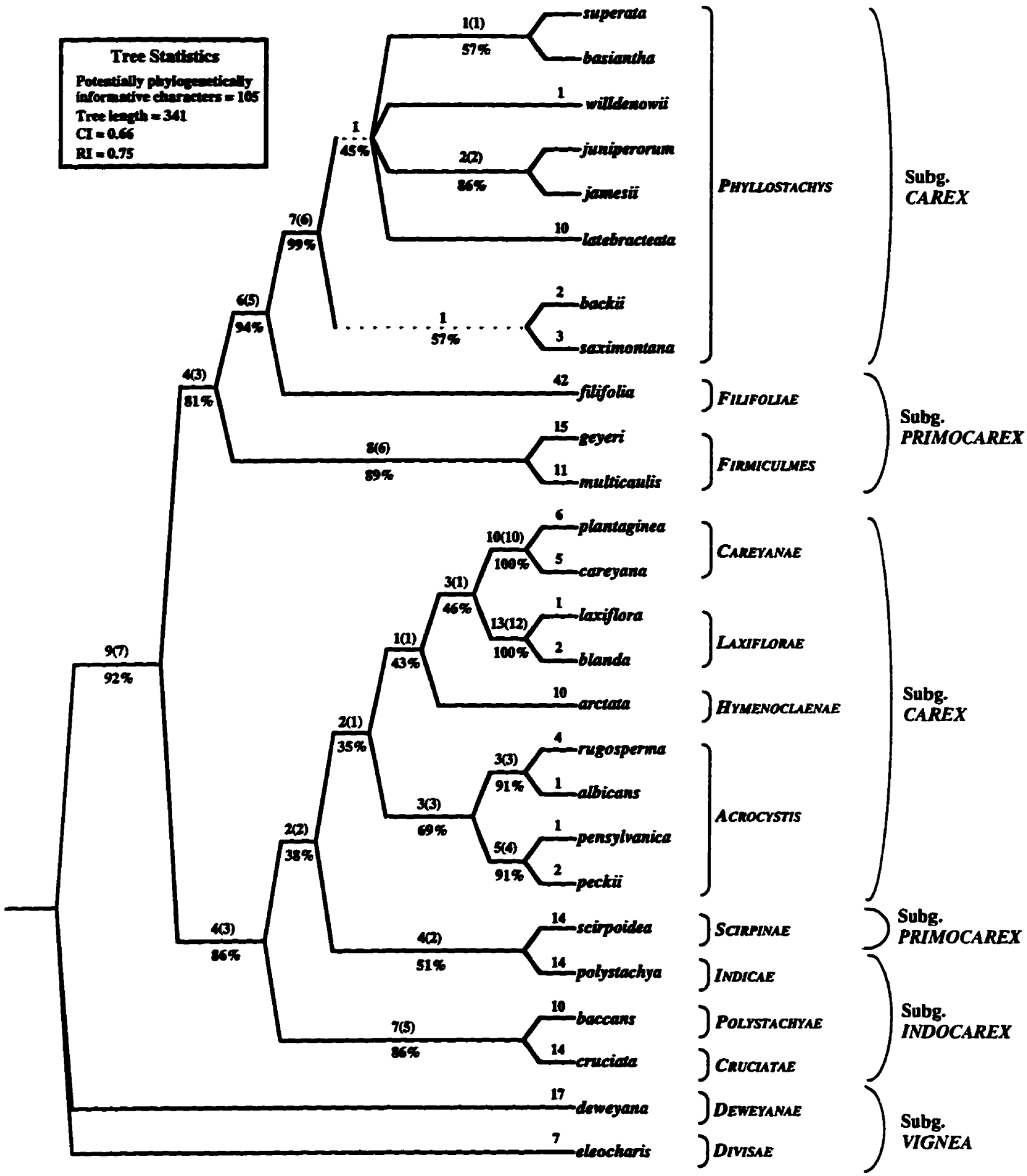


fig. 15. The single tree of five most parsimonious trees resulting from the phylogenetic analysis of *Carex* section *Phyllostachys* and ten putatively related sections that is topologically identical to the 50% majority rule tree. The members of subgenus *Vignea* were used as an outgroup. The names of the sections and subgenera are given in succession after the brackets to the right of the specific epithets. Branches that collapse in the strict consensus of the five most parsimonious trees are dotted. Unambiguous base pair changes and decay indices (in parentheses) are given above the branch, bootstrap values below the branch. The consistency index (CI), retention index (RI), tree length, and the number of potentially phylogenetically informative characters are given in the box at the top left.

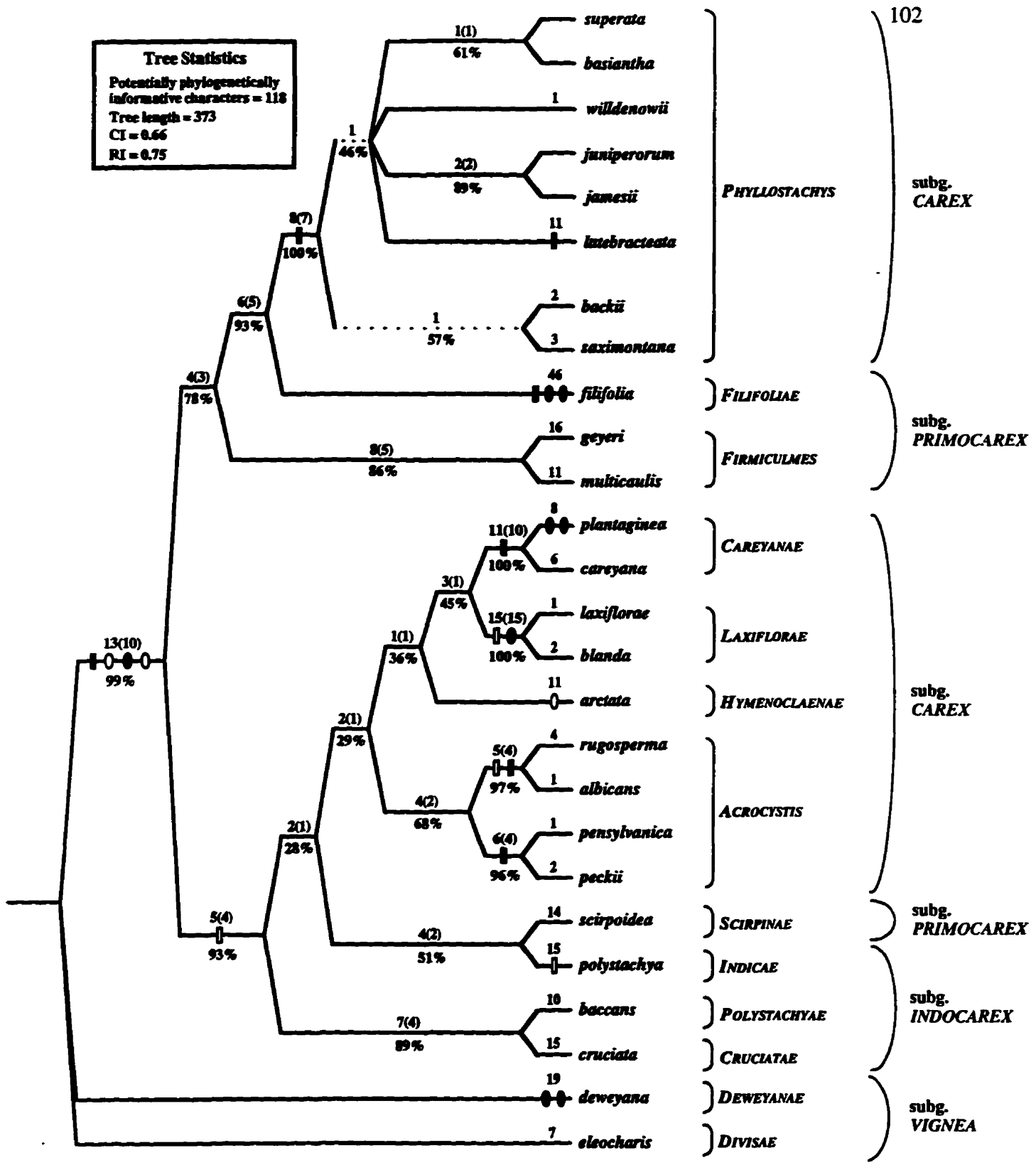


fig. 16. The only tree of five most parsimonious trees resulting from the phylogenetic analysis of *Carex* section *Phyllostachys* and ten putatively related sections that is topologically identical to the 50% majority rule tree. Insertion/deletion events (indels) and 17 bp at the 3' end of the 5.8S gene were included in this analysis. Unambiguous insertions are represented by bars, and unambiguous deletions by ellipses. Solid bars and ellipses represent indels with a CI=1; all other indels are homoplastic (i.e., CI<1). The names of the sections and subgenera are given in succession after the brackets to the right of the specific epithets. Branches that collapse in the strict consensus of the five most parsimonious trees are dotted. Unambiguous base pair changes and decay indices (in parentheses) are given above the branch, bootstrap values below the branch. The consistency index (CI), retention index (RI), tree length, and the number of potentially phylogenetically informative characters are given in the box at the top left.

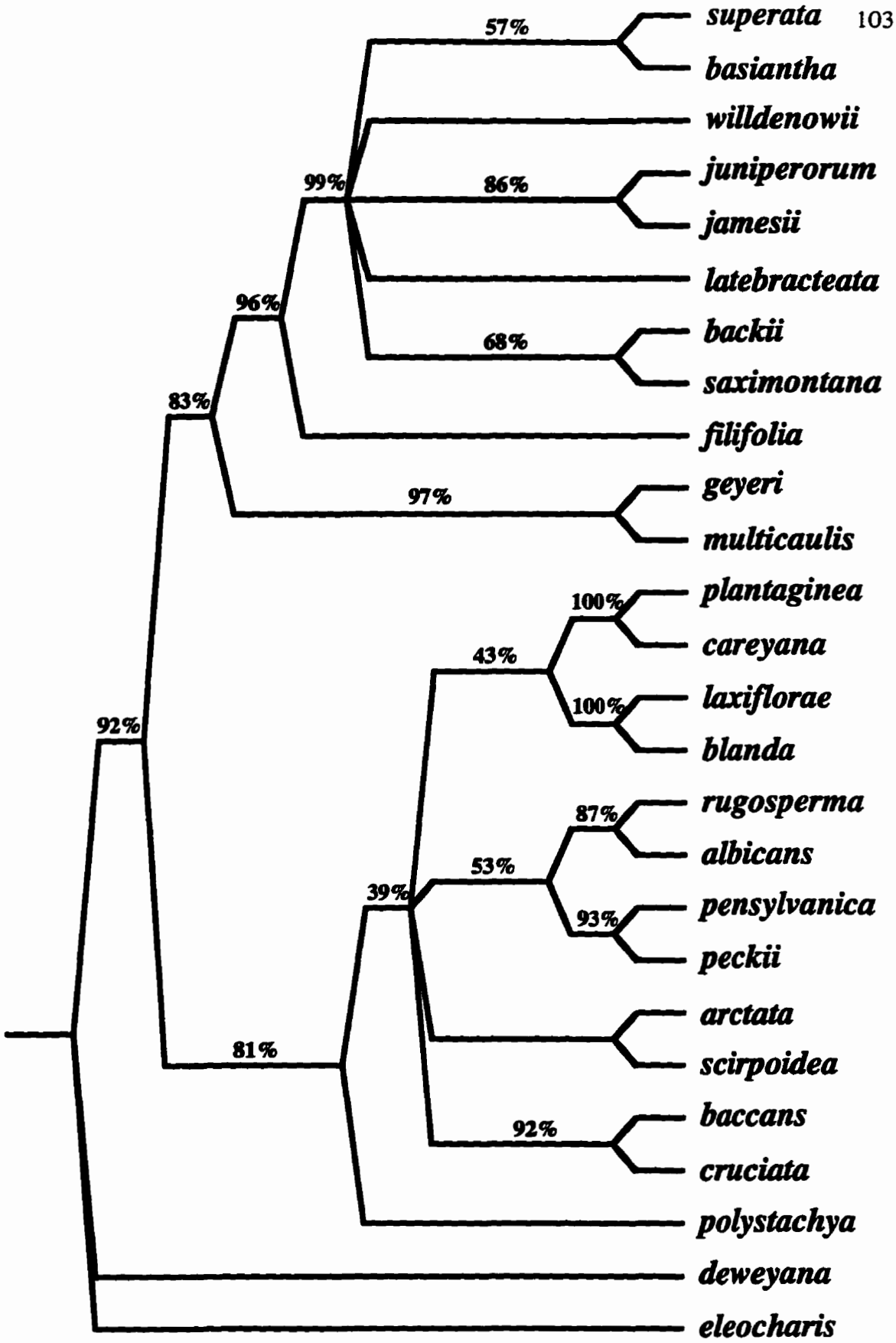


Fig. 17. The strict consensus tree of eight most parsimonious trees resulting from the phylogenetic analysis of *Carex* spp. in which transitions were favoured over transversions 2.2:1. Bootstrap values were determined from 500 replicates.

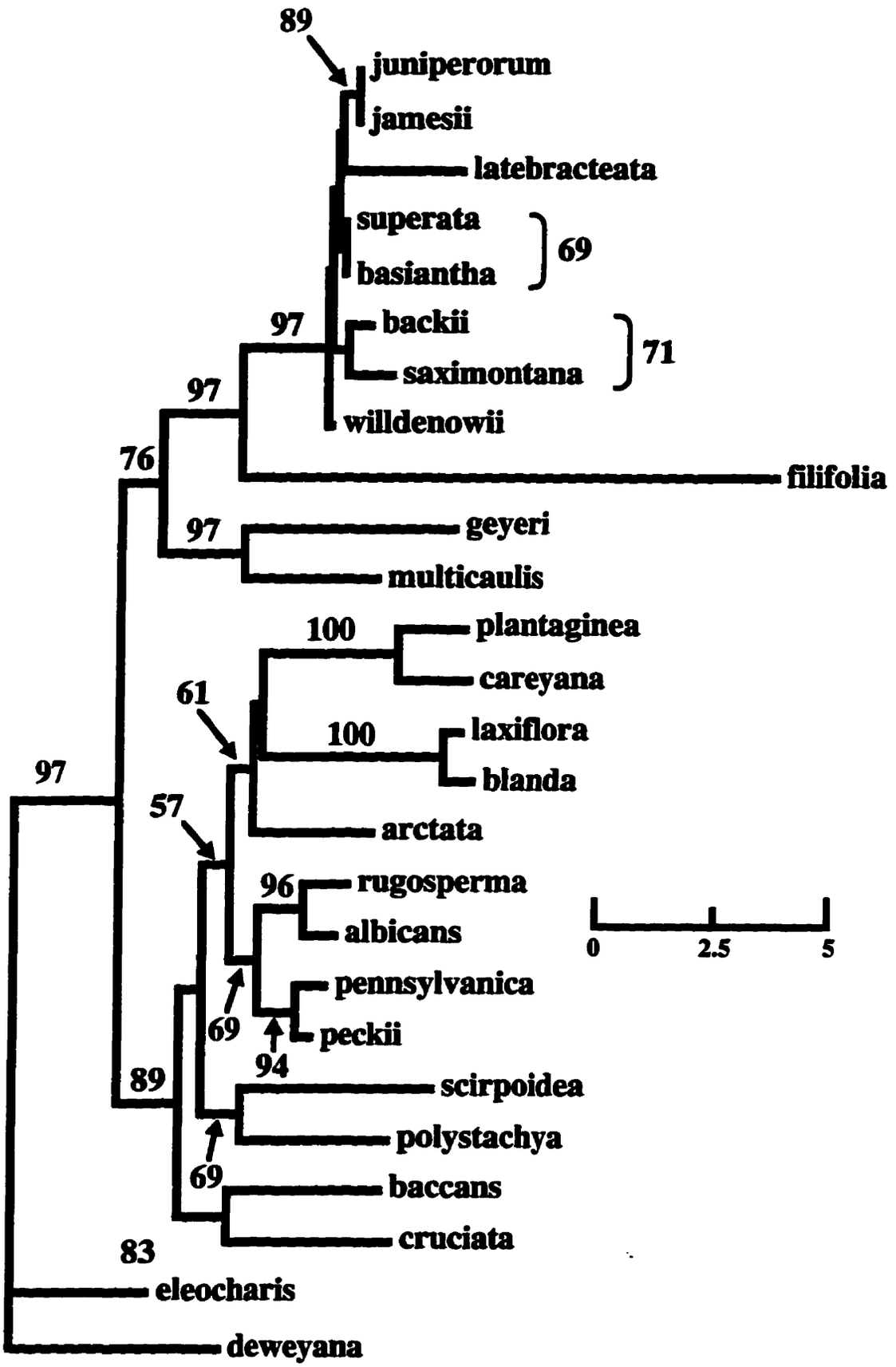


fig. 18. Minimum evolution tree constructed using a Jukes-Cantor model of sequence evolution for the ITS region in *Carex*. Bootstrap values (500 replications) are only given for those clades with >= 50% support. The scale represents 100 X the observed branch length value.

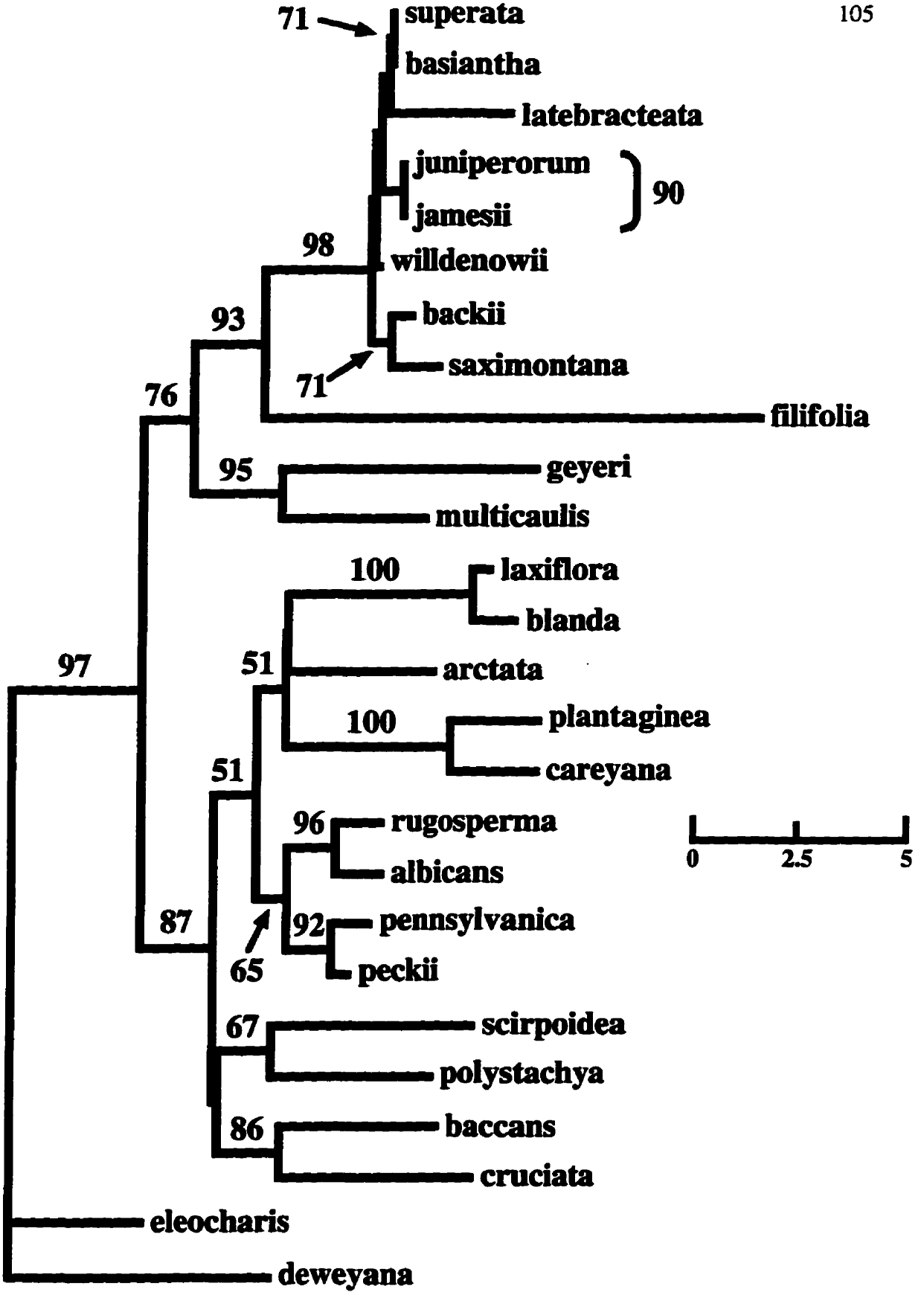


fig. 19. Minimum evolution tree constructed using a Log/Det model of sequence evolution for the ITS region in *Carex*. Bootstrap values (500 replications) are only given for those clades with >=50% support. The scale bar represents 100 X the observed branch length value.

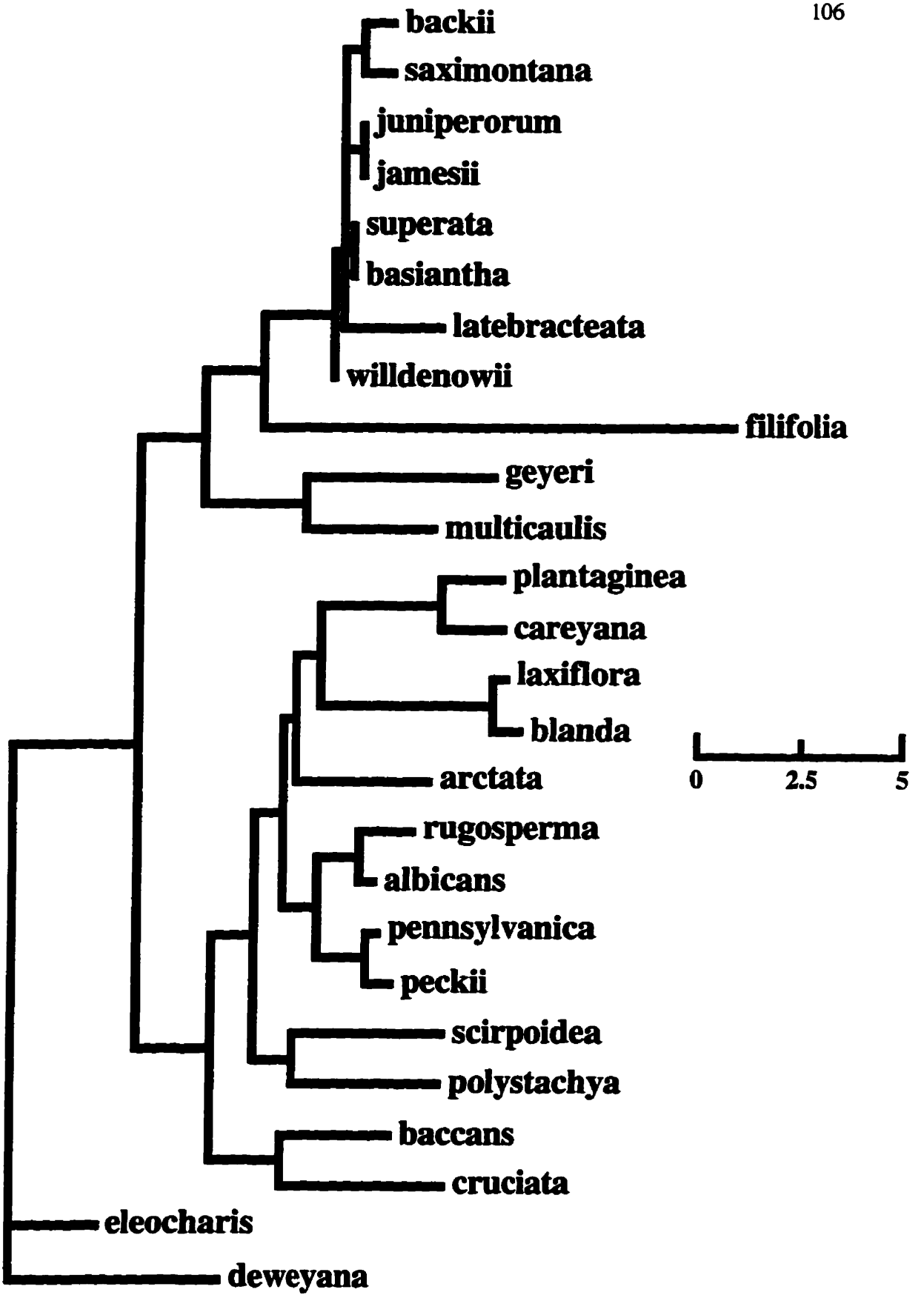


fig. 20. Maximum likelihood tree constructed using a Jukes-Cantor (JC) model of sequence evolution for the ITS region in *Carex*. Scale distance equals 100 X the observed branch length values.

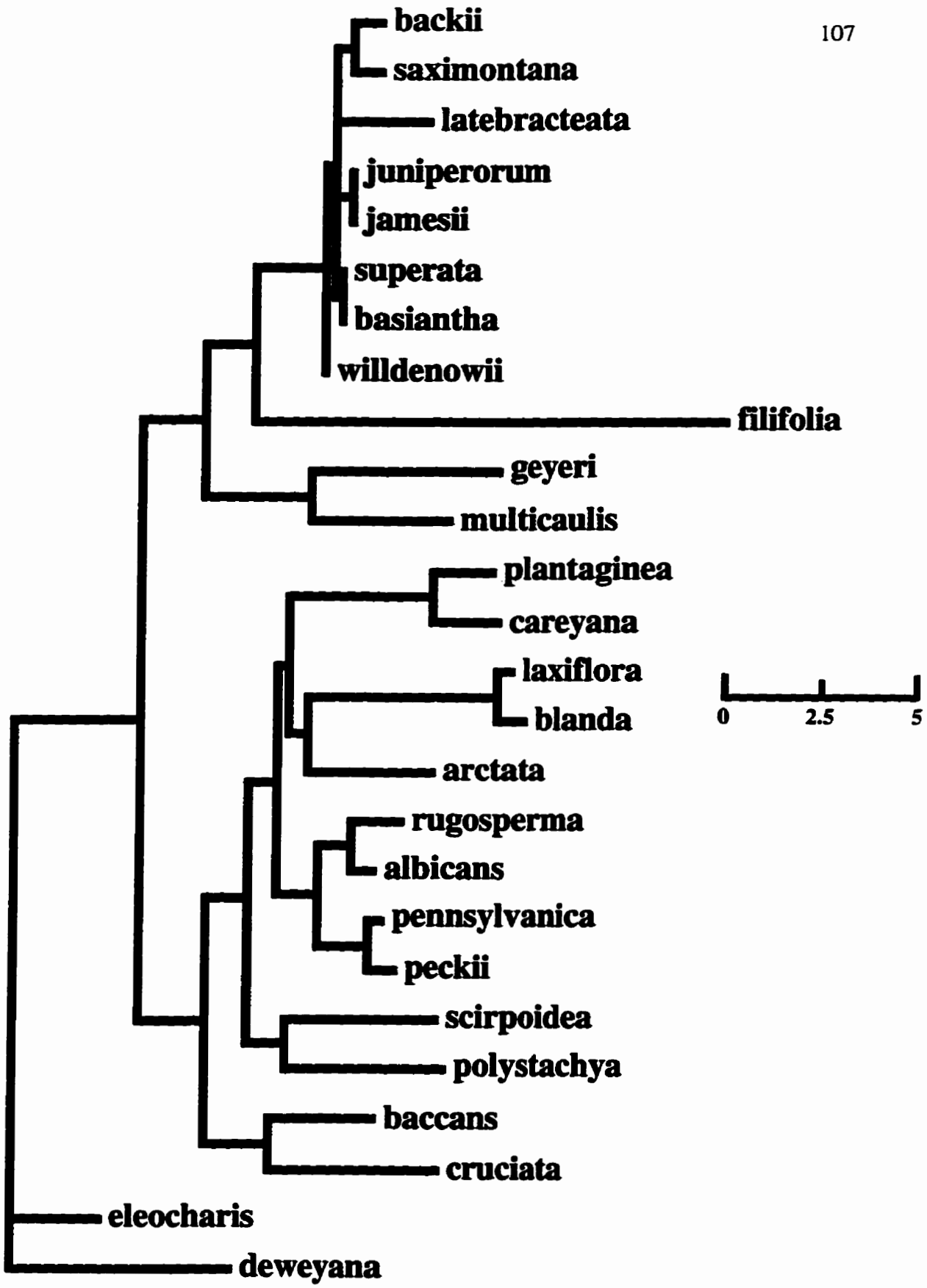


fig. 21. Maximum likelihood tree constructed using a Hasegawa-Kishino-Yano (HKY85) model of sequence evolution for the ITS region in *Carex* with a correction for rate heterogeneity across sites (Gamma distribution; Yang, 1993). Scale distances are equal to 100 X the observed value.

# CHAPTER 4

## A PHYLOGENETIC ANALYSIS OF *CAREX* SECTION *PHYLLOSTACHYS* (CYPERACEAE) BASED ON MORPHOLOGICAL, ANATOMICAL, AND MOLECULAR DATA.

Phylogeny within the large, cosmopolitan genus *Carex* L. is poorly understood at all levels. The genus' vast size (c. 2000 spp.) and global distribution, its subtle diagnostic characters that differentiate between species, and its lack of critical systematic research has led to the recognition of many artificial taxa (Naczi, 1992). These artificial groups have hindered phylogenetic research by obscuring evolutionary trends, confusing similarities, and by hiding taxa that might shed light on the phylogeny of this genus. This helps explain why only four previous cladistic studies have been attempted in *Carex* (Crins and Ball 1988; Crins 1990; Naczi 1992; chapter 3).

One solution to the problem of poor circumscription is to focus evolutionary studies on a number of small subgroups in the genus that appear to be distinct and well circumscribed (Crins 1990). One such taxon is *Carex* section *Phyllostachys* (J. Carey) L.H. Bailey, a clearly defined group of 8 taxa (*C. backii* Boott, *C. basiantha* Steudel, *C. latebracteata* Waterfall, *C. jamesii* Schweinitz, *C. juniperorum* Catling, Reznicek, & Crins, *C. superata* Naczi, Reznicek, & Ford, and *C. willdenowii* Willdenow) confined to North America, north of Mexico. Described by Mackenzie (1935) as "a very strongly marked group", the section is easily distinguished from other Carices by its foliaceous pistillate scales, few-flowered androgynous spikes, and its apically dilated and winged culms (Catling, Reznicek, and Crins, 1993). The eight species currently recognized in section *Phyllostachys* represent a group of geographically and reproductively dissimilar taxa. The species vary from narrowly restricted endemics (*C. juniperorum* and *C. latebracteata*) to far-ranging taxa (e.g., *C. backii* and *C. jamesii*). Many of the

distributions are confined to regions that were either glaciated or unglaciated during the Pleistocene, and a variety of reproductive characters such as flower number and position, and variability in the size of the pistillate scales, suggest that differences in breeding systems may have evolved. Chromosome numbers show a very wide range both within and between the species in section *Phyllostachys*, suggesting that the unusual cytology of the genus may have been a contributing factor to speciation in this section.

Because of its apparent monophyly, Crins (1990) used the five species then known in section *Phyllostachys* (i.e., *C. backii*, *C. saximontana*, *C. latebracteata*, *C. jamesii*, and *C. willdenowii* s.l.) to demonstrate the utility of character compatibility analysis for reconstructing infrasectional phylogenies in *Carex*. Because no obviously related sections were available to use as outgroups, Crins (1990) attempted to root his trees *a posteriori* using the assumption that chromosome evolution in *Carex* proceeds by simple agmatoploidy; i.e., speciation is accompanied by an increase in chromosome number (Davies, 1956; Faulkner, 1972; Whitkus, 1987). Although two major clades were identified, chromosome evolution within the group did not appear to follow simple agmatoploidy, and as a result his unrooted trees were unable to convey much information regarding character evolution or phylogeny.

In order to clarify the phylogenetic position of section *Phyllostachys* within the genus *Carex*, chapter 3 used the ITS region of nrDNA to test the various disparate hypotheses of relationship that had been proposed for sect. *Phyllostachys*. The results of this analysis indicated that sect. *Phyllostachys* (subg. *Carex*) along with two sections from subg. *Primocarex* Kükenth. (sects. *Filifoliae* Tuckerm. and *Firmiculmes* Kükenth.) were part of an evolutionally "reduced" clade that was phylogenetically separate from a larger "compound" clade composed of sections from subg. *Primocarex* Kükenth., *Carex*, and *Indocarex* Baillon. Unfortunately, variation within the ITS region was not sufficient to produce a robust phylogenetic hypothesis for the section although it did provide strong

support for potential outgroups that could be used in subsequent phylogenetic studies (i.e., sections *Filifoliae* and *Firmiculmes*).

In this study I explore evolution within *Carex* section *Phyllostachys* by combining the molecular data from the study presented in chapter 3 with morphological and anatomical characters (chapter 2). The objectives of this study were: (1) to assess whether molecular characters could be useful for uncovering subtle taxonomic variation within *Carex* section *Phyllostachys* that had already been recognized using traditional characters (viz., anatomy, micromorphology, morphology); (2) to explore theories on chromosome evolution in *Carex* within the context of an established phylogeny; (3) to reconstruct a rigorous phylogeny based on several lines of evidence, and (4) to use the hypothesized phylogeny to investigate trends in the biology and biogeography of section *Phyllostachys*.

## 4.1 Materials and Methods

### *Ingroup and Outgroup Selection*

All eight of the species currently recognized in *Carex* section *Phyllostachys* were used as the ingroup in this analysis. Trees were polarized by species representative of the two nearest outgroups to sect. *Phyllostachys* (sections *Firmiculmes* and *Filifoliae*) as determined in chapter 3. Morphological characters were scored for only two species in section *Firmiculmes* (*C. multicaulis* and *C. geyeri*), and one (*C. filifolia*) in sect. *Filifoliae* because these were the only species in these sections for which ITS sequences were currently available (chapter 3). It should be noted, however, that examinations of the literature and herbarium specimens revealed that further additions to the morphological data set of species from these sections did not affect the polarity decisions made in this analysis.

### *DNA Sequences*

All of the spacer sequences used in this study were taken from the ITS study of *Carex* presented in chapter 3. Detailed methods for DNA Isolation, PCR amplification, and sequencing of the ITS region in *Carex* are provided in that chapter.

### *Macromorphology and Anatomy.*

Morphological characters were critically assessed from live and pressed plants collected in the field, FAA preserved material, and from specimens observed or borrowed from the following herbaria; MICH, DAO, WIN, KNK and ALTA (herbarium abbreviations according to Holmgren et al., 1990). The characters examined were selected from the literature, and from observations of specimens in the lab and in the field. Anatomical characters were determined from cross-sectional studies of the leaf and culm, and from scrapes of the epidermal surfaces of the leaves of all species including the outgroups. All of the anatomical characters used in the phylogenetic analyses were

identified in a prior anatomical and micromorphological investigation of the section (chapter 2). Because of the difficulty of scoring micromorphological characters into discrete characters states, these characters were used as an external data set for assessing the robustness of the final phylogenies. Vouchers and detailed methods for the anatomical portion of this study are found in chapter 2.

All characters used in the phylogenetic analyses were selected on the basis of whether they possessed discontinuities in morphology or anatomy that could be categorized into discrete character states (Stevens, 1991). Of the 67 characters examined, only 26 (3 anatomical; 22 morphological) met this criterion. A description of characters and character states used in the final analysis is given in TABLE 12.

Of the 67 characters examined during the initial character search, 17 were previously used in the phylogenetic analysis of section *Phyllostachys* by Crins (1990). Characters 3, 4, 5, 8, 9, and 13 (numbers are those of Crins, 1990) did not possess consistent discontinuities and were discarded from this study. Characters 12, 14, and 17, were redefined after herbarium material indicated that a better description of their character states could be made. All of the remaining characters (1, 6, 7, 11, 15, and 16) were used in the final analysis without modification. For character number 16 (leaf indument) *C. saximontana* was scored as possessing a glaucous (1; TABLE 13) indument contrary to Crins (1990). Although not all dried specimens readily display this character, it is a distinctive and consistent character in the field and in live plants kept in a greenhouse.

### *Sequence Analysis*

The boundaries of coding (18S, 5.8S, and 26S rDNA), and non-coding sequences (ITS 1 and ITS 2) in the ITS region were determined by comparison to the published sequences for rice (Takaiwa, Oono, and Sugiura, 1985). Only sequences from the two internal transcribed spacers (ITS 1 and ITS 2) were used in this analysis. ITS sequences

were aligned initially using CLUSTAL V (Higgins et al. 1992), then manually adjusted to account for algorithmic anomalies using SeqApp version 1.8a (Gilbert, 1992). In order to reduce systematic error, all regions of ambiguous alignment were excluded from distance calculations and phylogenetic analyses (Swofford et al., 1996). Unambiguous indels were scored as either bases present=1 or absent=0, according to the coding strategy proposed by Barriel (1994). Absolute distances between sequences were calculated using the DISTANCE MATRIX option in PAUP 4.xx (Phylogenetic Analysis Using Parsimony; Swofford, 1997). Gaps were treated as "missing" during these calculations. Sequence lengths and GC contents were determined in Amplify 1.2 (Engels, 1993). Because this program recognizes only nucleotide base letters (i.e., A, G, C, T), some sequence lengths and GC contents had to be recalculated due to the presence of ambiguous nucleotide characters (e.g., N, Y, R). The ratio of transitions to transversions was estimated in MacClade 3.0 (Maddison and Maddison, 1992) using trees produced in PAUP 4.xx. This ratio was determined from tree topologies that were produced using a combined molecular and morphological data set, as well as from data sets exclusively composed of either morphological or molecular characters alone.

***Phylogenetic Analysis***

Phylogenetic reconstructions were performed using PAUP 4.xx via branch-and-bound searches of unweighted characters while using Fitch parsimony (Fitch, 1971) and a simple stepwise addition of taxa. All tree searches employed the "save all minimal trees" (MULPARS), and "accelerated transformation" (ACCTRAN) options provided by PAUP. Clade support in the most parsimonious trees was assessed via bootstrap (Felsenstein, 1985) and decay analyses (Bremer, 1988; Donoghue et al., 1992). Bootstrap values were determined from 500 replicates using branch-and-bound searches and a SIMPLE stepwise addition of taxa. Decay analysis was performed on trees 1 to 7 steps greater than the most parsimonious trees according to the method outlined in Bremer

(1988). In addition to decay and bootstrap values, the consistency index (CI; Kluge and Farris, 1969) and retention index (RI; Farris, 1989), were used to assess the amount of phylogenetic information in parsimony analyses. The $g_1$ statistic was also determined from the tree-length distribution of 10 000 random trees in order to evaluate the level of non-random structure in the molecular data set (Hillis and Huelsenbeck, 1992). The number of unambiguous character state changes along branches was determined in MacClade 3.0. As previously determined (chapter 3), intraspecific variation had no effect on parsimony analyses, therefore only the first individual representative of each species was used during searches to reduce computational time.

In order to determine whether the molecular and the morphological/anatomical data sets should be combined, and to assess their relative contribution to the final reconstruction, these data sets were analyzed both separately and in combination. Three distinct analyses were performed during phylogenetic reconstruction. In the first analysis, trees were constructed using only point mutations. In the second analysis, both point mutations and insertions/deletions (indels) events were used to construct trees, and these trees were then compared to the trees produced from point mutations alone. Since the addition of indels only improved the resolution of the trees and these characters displayed no homoplasy in these analyses, they were included in the final analysis of both the molecular and combined data sets.

In the third analysis, the results of the molecular reconstruction were compared to the results of the separate analysis of morphological and anatomical characters. Since support for the molecular analysis was weak and the results of the two analyses were congruent, the two data sets were combined to produce a more robust phylogeny (Donoghue and Sanderson, 1992).

***Biological and Phytogeographical Analysis***

The distribution of chromosome counts from published and unpublished sources was examined on the most parsimonious tree of the combined analysis. This was done to determine whether evolutionary trends followed current concepts of chromosome evolution in the genus (TABLE 14). The geographical distributions of the species in section *Phyllostachys* relative to the greatest extent of the Pleistocene ice sheets (TABLE 14) was plotted on this tree to ascertain whether a correlation between present species ranges, branching patterns, and ice distributions was apparent. The final tree was also used to assess the utility of micromorphological characters for estimating relationships in the section, and to examine whether trends in character evolution and flower number appeared to be related to breeding systems.

## 4.2 RESULTS

***Sequence Analysis***

Aligned sequences for both ITS 1 and ITS 2 in all of the taxa used in the phylogenetic analysis are presented in fig. 22. Summary statistics for these sequences are given in TABLE 15.

Spacer lengths for all Carices examined ranged from 220 bp to 222 bp for ITS 1, and from 211 bp to 223 bp for ITS 2. The large range in size of the ITS 2 spacer was due to a unique 10 bp deletion in *C. filifolia*. All other sequences differed by no more than two base pairs (i.e., 221-223 bp). The alignment of spacer sequences resulted in 448 characters of which 111 (24.8%) were variable, and 30 (6.7%) were phylogenetically informative.

Within sect. *Phyllostachys* spacer lengths were fairly uniform. The ITS 1 spacer length for all members examined ranged from 220 bp to 222 bp. Within the ingroup only 12 (5.3%) sites were variable in the molecular analysis. Of these 12, only 3 provided phylogenetic information that helped to resolve ingroup relationships while the other eight were autapomorphies seen in a single taxon, *C. latebracteata*. When outgroups were considered, however, the number of variable sites increased enormously. Sixty-one sites (27.2%) were variable when the members of sections *Firmiculmes* and *Filifoliae* were included in the analysis. Seven of these sites provided synapomorphies that supported the strong monophyly of sect. *Phyllostachys* (see below).

ITS 2 spacer lengths within the section varied even less than ITS 1 with all of the sequences differing by no more than a single base pair (222 bp to 223 bp). Fifty sites (22.4%) were variable, but only two of these supported ingroup relationships. None of the phylogenetically informative characters in ITS 2 supported the monophyly of sect. *Phyllostachys*.

Sequence divergence within section *Phyllostachys* was extremely low. Except for comparisons involving *C. latebracteata*, pairwise divergence values in the section varied

from complete identity at all sites (*C. superata* vs. *C. basiantha*; *C. juniperorum* vs. *C. jamesii*) to differences of up to 1.6% (*C. backii* or *C. saximontana* vs. *C. jamesii* or *C. juniperorum*; TABLE 16). Divergence values between *C. latebracteata* and the rest of the section (3.0-4.2%) were nearly twice as high as the difference between any other two species compared. Sequence divergence with the outgroup was considerably higher, ranging from 9.2-17.4% of the sequence. The range in sequence divergence within section *Phyllostachys* is very similar to that seen in the genus *Dendroseris* D. Don. (0.0-4.1%; Sang et al., 1994). If the average mutation rate estimated for this genus ($3.94 \times 10^{-9}$ per site per year) is used to determine divergence times in sect. *Phyllostachys*, this would suggest that the ancestors of the *C. juniperorum*/*C. jamesii* and the *Carex willdenowii* complex clades diverged 1.74 mya, and that the split between *Carex willdenowii* and the ancestor of *C. superata* and *C. basiantha* clade occurred approximately 1.16 mya. This would also suggest that the two species pairs that have identical ITS sequences speciated less than 0.58 mya, and that *C. backii* and *C. saximontana* diverged around 3.48 mya.

With the exception of the unique 10 bp deletion in *C. filifolia*, insertion-deletion (indels) events were never greater than 2 bp in size (fig. 22). Of the 9 unambiguous indels that were scored, three were synapomorphies and seven were autapomorphies. All indels had a consistency index (CI) equal to 1.0.

The use of only the two nearest outgroups to sect. *Phyllostachys* (chapter 3) had a beneficial effect on the phylogenetic analysis. Only 1.3% of the positions had to be excluded due to alignment ambiguity as opposed to 6.2% in the previous analysis (chapter 3). As a result, a phylogenetically informative deletion (indel 9; figs. 24 and 25) located in a highly variable portion of ITS 2 that had to be excluded in chapter 3 was used in this analysis. This proved to be the only molecular character that supported the monophyly of the narrow-scaled clade.

***Morphological and Anatomical Analysis***

An examination of the anatomy and morphology of section *Phyllostachys* revealed 25 discontinuous characters (TABLE 12) which were polarized using sections *Firmiculmes* and *Filifoliae* as outgroups. Of the 25 characters used in the analysis, 17 (68%) were phylogenetically informative (i.e., RI>0), and 12 (48%) had a CI equal to 1.0.

The phylogenetic analysis produced 11 trees, 42 steps in length with a consistency index (CI) of 0.64 and retention index (RI) of 0.66. The one tree topologically identical to the 50% majority rule tree (Margush and McMorris, 1981) is presented in fig. 23 along with bootstrap values, decay indices, and unambiguous character changes per branch. Two major clades were distinguished in this analysis: (1) a wide-scaled clade consisting of *Carex backii*, *C. saximontana*, and *C. latebracteata*; and (2) a narrow-scaled clade comprising *C. jamesii*, *C. juniperorum*, *C. willdenowii*, *C. superata*, and *C. basiantha*.

The wide-scaled taxa formed the strongest clade in the section (five apomorphies, decay index (DI) = 4; fig. 23), and they were the only statistically significant group (95% bootstrap value; fig. 23). *Carex backii*, *C. saximontana*, and *C. latebracteata* formed a clade based on the shared possession of chlorophyllous pistillate scale margins [morphological character m1; CI = 0.50], short thickened stigmas [m8; CI = 1.0], fused staminate scale margins [m14; CI = 0.50], leaves greater than 3 mm [m15; CI = 0.50], and pistillate scales that entirely conceal the perigynia [m2; CI = 1.0]. The most basal taxon on the cladogram, *C. latebracteata*, was found within this clade (two nodes from the ancestor). Within the wide-scaled taxa, *C. backii* and *C. saximontana* formed a weak clade (53% bootstrap value) based on the shared derivation of leaf-like pistillate scales (i.e., uppermost) [m3; CI = 0.50], and acuminate [m9; CI = 1.0] and erect stigma lobes [m10; CI = 1.0]. Both *C. backii* and *C. saximontana* possessed distinct morphological apomorphies that distinguished the two from each other (*C. saximontana* [m5]; *C. backii* [m7]).

The narrow-scaled taxa (*C. jamesii*, *C. juniperorum*, *C. willdenowii*, *C. superata*, and *C. basiantha*) formed a weak monophyletic group (37% bootstrap value) based on a single synapomorphy, swollen achene bases [m13; CI = 1.0).

Within the narrow-scaled taxa, *C. juniperorum* and *C. jamesii* formed a small, moderately robust clade (DI = 1, 76% bootstrap). This monophyletic group was supported by contracted beaks [m6; CI = 1.0], reddish-brown basal sheaths [m23; CI = 1.0], and staminate scales that are both fused [m14; CI = 0.50] and truncate [m15; CI = 1.0]. A considerable amount of parallel evolution was seen between *C. juniperorum* and the rest of section *Phyllostachys*. This species shared apomorphies with members of both the wide-scaled [m1, m3, and anatomical character a19] and narrow-scaled [m20] clades. The *C. willdenowii* complex (*C. willdenowii*, *C. superata*, and *C. basiantha*) formed a weak clade within the narrow-scaled group (DI = 1, 57% bootstrap). A single character state change from synchronous to asynchronous flowering periods [m25; CI = 1.0], supported the monophyly of this group. Within the complex, *C. superata* and *C. basiantha* formed a clade (DI = 1, 74% bootstrap) supported by a single synapomorphy, seasonal dimorphism in the inflorescence [m24; CI = 1.0]. Although the topology of the tree suggested that these taxa formed the most derived clade within the section, they possessed fewer apomorphies than both the clade consisting of *C. jamesii* and *C. juniperorum* (five), and the clade consisting of *Carex backii* and *C. saximontana* (eight).

Many of the clades in the morphological and anatomical analysis possessed very few apomorphies and were statistically weak. A strict consensus of the 11 most parsimonious trees resulted in the collapse of the wide-scaled clade, the *C. backii* and *C. saximontana* clade, and even the clade supporting the monophyly of section *Phyllostachys*. The single morphological character (m21; stems apically dilated) that differentiated the ingroup from the outgroup was not sufficient to prevent outgroup species from entering the ingroup during parsimony analysis (the *Phyllostachys* clade collapsed in the 50% majority-rule tree).

***Molecular Analysis***

The alignment of ITS sequences produced a matrix of 447 characters of which only 3 (1.3%) positions at the 3' end of ITS 1 had to be excluded due to ambiguity in the alignment (marked by asterisks in fig. 22). Of the remaining 444 characters, 111 positions were variable (24.8%), but only 30 (6.7%) of these were potentially informative (i.e., shared by more than one species). The phylogenetic analysis of molecular characters using both point mutations and insertion/deletion events produced only 2 trees, 139 steps in length. Both trees had a CI of 0.92 (0.78 excluding uninformative characters) and an RI of 0.81. A value of -2.353170 was calculated for the $g_1$ statistic from the random distribution of 10 000 trees. This value was considerably lower than the critical value of -0.33 ($P<0.01$ level; 100 variable characters, 10 taxa) indicating that the sequences had not been saturated by mutations and were appropriate for phylogenetic analysis (Hillis and Huelsenbeck, 1992).

The more resolved of the two most parsimonious trees obtained during parsimony analysis is presented in fig. 24. The topology of this tree was highly congruent with the morphological and anatomical tree, and only differed in the placement of *C. latebracteata* as sister to the narrow-scaled taxa, and in the lack of support for the *C. willdenowii* clade. As in the morphological and anatomical analysis, most of the clades produced from molecular data in sect. *Phyllostachys* had few synapomorphies and poor branch support. The clade comprising *C. jamesii* and *C. juniperorum* (DI = 1; 89% bootstrap), was the only exception to this rule. One significant difference between the two analyses was seen in character support for the monophyly of sect. *Phyllostachys*. While only a single character could be found to separate the ingroup from the outgroup in the morphological and anatomical analysis, 7 unambiguous point mutations and an insertion/deletion [4] event strongly supported the recognition of sect. *Phyllostachys* as a natural group (DI = 7; 99% bootstrap). Nonetheless, the estimates of phylogeny drawn from these trees were very similar. The only significant topological difference was isolated to a single

homoplastic transition (character 295; fig. 24) in the molecular tree which weakly supported a sister relationship between *C. latebracteata* and the wide-scaled clade. The two data sets were thus combined to further resolve and support the relationships suggested in these trees (Donoghue and Sanderson, 1992).

### *Combined Analysis*

The phylogenetic analysis of a combined morphological, anatomical, and molecular data set produced a single tree, 182 steps in length (fig. 25). This tree had a CI of 0.86 (0.70 excluding uninformative characters) and an RI of 0.73, and it was topologically identical to the tree produced in the morphological analysis. Combining data sets produced a stronger and more resolved phylogenetic hypothesis than the analyses of separate data sets. Statistical support for clades present in the combined analysis either remained stable or increased significantly as compared to the support for these clades in separate data analyses. Examinations that used a different weighting scheme for transitions and transversions (Ti/Tv=2.5) produced the same most parsimonious tree, and showed little change in support for clades (cf. figs. 25 and 26). Because data sets did not conflict significantly, and because combining data sets led to improved resolution and increased statistical support for clades, the combined analysis tree was considered the best estimate of phylogeny for section *Phyllostachys*. Character evolution, and biological and phytogeographical trends were interpreted within the context of this topology.

### *Biological and Phytogeographical Trends*

The distribution of chromosome counts on the combined analysis tree shows a pattern within the wide-scaled clade that is inconsistent with simple agmatoploidy (fig. 27). Speciation within this clade appears to have been accompanied by a reduction in chromosome numbers, not an increase (i.e., n = 49, for *C. latebracteata*, and n = 32 and

33 for the clade comprising *C. saximontana* and *C. backii*; fig. 27; TABLE 14). If chromosome evolution is assumed to proceed via agmatoploidy in the narrow-scaled clade, the overlapping aneuploid series between *C. jamesii* (n = 33, 35) and *C. willdenowii* (n = 31, 39) makes a determination of evolutionary direction ambiguous. The low (n = 33) and high chromosome counts (n = 35) for *C. willdenowii* implies that this species is both more primitive and more advanced than *C. jamesii*.

A relationship between present species ranges, phylogeny, and Pleistocene ice distributions is apparent in the branching patterns of the combined tree (fig. 27; TABLE 14). Among the wide-scaled taxa, the clade that comprises *C. saximontana* and *C. backii* prefers naturally disturbed, glaciated regions, while the most basal taxon in sect. *Phyllostachys*, *C. latebracteata*, is endemic to a glacial refugium, the Ouachita mountains. The main dichotomy in the narrow-scaled clade also divides along lines that appear to be related to the glacial epoch. The *Carex willdenowii* complex (*C. superata*, *C. basiantha*, and *C. willdenowii*; Naczi, Reznicek, and Ford, 1997) is found predominately on non-glaciated terrains, whereas the clade comprised of *C. jamesii* and *C. juniperorum* ranges widely on both glaciated and non-glaciated lands.

The micromorphological groups suggested in chapter 2 agree with the clades presented in the combined tree (fig. 26). All of the species pairs identified in the combined analysis correspond to those species with the most similar silica bodies. The clade comprising the three species of the *Carex willdenowii* complex is also supported by the presence of highly convex silica platforms.

Trends in character evolution and flower number in the combined tree appear to be related to the evolution of differences in breeding system between the wide-scaled and narrow-scaled clades. The derivation of large pistillate scales that conceal the perigynia and inflorescence, and a trend towards a reduction in the total number of flowers in the inflorescence with speciation (fig. 27; TABLE 14), points to the evolution of an autogamous breeding system in the wide-scaled clade. In the narrow-scaled clade,

speciation has been accompanied by an increase in flower number (fig. 27; TABLE 14), a loss of fusion in the staminate scales (*C. willdenowii* complex), the derivation of asynchronous spike production (*C. willdenowii* complex), and the development of seasonally dimorphic spikes in *C. basiantha* and *C. superata*. These trends appear to point to the evolution of mechanisms for increased outcrossing in the narrow-scaled clade.

## 4.3 Discussion

***Utility of Morphological, Anatomical, and Micromorphological Characters to Future Phylogenetic Studies in Carex.***

Conflicts with trends in morphological data sets have led several authors to suggest that homoplasy is common in anatomical (Akiyama, 1942; Standley, 1987; Standley, 1990a) and micromorphological (Rettig, 1986; Waterway, 1990a) characters. However, several of these studies have come to this conclusion while working on either paraphyletic groups (Standley, 1987), or large groups whose taxonomy is still highly confused (Standley, 1990a; Waterway, 1990a). Chapter 2 assessed the utility of these characters for inferring evolutionary relationships by comparison to trends in morphology, but these character types have never been evaluated within the context of an hypothesized phylogeny. The present phylogenetic analysis is thus a unique opportunity to evaluate the contribution of different character types to the analysis, and to suggest which characters may be the most rewarding to future phylogenetic studies below the sectional level in *Carex*.

Not surprisingly, almost all of the non-molecular characters used in this analysis were reproductive in nature (17 of 26 characters). These characters were also much more reliable for phylogenetic reconstruction than were either vegetative or anatomical characters. Of the nine vegetative (5) or anatomical (4) characters scored in this analysis only three had an RI greater than zero. These characters also accounted for four of the six completely homoplastic characters (i.e., CI = 0; RI = 0) used in the combined analysis.

Anatomical leaf and culm characters were particularly unreliable, and did not support any relationships in the final phylogenetic reconstruction. Only three characters [a18, a19, a22] could be scored discretely from chapter 2, and of these three, one was autapomorphic [a22], and two [a18, a19] were completely homoplastic. Studies that have broadly examined leaf and culm anatomy in *Carex*, such as Shepherd (1976) and Standley (1990a), have found some correlation between anatomical characters and

current morphological classifications. However, these studies have also concluded that the exclusive use of anatomical characters in *Carex* would result in the grouping of obviously disparate taxa. Presumably, this is due to high levels of homoplasy or stasis in anatomical characters (Standley, 1987; Standley, 1990a), as is suggested by the great uniformity of the anatomy of the genus and its tribe the *Cariceae* (Metcalf, 1971; Reznicek, 1990). Although there is little doubt that anatomical leaf and culm characters are of great value for species circumscriptions (e.g., Le Cohu, 1970; Metcalf, 1971; Shepherd, 1976; Standley, 1990a; chapter 2), my results indicate that their value for cladistic analyses within and between sections may be limited (chapter 2).

Micromorphological characters, on the other hand, are entirely congruent with the proposed phylogenetic hypothesis. All of the species pairs identified in this study also correspond to those species with the most similar silica bodies (chapter 2). The *Carex willdenowii* clade, for which only a single reproductive character could be found in this analysis, is supported in the micromorphological analysis by the shared possession of highly convex silica platforms. Within section *Phyllostachys*, it appears that micromorphological characters are superior to anatomical characters as indicators of relationship. However, as was previously pointed out in chapter 2, not only are silica body characters difficult to define, but the silica bodies seen in the section are of a type common to many Carices and cyperaceous genera. This suggests that high levels of homoplasy can be expected between higher taxa (Rettig, 1986), making them useless for polarizing characters using the outgroup method. Some authors have suggested that silica body characters are evolutionally conserved and can be used to circumscribe sections comprised of closely related species (e.g., Walter, 1975; Tallent and Wujek, 1983; Menapace and Wujek, 1987). Several studies on larger sections have contradicted this conclusion (e.g., Waterway, 1990a), although smaller groups within these sections appear to be supported. This suggests that micromorphological characters are appropriate indicators of relationship between closely related species and sections (Walter, 1975;

etc.), and between closely related species within large sections (Waterway, 1990a). Given the difficulties associated with using these characters directly in cladistic analyses (chapter 2) they are probably best used as external data sets for evaluating relationships hypothesized using other lines of evidence.

***Character Evolution and Breeding Systems***

Evolution within section *Phyllostachys* has resulted in the formation of two distinct clades: (1) a wide-scaled clade consisting of *Carex backii*, *C. saximontana*, and *C. latebracteata*; and (2) a narrow-scaled clade consisting of *C. willdenowii*, *C. superata*, *C. basiantha*, *C. juniperorum*, and *C. jamesii*. Character evolution within these two clades follows lines that would appear to suggest that differences in breeding systems have evolved between the wide- and narrow-scaled taxa.

In the wide-scaled clade, the large pistillate scales that conceal the perigynia and inflorescence, the short, thickened stigmas, highly congested inflorescences (< 20 flowers), and evolutionary trend towards a reduced number of flowers in the inflorescence (fig. 27), all suggest adaptations to an autogamous breeding system. In *Carex backii* and *C. saximontana*, the evolution of erect stigmas would seem to be a further refinement of the autogamous system. Although the inflorescences in section *Phyllostachys* are androgynous, the erect stigmas that have evolved in these two species often ensure that the stigmatic surfaces of several pistillate flowers either rest up against, or are in close proximity to, the staminate flowers of the inflorescence (Starr, unpublished data).

On the other hand, character evolution in the most derived members of the narrow-scaled clade points to the evolution of mechanisms for increased outcrossing. An asynchronous flowering period and a loss of fusion in the staminate scales of the *C. willdenowii* complex, appears to have been accompanied by an increasing trend in the number of staminate flowers (fig. 27), and the evolution of sexually dimorphic

inflorescences in *Carex basiantha* and *C. superata*. These two species produce two kinds of inflorescence: (1) a staminate inflorescence with aborted pistillate flowers that is generated only in the spring, and (2) a pistillate inflorescence with a small staminate portion that is produced throughout the growing season. If there is differential maturation in the two types of inflorescence this could be a mechanism for promoting outcrossing in the spring, while the asynchronous production of pistillate inflorescences could favour selfing or apomixis during the rest of the season.

While the hypothesis that the wide- and narrow-scaled clades may have different breeding systems seems plausible, isozyme analysis of sect. *Phyllostachys* does not support it (Ford et al., 1997a, 1997b). Outcrossing rates inferred from F values are highly negative indicating that all the species in the section display heterozygous excess. Ford et al. (1997b) suggested that processes such as selection, disassortative mating, and long distance seed dispersal might be responsible for maintaining the high levels of heterozygosity seen throughout the section. However, if disassortative mating and/or long distance seed dispersal were taking place this would still require a reasonable amount of outcrossing to maintain the levels of heterozygosity detected in sect. *Phyllostachys*. Within the narrow-scaled clade such an hypothesis is tenable, but it is not so easily applied to the wide-scaled taxa. In particular, it is difficult to understand how a species such as *Carex latebracteata*, whose pistillate scales completely envelop the entire inflorescence of the plant, could not be either autogamous or apomictic. Given the section's perennial habit, and the fact that apomixis is unknown in Carices (Whitkus, 1988), this would appear to favour the hypothesis that the species are predominantly selfers, and that heterozygous excess in sect. *Phyllostachys* is maintained by highly infrequent outcrossing events and selection for heterozygotes (Hamrick, 1989; Mitton, 1989). If these species are predominantly selfing this may also explain how species such as *C. backii* and *C. saximontana* are maintaining reproductive isolation despite mixed populations and a lack of ecological differentiation (Ford 1997a; see below).

***Phylogeny, Phytogeography, and Speciation***

The historical events of the Pleistocene have often been cited as having had a significant effect on the distribution and speciation of northern Carices (Kreczetovicz , 1936; Whitkus, 1981; Kukkonen and Toivonen, 1988; Ball, 1990). Kreczetovicz (1936) believed that many sections, such as the *Phyllostachys*, had arisen during the Pliocene or post-Pliocene epochs due to the increased selective pressures that would have accompanied the onset of a glacial period (cooling and xerothermization). Whitkus (1981) hypothesized that the preference for seral habitats in glaciated areas and close morphology of the *Carex macloviana* aggregate was a consequence of the fact that the group had speciated into the open habitats left by the retreating ice (c. 20 000 ybp). Similarly, Kukkonen and Toivonen (1988) and Ball (1990), felt that the advancing and retreating glacial front had promoted rapid speciation in *Carex* by splitting species ranges and by creating new habitats for novel species to establish. These authors also suggested that the unusual cytology of the genus may have been a contributing factor to such a rapid speciation process. Unfortunately, these hypotheses were merely speculative, and firm evidence for a connection between the glacial epoch and speciation in the genus has been lacking. If I superimpose phytogeography and past glacial movements upon the phylogeny of section *Phyllostachys* (fig. 27), it seems to indicate that the main dichotomies within both the narrow-scaled and wide-scaled clades are somehow correlated with the historical events of the Pleistocene.

The members of the *C. willdenowii* s.l. complex are almost exclusively confined to regions south of the former extent of the ice (see Naczi, Reznicek, and Ford, 1997, for distributions). Although the ranges of the most derived species in the *C. willdenowii* complex (*C. superata* and *C. basiantha*) never approach the past extent of the ice, the range of the most basal taxon in this complex, *C. willdenowii*, closely follows the southern edge of the Kansas ice sheet. This is in contrast to *C. jamesii*, which can be found both on and off formerly glaciated lands (see Crins, in press, for distributions), and

*C. juniperorum*, which is distributed on glaciated lands or on lands that were heavily influenced by glaciation (i.e., within kilometers of the ice front). *Carex juniperorum* is particularly interesting in that it is highly localized and restricted to two highly disjunct areas, where it typically grows in a specialized habitat (i.e., on alvars; Catling et al. 1993). It is possible that *C. jamesii* and *C. juniperorum* may represent a recent progenitor-derivative species pair where the movement north by *C. jamesii* after the retreat of the ice may have resulted in the speciation of *C. juniperorum* into marginal habitats. This hypothesis is supported by the complete identity of the ITS sequences in these two species, and by allozyme data which indicates that *C. juniperorum* possesses a subset of the allelic diversity present in *C. jamesii* (Ford et al., 1997b). Divergence time estimates based on the average mutation rate for the ITS region in the genus *Dendroseris* (Sang et al, 1994), suggest not only that *C. juniperorum* has speciated within the last 0.58 million years, but that speciation as a whole within the narrow-scaled clade has occurred within the last 1.7 million years (i.e., within the Pleistocene epoch). The present disjunct populations of *Carex juniperorum* may represent the remnants of a wider species range that has contracted as succession replaced the open habitats left by retreating glaciers.

Within the wide-scaled taxa, the dichotomy that separates *C. saximontana* and *C. backii* from *C. latebracteata* also appears to be associated with the glacial events of the Pleistocene (fig. 27). While *C. saximontana* and *C. backii* are predominantly found on areas that were formerly glaciated, the most basal taxon in this clade, *C. latebracteata*, is confined to a known glacial refugium (i.e., the southeastern United States).

Within *Carex*, the occurrence of closely related sympatric species appears to be a fairly common phenomenon (Ball, 1990). The close species pair of *Carex backii* and *Carex saximontana* appears to represent just such an example. Little ecological differentiation appears to have occurred between these two taxa. Both species prefer the naturally disturbed slopes of moist coniferous or deciduous forests, and both are known to occur in close proximity (e.g., Hudson, 1977; Starr, unpublished data), and even in

mixed populations (e.g., *C. saximontana*, Ford 9501 & Starr WIN; *C. backii*, Ford 9502 & Starr WIN, Treesbank, Manitoba). The species range of *C. backii* significantly overlaps that of *Carex saximontana*, although populations of *C. backii* occur less frequently in the west than they do in the east. Nevertheless, both isozyme (Ford et al., 1997a) and ITS sequence data suggest that these species are reproductively isolated, as does their marked anatomical and morphological differences. If the events of the glacial period had an effect on speciation, an hypothesis similar to that of Kukkonen and Toivonen (1988) would best explain all available data. In such an hypothesis, the fragmentation of an ancestral population with ice movements would be accompanied by the fixation of few characters and the derivation of reproductive barriers. No ecological differentiation would have occurred and the present sympatric distribution would only be of recent origin. This would explain why the two are distinguished by unique alleles at only one locus (DIA-1), have such a high genetic identity (I=0.854), and yet do not produce intermediates within mixed populations (Ford et al., 1997a).

The same hypothesis was used by Ball (1990) to explain how the numerous wind-pollinated, more or less sympatric species of *Carex* section *Laxiflorae* may have evolved in eastern North America. Like sect. *Phyllostachys*, the species in this section are very closely related (Manhart, 1984), poorly differentiated ecologically, and sympatric across large portions of their ranges. Based mainly on their low chromosome counts, Ball (1990) hypothesized that *C. purpurifera* Mackenzie and *C. manhartii* Bryson, two narrow endemics to the southern Appalachian region, were relictual species that could be close to the ancestors of the section.

Naczi (1990) also detected the same type of evolutionary trends in his phylogenetic analysis of *Carex* section *Griseae* (Naczi, 1992). Several of the species belonging to the most basal clade in the section are either narrow endemics, such as *C. asynchrona* Naczi and *C. brysonii* Naczi, or had small, often disjunct geographic ranges, such as *Carex ouachitana* Kral, Manhart, & Bryson. Five of the six species found within

the basal clade were either restricted to the southeastern United States or northeastern Mexico, while the sixth was found within this region but was not restricted to it. Naczi (1992) thus concluded that the most basal taxa within section *Griseae* were relicts, and that the section had an origin somewhere in the southeastern United States.

The evolutionary pattern displayed by all three of these sections appears to be the same. The most primitive members are narrowly distributed relicts located within regions that are known to be glacial refugia, they are very closely related, and they are poorly differentiated ecologically. Chromosome evolution within sections *Griseae* and *Phyllostachys* does not appear to follow simple agmatoploidy, possibly indicating that the cytological flexibility of Carices may have played a part in their evolution (see below). Future phylogenetic studies should try to determine whether the evolutionary patterns described here are restricted to these three sections or whether they might represent a wider evolutionary trend in the genus. It is interesting to note that Crins (1990) also found that the most primitive members of section *Limosae* were those that had the most restricted contemporary ranges.

### *Taxonomic Implications*

The results of this analysis support not only the clade comprising *C. backii* and *C. saximontana* (4 synapomorphies, 71% bootstrap), but also the separation of these taxa into two distinct species. Taxonomists such as Gleason and Cronquist (1991) and Boivin (1992), have treated *C. saximontana* as a variety of *C. backii*, while others such as Taylor (1983), have failed even to recognize the morphological distinctiveness of these two species. The present study reveals at least two molecular autapomorphies in *C. saximontana* [246, 429], and one in *C. backii* [434] that are unique to all the populations sampled for these species including a mixed population from Manitoba (*C. saximontana* Ford 9501 & Starr WIN; *C. backii* Ford 9502 & Starr WIN). Several consistent morphological characters such as the much shorter perigynium (< 4.5 mm), the small

contracted beaks (< 1 mm), and the tight investment of the achene by the perigynium are useful characters that distinguish *Carex saximontana* from *Carex backii* (see Crins, in press). The most convincing evidence for their separation, however, comes from anatomy (chapter 2). A glaucous indument, a revolute leaf, hyaline margins, and papillation on both the adaxial and abaxial surfaces of the leaf and on the entire epidermis of the culm, are all characteristics that *C. saximontana* does not share with *C. backii*. This evidence, in combination with the allozyme divergence demonstrated by Ford et al. (1997a), clearly supports the recognition of two distinct species.

The results of the phylogenetic analysis do not support Hermann's (1954) ranking of *C. superata* as a variety of *C. willdenowii* s.l. The topology of the cladogram suggests that at least one speciation event separates *C. willdenowii* from *C. superata* and *C. basiantha*. This conclusion is concordant with the recent morphological and molecular evidence presented by Naczi, Reznicek, and Ford (1997), and by Ford et al. (1997c) which suggests that *C. willdenowii* s.l is a complex of three distinct species. These two studies have found that the three members of the complex (i.e., *C. willdenowii*, *C. superata*, and *C. basiantha*), can be separated not only by distinct morphological traits, but also by unique alleles at multiple loci. This conclusion is also strongly supported by the anatomical and micromorphological survey of the section (chapter 2). The distinctive conical satellite bodies of *C. willdenowii* and the roughened silica platforms of *C. basiantha*, clearly distinguish these taxa from each other and the rest of the section. Characters such as the short, highly sclerified culms of *C. superata*, and weakly sclerified margins of the stems of *C. basiantha*, are further evidence that *Carex willdenowii* s.l. is a complex of three distinct species.

The other three species in section *Phyllostachys* (*C. latebracteata*, *C. juniperorum*, *C. jamesii*) are also distinguished by a number of morphological, anatomical, and micromorphological differences, however, only *C. latebracteata* possesses species-specific molecular autapomorphies.

***Chromosome Evolution***

The genus *Carex* is remarkable for its long and nearly continuous aneuploid series. Chromosome numbers in the genus range from n=6 to n=56, and every number from n=12 to n=43 is found in at least one species (Wahl 1940). The way in which this aneuploid series evolved, however, is quite controversial. At least some of the observed aneuploidy in *Carex* can be attributed to the effects of polyploidy (Heilborn, 1932; Tanaka, 1940; Wahl 1940; Davies, 1956; Löve, Löve, and Raymond, 1957; Faulkner, 1972); however, several authors have recognized that chromosome size decreases with increasing number, and they have thus suggested that an increase in chromosome number, via fragmentation (i.e., agmatoploidy), accompanies speciation (e.g., Davies 1956; Nishikawa, Furuta, and Ishitobi, 1984; Whitkus, 1987). This is supported by the fact that like many members of the Cyperaceae (e.g., *Eleocharis palustris*; Håkansson 1954, 1958), the genus *Carex* possesses polycentric chromosomes with diffuse centromeres (Grant 1981) which allow chromosomal fragments to act like independent chromosomes during meiosis.

The superimposition of chromosome numbers on the cladograms of sections *Ceratocystis* and *Limosae* seems to support the hypothesis that chromosome evolution in *Carex* is unidirectional and ascending (Crins 1990). However, authors such as Faulkner (1972) and Reznicek (1990) have suggested that the same cytological peculiarities that allow for the fragmentation of chromosomes in *Carex*, could also be involved in their fusion. Such an hypothesis would explain why a presumably highly derived, xeromorphic section with a reduced inflorescence, such as section *Acrocystis* (n=15-18; Wahl 1940), could possess such low chromosome counts (Reznicek, 1990). A reason as to why fusion should occur is given by Stebbins (1974). He believes that the aneuploid series that have arisen in many species adapted to open or semi-arid pioneer habitats are related to the genetic linkage of adaptive gene combinations (e.g., *Astragalus*, *Crepis*).

As in section *Griseae* (Naczi 1992), chromosome evolution within section *Phyllostachys* does not always appear to follow simple agmatoploidy (fig. 27). Within the wide-scaled clade the most primitive member of the group, *C. latebracteata*, possesses a haploid chromosome count of n=49 (Naczi, unpublished), whereas the predominantly on-ice species pair of *Carex backii* and *C. saximontana* possesses counts of n=33 and n=32 respectively (Löve and Löve, 1981). This may represent an instance where a fixing of favourable gene combinations has resulted in a reduction in chromosome numbers with speciation into an open habitat (Stebbins, 1974). The use of chromosome number to polarize phylogenetic trees may thus be inappropriate in many sections and could lead to a false sense of evolutionary direction.

Chromosome evolution within the narrow-scaled taxa is ambiguous not only due to a lack of counts, but also due to overlapping series. Naczi (unpublished) reports chromosome counts of n=33 and n=39 for *C. jamesii* and *C. willdenowii* respectively, whereas Wahl (1940) reports values of n=35 and n=31 for the same species. The great disparity between the extreme counts for *C. willdenowii* has never been recorded within a single species of *Carex*, but it is approached in *C. oligocarpa* (n=20-27; Naczi, 1992). The differences seen in the counts for *C. jamesii*, however, are not inconsistent with the range in counts given for other species of *Carex* by previous authors (e.g., Wahl, 1940; Faulkner, 1972; Naczi, 1992). Although the numbers for *C. willdenowii* need to be confirmed, overlapping chromosome series are also seen in section *Griseae* (Naczi, 1992), further highlighting the caution that needs to be exercised when inferring evolutionary direction from chromosome counts. Even if chromosomal evolution is unidirectional and ascending, the extinction or incomplete sampling of one end of an aneuploid series within a species could lead to a false interpretation of phylogeny due to overlapping series between species (cf. *C. jamesii* and *C. willdenowii*).

## 4.4 CONCLUSIONS

ITS sequences have been very useful in resolving phylogenetic questions within sect. *Phyllostachys*. Even though variation within the section was low, the ITS region was able to uncover hidden diversity within sect. *Phyllostachys* that had previously been identified using traditional methods. Although ITS was not informative enough to resolve ingroup relationships alone, congruent data sets of both molecular and morphological characters in combination compensated for the variable rates in the evolution of each character type (Donoghue and Sanderson, 1992). This study like several other studies between very closely related species (e.g., Sang et al., 1994; Bain and Jensen, 1995; Bayer, Soltis, and Soltis, 1996) has pushed the lower limits of the ITS region's ability to resolve relationships. New sequences are needed not only to explore species level questions but also to take advantage of the numerous practical and theoretical benefits of molecular data (e.g., Sytsma, 1990; Donoghue and Sanderson, 1992).

The evolutionary similarities between section *Phyllostachys* and section *Griseae* suggest that the historical and environmental pressures placed on these sections may have been similar. The cladistic analysis of these sections also suggests that chromosome evolution within these taxa is not unidirectional and ascending. Both sections appear to have evolved from the southern United States, and both possess basal taxa that are either narrow endemics or are found in small disjunct populations within areas that are known glacial refugia. Some of the same evolutionary trends are also seen in *Carex* section *Laxiflorae*.

The impact of the Pleistocene glaciations upon the distribution of plant species in North America has been well documented through the study of pollen records (Pielou, 1991). This evidence suggests that migration northward after the retreat of ice sheets was rapid (Davis, 1976), and it is believed that this played a significant role in the population genetics of many species from eastern North America (Lewis and Crawford, 1995). This study suggests that the powerful effects that the events of the Pleistocene appear to have

had on the distribution and genetic diversity of many species (e.g., *Pinus resinosa* Aiton, Fowler, and Morris, 1977; *Thuja plicata* Donn ex D. Don in Lambert, Copes, 1981; *Polygonella* Michx. spp., Lewis and Crawford, 1995) may also have resulted in cladogenesis. The evolutionary similarities between sections *Phyllostachys*, *Griseae*, and *Laxiflorae* may point to a wider evolutionary trend in the genus of recent speciation promoted by Pleistocene glaciation events.

TABLE 12. Morphological characters and character states used in the phylogenetic analysis of *Carex* section *Phyllostachys*. Those character states marked by zeros are primitive. Characters 18, 19, and 22 were determined from the previous anatomical study of the section by Starr (chapter 2).

1. **Pistillate scale** (margins): prominently hyaline (0); chlorophyllous (1)
2. **Pistillate scale** (2nd lowest): not concealing(0); concealing perigynia (1)
3. **Pistillate scale** (uppermost): scale-like (0); leaf-like (1)
4. **Pistillate flowers** (number): <=5 (0); usually >5 (1)
5. **Perigynium** (length): >=4.5mm (0); <4.5mm (1)
6. **Perigynium** (beak base): tapering (0); contracted (1)
7. **Perigynium** (inflation): filled by achene (0); loose (1)
8. **Stigma** (lobe): long, filiform (0); short, thickened (1)
9. **Stigma** (lobe shape): linear (0); acuminate (1)
10. **Stigma** (lobe orientation): reflexed (0); erect (1)
11. **Achene** (carpels): convex (0); concave (1)
12. **Achene** (shape): orbicular to elliptical (0); obovate to obpyriform (1)
13. **Achene** (base): non-swollen (0); round, swollen (1)
14. **Staminate scale** (margins): free (0); fused (1)
15. **Staminate scale** (apex): obtuse to acute (0); truncate (1)
16. **Leaf** (width): <3mm (0); >3mm (1)
17. **Leaf** (indument): green (0); glaucous (1)
18. **Leaf** (shape): V-shaped (0); revolute (1); involute (2)
19. **Leaf** (margin): chlorophyllous (0); adaxial sclerenchyma (1); hyaline, sclerified (2)
20. **Stem** (length): > half the height of leaves (0); < half the height of leaves (1)
21. **Stem** (apex): linear (0); dilated (1)
22. **Stem** (sclerification): weakly sclerified (0); sclerified (1); highly sclerified (2)

TABLE 12. Continued.

| |
|---|
| 23. **Pseudoculm** (basal sheath): light to dark brown (0) reddish brown (1) |
| 24. **Inflorescence**: spikes monomorphic (0) seasonally dimorphic (1) |
| 25. **Inflorescence** (Flowering period): synchronous (0) asynchronous (1) |

TABLE 13. Data matrix of morphological characters used in the phylogenetic analysis of *Carex* section *Phyllostachys*. See TABLE 12 for a description of characters and character states.

| | Characters | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** |
| *Carex backii* | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| *C. saximontana* | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| *C. latebracteata* | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| *C. juniperorum* | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 |
| *C. jamesii* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| *C. willdenowii* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| *C. superata* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 1 | 1 |
| *C. basiantha* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| *C. filifolia* | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. geyeri* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. muticaulis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

TABLE 14. Haploid chromosome counts, total flower numbers in the inflorescence, and present distributions of species relative to the greatest extent of the Pleistocene ice sheets in section *Phyllostachys*.

| Species Studied | Haploid Chromosome Number | Distribution Relative to Pleistocene Glaciers | Flower Numbers |
|---|---|---|---|
| 1. *C. backii* Boott | 33[a] | On Ice | 1-3 male; 2-5 female[d] |
| 2. *C. saximontana* Mackenzie | 32[a] | Predominately On Ice | 2-3; 2-6[d] |
| 3. *C. latebracteata* Waterfall | 49[b] | Off Ice | 2-5 male; 3-14 female[d] |
| 4. *C. jamesii* Schweinitz | 33[b], 35[c] | On/Off Ice | 3-13 male; 1-4 female[d] |
| 5. *C. juniperorum* Catling, Reznicek, & Crins | na | On Ice | (5-) 7-15 (-21) male; (2-) 4-9 female[d] |
| 6. *C. willdenowii* Willdenow | 31[c], 39[b] | Predominately Off Ice | 3-29 male; 3-8 (-9) female[e] |
| 7. *C. superata* Naczi, Reznicek, & B. A. Ford | na | Off Ice | 7-56 male; 2-4 (-6) female[e] |
| 8. *C. basiantha* Steudel | na | Off Ice | 4-61male; 2-4 (-5) female[e] |

[a] Löve and Löve (1981)

[b] Naczi (unpublished)

[c] Wahl (1940)

[d] Crins (in press)

[e] Naczi, Reznicek, and Ford (1997, in press)

TABLE 15. General sequence statistics for ITS 1 and ITS 2 (alone and combined).

| | ITS 1 | ITS 2 | Combined (ITS 1 + ITS 2) |
|---|---|---|---|
| Length range (bp) | 220 - 222 | 221 - 223 (211) | 431 - 445 |
| Length mean (bp) | 221.0 | 220.6 | 441.7 |
| Aligned length (bp) | 224 | 223 | 447 |
| G+C content range (%) | 56.4 - 66.8 | 58.8 - 69.8 | 57.5 - 68.3 |
| G+C content mean (%) | 61.8 | 66.6 | 64.2 |
| Sequence divergence all taxa (%) | 0.0 - 18.1 | 0.0 - 15.6 | 0.0 - 17.4 |
| Sequence divergence ingroup (%) | 0.0 - 4.1 | 0.0 - 4.0 | 0.0 - 4.2 |
| Number of excluded sites | 3 (1.3%) | 0 | 3 (0.7%) |
| Number of indels | 5 | 4 | 9 |
| Number of variable sites | 61 (27.2%) | 50 (22.4%) | 111 (24.8%) |
| Number of potentially informative sites | 19 (8.5%) | 11 (4.9%) | 30 (6.7%) |
| Number of informative sites that unambiguously support ingroup relationships | 3 (1.3%) | 2 (0.9%) | 5 (1.1%) |
| Number of constant sites | 161 (71.9%) | 173 (77.6%) | 334 (74.7%) |
| Number of autapomorphic sites | 42 (18.8%) | 39 (17.5%) | 81 (18.1%) |
| Skewness of tree-length distribution ($g_1$ value for 10 000 random trees) | -2.088972 | -1.724172 | -2.353170 |

TABLE. 16. Sequence divergence values between species of section *Phyllostachys* and the outgroup. Percent divergence is given above the diagonal, absolute differences below the diagonal.

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. *backii* | - | 1.4% | 4.1% | 1.6% | 1.6% | 1.4% | 1.4% | 1.4% | 12.8% | 9.6% | 9.1% |
| 2. *saximontana* | 6 | - | 4.1% | 1.6% | 1.6% | 1.4% | 1.4% | 1.4% | 13.5% | 10.5% | 10.0% |
| 3. *latebracteata* | 18 | 18 | - | 3.2% | 3.2% | 2.9% | 2.9% | 2.9% | 15.2% | 12.1% | 11.4% |
| 4. *juniperorum* | 7 | 7 | 14 | - | 0.0% | 0.7% | 0.7% | 0.7% | 13.3% | 10.0% | 9.1% |
| 5. *jamesii* | 7 | 7 | 14 | 0 | - | 0.7% | 0.7% | 0.7% | 13.3% | 10.0% | 9.1% |
| 6. *willdenowii* | 6 | 6 | 13 | 3 | 3 | - | 0.5% | 0.5% | 12.6% | 10.0% | 8.7% |
| 7. *superata* | 6 | 6 | 13 | 3 | 3 | 2 | - | 0.0% | 12.6% | 9.6% | 9.1% |
| 8. *basiantha* | 6 | 6 | 13 | 3 | 3 | 2 | 0 | - | 12.6% | 9.6% | 9.1% |
| 9. *filifolia* | 55 | 58 | 65 | 57 | 57 | 54 | 54 | 54 | - | 17.6% | 17.5% |
| 10. *geyeri* | 42 | 46 | 53 | 44 | 44 | 44 | 42 | 42 | 75 | - | 7.5% |
| 11. *multicaulis* | 40 | 44 | 50 | 40 | 40 | 38 | 40 | 40 | 75 | 33 | - |

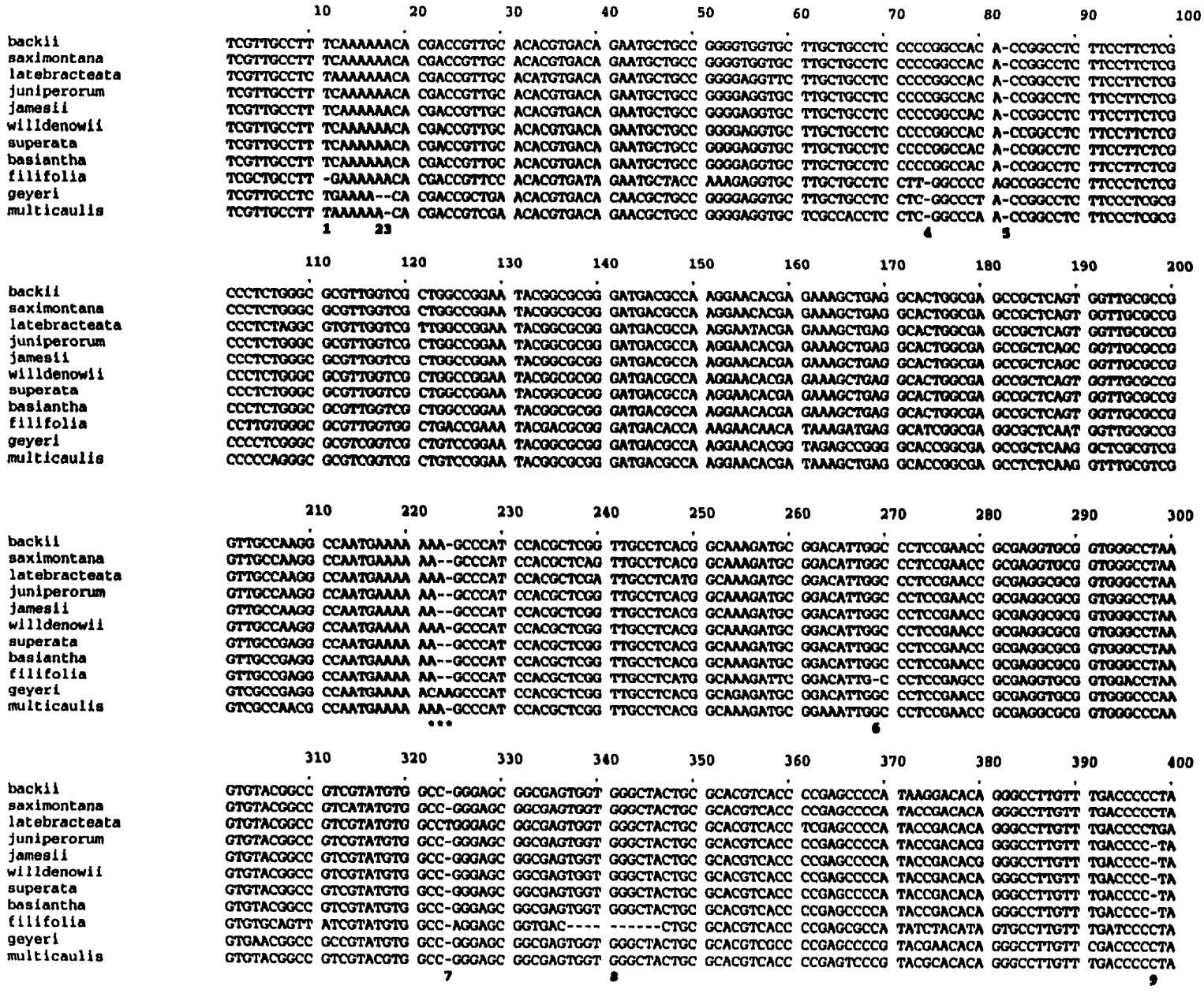


*fig. 22.* Aligned sequences for ITS 1 (positions 1-224) and ITS 2 (positions 225-447) of all taxa used in the phylogenetic analysis of *Carex* section *Phyllostachys*. Regions of ambiguous alignment that were not used in analyses are indicated by asterisks below the sequence. Insertions/deletions (indels) used in the analysis are indicated by number below the sequence. The complete length of the combined ITS 1 and ITS 2 spacers for each taxon is given in brackets at the end of each sequence.

```
                    410         420         430         440
                     .           .           .           .
backii              ACGAGGAGCA TGCTGTCGCG GCTTGTGCTG TGCGGCACCT TCGGACC  [444]
saximontana         ACGAGGAGCA TGCTGTCACG GCCTGTGCTG TGCGGCACCT TCGGACC  [443]
latebracteata       ACGAGGAGCA TGCTGTCGCG GCCTGTGCTG TGCGGCACCT TCGGACC  [445]
juniperorum         ACGAGGAGCA TGCTGTCGCG GCCTGTGCTG TGCGGCACCT TCGGACC  [442]
jamesii             ACGAGGAGCA TGCTGTCGCG GCCTGTGCTG TGCGGCACCT TCGGACC  [442]
willdenowii         ACGAGGAGCA TGCTGTCGCG GCCTGTGCTG CGCGGCACCT TCGGACC  [442]
superata            ACGAGGAGCA TGCTGTCGCG GCCTGTGCTG TGCGGCACCT TCGGACC  [443]
basiantha           ACGAGGAGCA TGCTGTCGCG GCCTGTGCTG TGCGGCACCT TCGGACC  [443]
filifolia           ACAATGAGCA TGCTGTCGCG GCTTATGCTG CGCAGCACCT TCGAACC  [431]
geyeri              ACGAGGAGCA TGCCGTCGCG GCTTGCGCCG TGCGGCACCT TCGGACC  [442]
multicaulis         ACGAGGAGCA TGCCGCCGCG GCTCGCGCCG CGCGGCACCT TCGGACC  [442]
```

fig. 22. Continued.

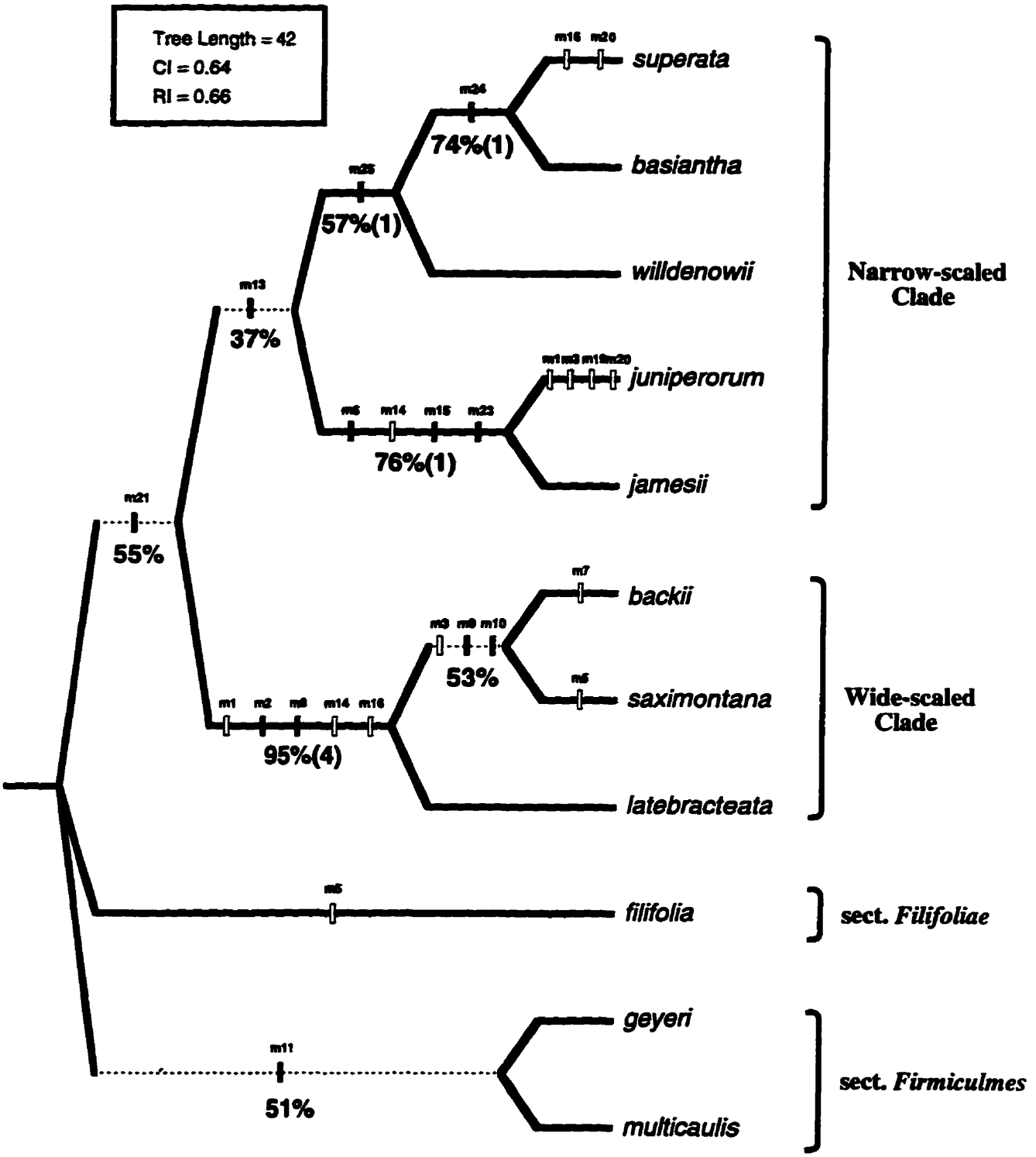


fig. 23. The single tree topologically identical to the 50% majority rule tree of 11 most parsimonious trees found in the phylogenetic analysis of*Carex* section *Phyllostachys* using morphological characters. Unambiguous characters are indicated by bars. Character numbers are given above bars and correspond to those given in TABLE 12. Characters with a CI=1.0 are represented by solid bars, homoplastic characters by open bars. Bootstrap (in percent %) and decay indices (in parentheses) are given below the branch. Branches that collapse in the strict consensus tree are dotted. Tree statistics are given in the top left hand corner.

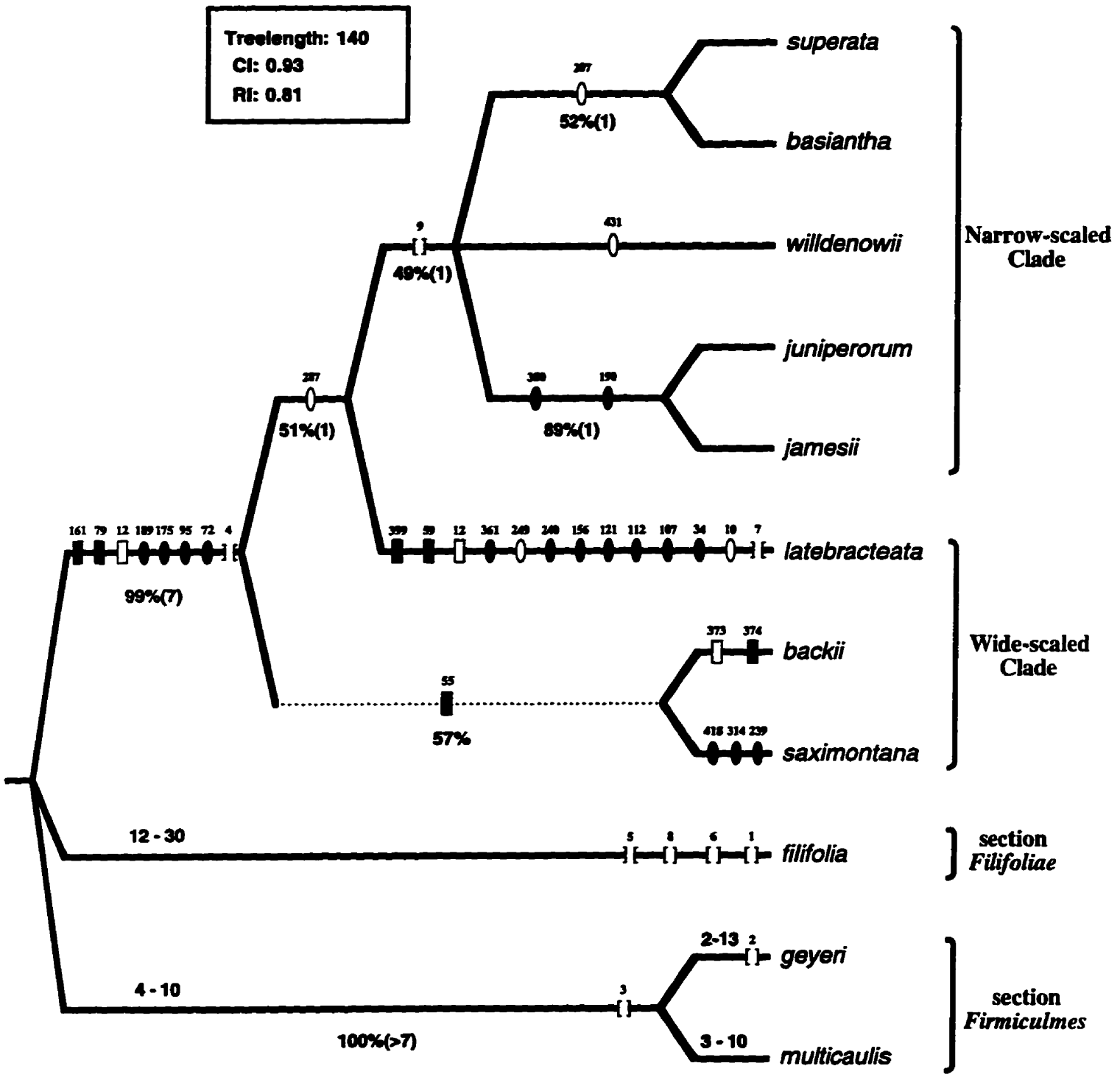


fig. 24. The most resolved of two most parsimonious trees found in the phylogenetic analysis of *Carex* section *Phyllostachys* using molecular characters. Unambiguous transversions are represented by rectangles, transitions by ellipses. Insertions are represented by ] [, and deletions by [ ] (the number in brackets corresponds to the number of the indel in fig. 22). Characters with a CI=1.0 are solid, homoplastic characters are open. Bootstrap (in percent %) and decay indices (in parentheses) are given below the branch. Tree statistics are given in the left-hand corner of the figure. Character numbers are placed above their respective symbols and correspond to those given in figs. 22. Branches that collapse in the strict consensus tree are dotted.

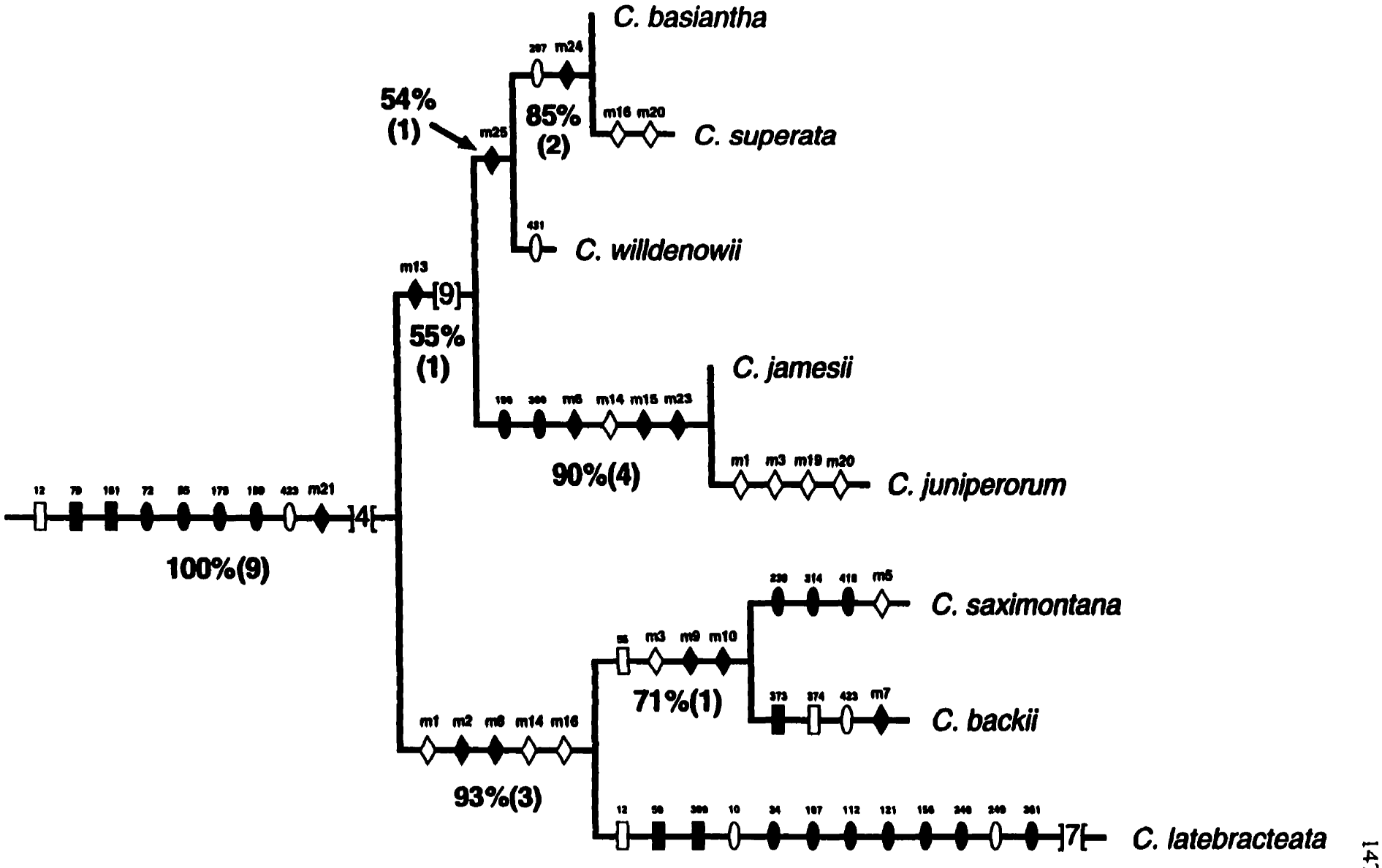




fig. 25. The single most parsimonious tree obtained from the phylogenetic analysis of *Carex* section *Phyllostachys* using morphological, anatomical, and molecular characters (outgroup not shown). The tree is 182 steps long, with a CI of 0.86 (0.70 excluding uninformative characters), and an RI of 0.73. Transversions are represented by rectangles, transitions by ellipses, and morphological characters by diamonds. Insertions are represented by ] [, and deletions by [ ] (the number in brackets corresponds to the number of the indel in fig. 22). Characters with a CI=1.0 are solid, homoplastic characters are open. Bootstrap (in percent %) and decay indices (in parentheses) are given below the branch. Character numbers are placed above their respective symbols and correspond to those given in fig. 22 and TABLE 12. Morphological characters are preceded by a lowercase m (e.g., m14).

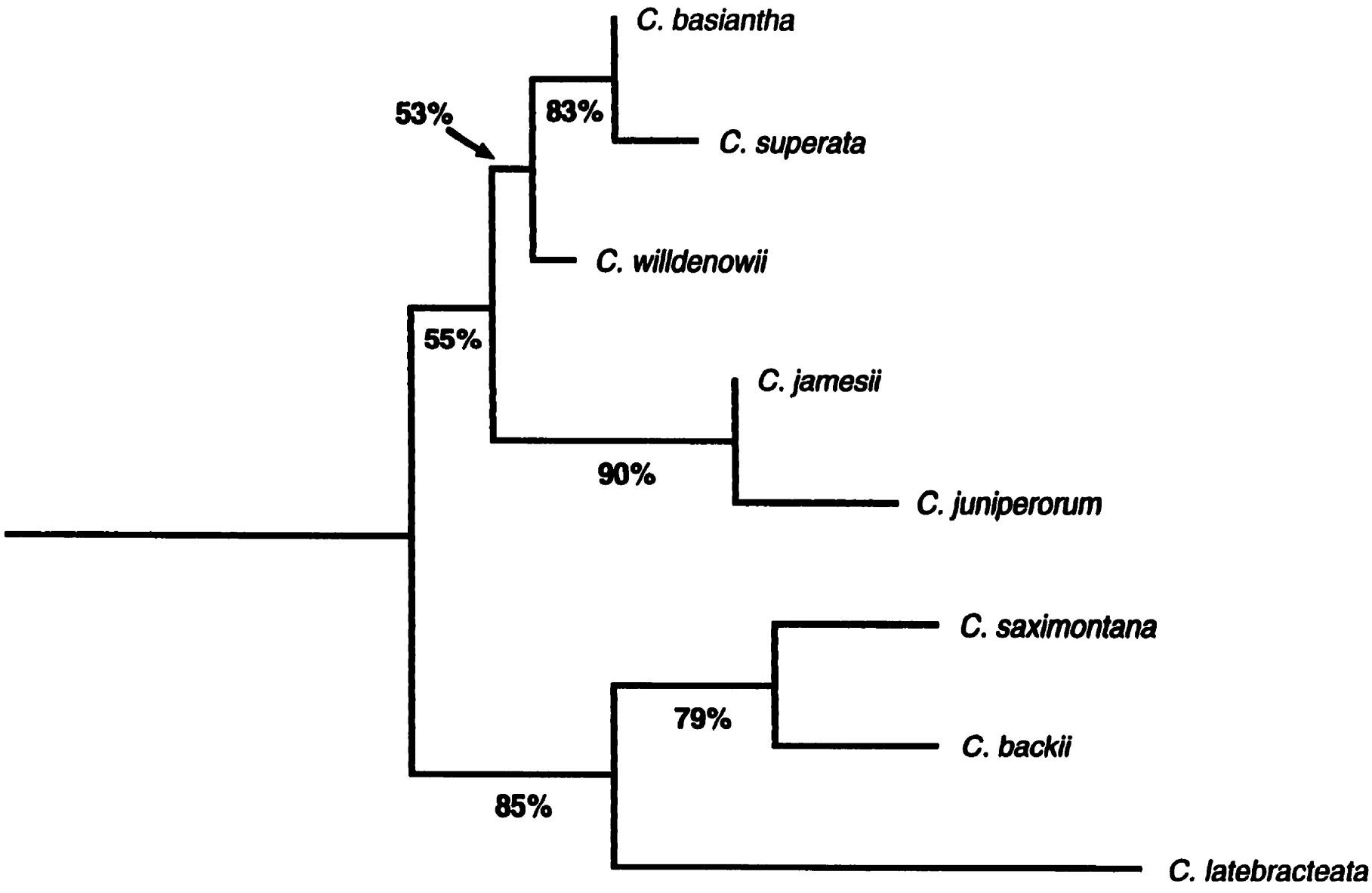


fig. 26.The single most parsimonious tree obtained from the phylogenetic analysis of *Carex* section *Phyllostachys* using morphological, anatomical, and molecular characters (outgroup not shown). A transition to transversion ratio of 2.5 was used during these searches. Bootstrap (in percent %) values are given below branches..

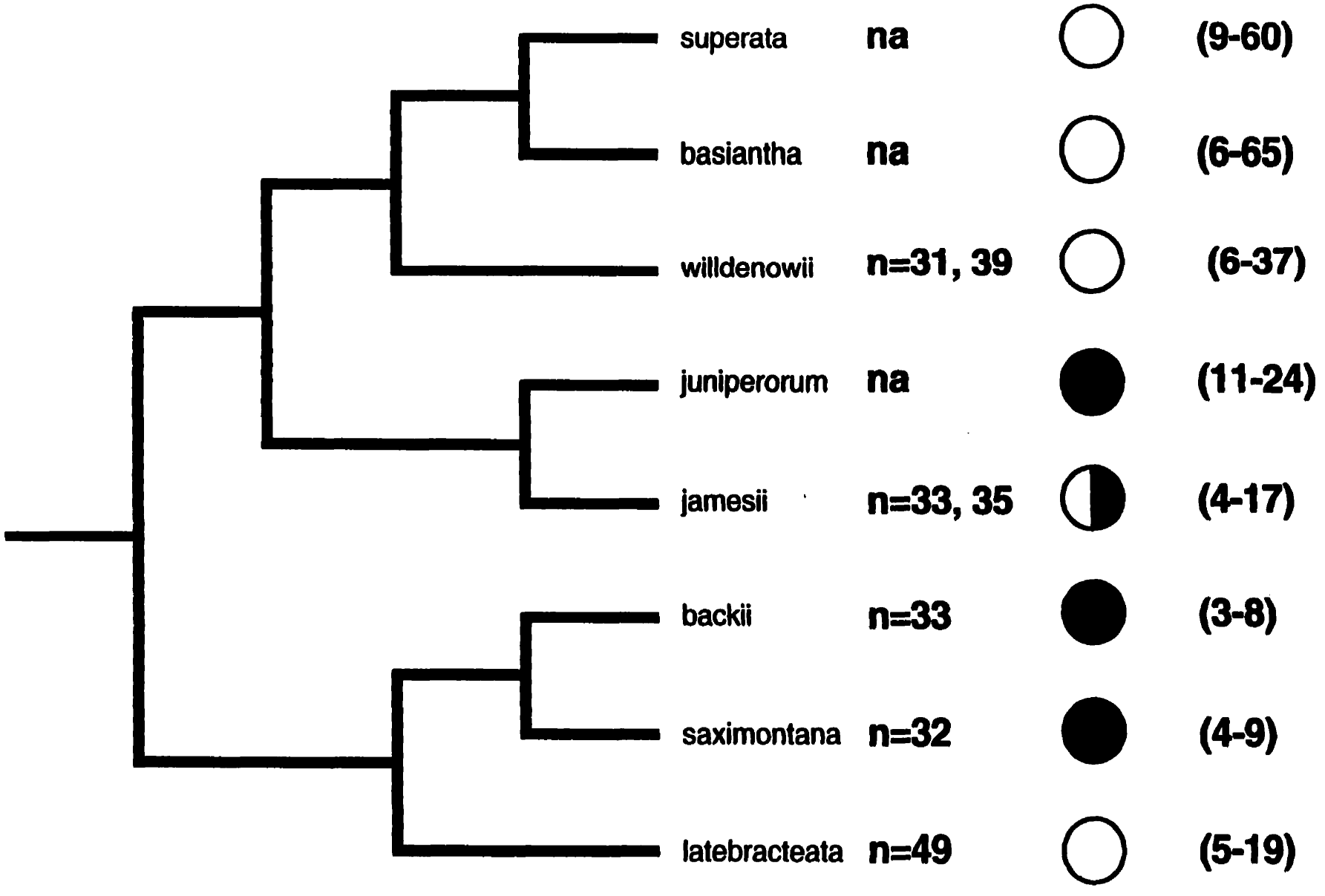


fig. 27. The distribution of haploid chromosome counts, contemporary species ranges relative to the greatest extent of the Pleistocene ice sheets, and total flower numbers on the most parsimonious tree of the combined analysis. Species ranges that are predominantly on formerly glaciated lands are represented by solid circles, ranges predominantly or completely off glaciated lands by open circles, and ranges both on and off ice by half solid circles. Chromosome counts are given to the left of range circles, total flower numbers to the right.

# CHAPTER 5

## CONCLUSIONS

Anatomical, micromorphological, and molecular characters support the recognition of eight species within *Carex* section *Phyllostachys*. Numerous anatomical characters and several molecular autapomorphies clearly separate the close species pair of *C. saximontana* and *C. backii*, although micromorphologically they are alike. Micromorphological and anatomical characters support the recognition of three species within the *C. willdenowii* complex as suggested by Naczi, Reznicek, and Ford (1997) and Ford et al. (1997c).

*Carex* section *Phyllostachys* is a strong monophyletic group that is part of an evolutionally "reduced" clade that includes two sections from subgenus *Primocarex*: *Filifoliae* and *Firmiculmes*. This clade is separate from a larger "compound" clade in the genus that comprises sections from subgenus *Indocarex*, *Carex*, and *Primocarex*. This indicates that all of the subgenera in *Carex* as they are presently circumscribed are artificial, except for subgenus *Vignea*. The presence of the reduced section *Scirpinae* (subgenus *Primocarex*) in the "compound" clade suggests that extreme reduction has occurred along several different lineages within *Carex* as former authors have hypothesized.

The ITS region is highly useful for circumscribing sections and estimating their relationships in *Carex*, but it is not variable enough to fully resolve relationships below the sectional level. Nevertheless, congruent data sets of both molecular and morphological characters, in combination, compensated for the variable rates seen in the evolution of each character type to produce a fully resolved phylogeny. The low levels of infraspecific variation and species-specific mutations further suggest that with a small amount of sampling, this region can sometimes provide useful characters to help circumscribe species in critical groups below the sectional level.

Micromorphological characters are generally more conserved than anatomical characters and are probably good indicators of evolutionary relationship between closely related species and sections. The problems with scoring, polarization, and character numbers indicate, however, that these characters would be best used for suggesting relationships and as external data sets for assessing the strength of a phylogeny constructed using other more conserved characters. Relationships inferred from anatomical leaf and culm characters conflict significantly with morphological and molecular phylogenies, and with trends in micromorphology. This suggests that the contribution of these anatomical characters to future phylogenetic reconstructions in *Carex* may be limited.

Section *Phyllostachys* can be divided into two major clades: (1) a wide-scaled clade consisting of *Carex backii*, *C. saximontana*, and *C. latebracteata*; and (2) a narrow-scaled clade consisting of *C. willdenowii*, *C. superata*, *C. basiantha*, *C. juniperorum*, and *C. jamesii*. Trends in character evolution in the wide-scaled clade point to the refinement of an autogamous breeding system, whereas character trends in the narrow-scaled clade suggest mechanisms to increase outcrossing. However, this pattern is not supported by isozyme studies of the section. The direction of chromosome evolution in sect. *Phyllostachys* does not appear to follow simple agmatoploidy suggesting that chromosome fusion might have occurred. The role that chromosome number changes play in the evolution of Carices is poorly understood, and it does not provide a reliable means for inferring evolutionary direction.

Phytogeographic and glacial evidence suggest that speciation in the section may have been influenced by the events of the Pleistocene. The most basal species in sect. *Phyllostachys* is *C. latebracteata*, a narrow endemic of the Ouachita Mountains of Arkansas and Oklahoma - a known glacial refugium. Similar patterns of evolution within sections *Phyllostachys*, *Griseae*, and *Laxiflorae* may point to the recent diversification of all three of these taxa under the influence of historical events in the Pleistocene.

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