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ERAGROSTIS (POACEAE): MONOPHYLY AND INFRAGENERIC CLASSIFICATION

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ABSTRACT

Eragrostis is a large genus in subfamily Chloridoideae of Poaceae. Recent phylogenetic analyses have suggested that the genus may not be monophyletic, that some of its segregate genera may be better placed within *Eragrostis,* and that current infrageneric classifications may not represent monophyletic groups. We have used molecular sequence data from the plastid locus *rps*16 and the nuclear gene *waxy* from a broad sample of *Eragrostis* species and representatives of six of the seven segregate genera to address these issues. We found that *Eragrostis* is monophyletic with the inclusion of several of the segregates, including *Acamptoclados, Diandrochloa,* and *Neeragrostis.* The placement of *Cladoraphis* and *Stiburus* is uncertain. *Thellungia* does not belong in *Eragrostis* and is actually more closely related to *Sporobolus.* These data also suggest that existing infrageneric classifications are inadequate and do not correspond to monophyletic groups within *Eragrostis.*

Key words: *Acamptoclados, Cladoraphis, Diandrochloa, Eragrostis, Neeragrostis, Pogonarthria, rps*16, *Stiburus, Thellungia, waxy.*

INTRODUCTION

Eragrostis Wolf is a genus of approximately 350 species in Poaceae (Watson and Dallwitz 1992). It is the largest genus in subfamily Chloridoideae, a group comprising about 1500 species (Van den Borre and Watson 1997). Members of *Eragrostis* generally are characterized by paniculate inflorescences, multi-floreted spikelets, glabrous three-nerved lemmas, ciliate ligules, and C_4 photosynthesis. The genus is morphologically and anatomically diverse, however, and exhibits a wide range of variation in many characters. For instance, the panicles range from very loose and open to highly contracted spicate structures (Watson and Dallwitz 1992). NAD-ME, PCK-like, and intermediate forms of leaf blade anatomy are found in the genus (Van den Borre and Watson 1994). Several major types of the bicellular microhairs common to Chloridoideae are found in *Eragrostis,* including the chloridoid type, the panicoid type, the *Pappophorum* type, and intermediates (Prendergast et al. 1986; Amarasinghe and Watson 1990). *Eragrostis* species range throughout the world's tropical and subtropical regions, and they are most commonly found in weedy disturbed areas and in dry habitats. Most of the species are of little economic importance, but one species (*E. tef*; tef) is cultivated as a major cereal crop in Ethiopia. This species is also an important forage grass, as are several other species, including *E. cilianensis* and *E. curvula.* Due in part to its large size and wide geographic distribution, there has been no comprehensive taxonomic treatment of *Eragrostis,* and there has been some debate in the recent literature as to whether the genus is monophyletic (Van den Borre and Watson 1997; Hilu and Alice 2001) and how infrageneric groups should be delimited (reviewed in Van den Borre and Watson 1994).

Eragrostis was first described by Wolf (1776) from material of *E. minor* (for a detailed taxonomic history of the genus, see Van den Borre and Watson 1994). Since the original description there has been little agreement as to which species actually belong in the genus and how they are related to each other. Species in *Acamptoclados* Nash, *Cladoraphis* Franch., *Diandrochloa* De Winter, *Eragrostiella* Bor, *Neeragrostis* Bush, *Stiburus* Stapf, and *Thellungia* Stapf have been included in *Eragrostis* at various times. Clayton and Renvoize (1986) also suggested a close relationship between *Eragrostis* and *Pogonarthria* Stapf.

The various modes of spikelet disarticulation that are seen in the genus have been the most common source of characters for delimiting infrageneric groups. Spikelets may disarticulate from the top, from the bottom, or as a unit. Additionally, whether the paleas are retained on the rachilla or fall with the lemmas can be an important character, as can the persistence of the rachilla. These characters are quite variable in *Eragrostis* and can be seen in a number of different combinations. Unfortunately, these characters are not generally as useful as one might hope. There may be temporal variation that is not always obvious at particular stages in the life cycle (e.g., the paleas may be retained slightly longer than the lemmas but are still deciduous). From a practical standpoint, this means that herbarium material may be impossible to score or misleading if not collected at precisely the correct stage. These intermediacies also cloud the distinction of the character states. The infrageneric classifications proposed by Koch (1848) and numerous later botanists relied heavily on these characters (see Van den Borre and Watson 1994). More recent classifications have included some other morphological characters, such as spikelet shape (Lazarides 1997), pubescence on the palea keels, panicle branching, lemma keel and margin shape and curvature, and floret fertility (Cope 1998).

A recent phylogenetic analysis of anatomical and morphological data from 56 species of *Eragrostis* and two segregate genera by Van den Borre and Watson (1994) led the authors to conclude that divisions based on these spikelet

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disarticulation characters did not represent natural groups. They instead suggested that the genus could be divided into two subgenera based on a number of correlated anatomical and morphological characters. However, no uncontradicted synapomorphies were identified for these groups, and there are numerous exceptions to most of the characters proposed as diagnostic of the subgenera. The results from the phylogenetic analyses in their study may also have been affected by the frequent occurrence of allopolyploidy in the genus. Approximately 69% of the species in the genus are polyploids (Hunziker and Stebbins 1986), and many of the taxa included in the Van den Borre and Watson (1994) study are known allopolyploids. Taxa of hybrid origin can exhibit unique combinations of morphological and anatomical character states and when included in phylogenetic analyses can produce misleading hypotheses of relationships (McDade 1990, 1992).

The monophyly of *Eragrostis* has been brought into question by recent phylogenetic analyses of subfamily Chloridoideae. Van den Borre and Watson (1997) analyzed a data set of anatomical and morphological characters from all 166 chloridoid genera (as recognized by Watson and Dallwitz 1992). The authors used the two subgenera of *Eragrostis* identified in their 1994 analysis of the genus as terminals for their phylogenetic analyses and found that they were not sister taxa and were in fact found in two widely divergent clades. Hilu and Alice (2001) conducted a phylogenetic analysis of sequence data from the plastid locus *mat*K from 74 species in 56 chloridoid genera. Included in the sample were seven *Eragrostis* species, and the authors also found that these species did not form a monophyletic group. Five of the species grouped together in a clade sister to *Eragrostiella,* a genus thought to be closely related to *Eragrostis,* but two other species were found in a different clade. Even more surprisingly, *Pappophorum bicolor* was embedded within the larger *Eragrostis* clade. This result was unexpected because *Pappophorum* Schreb. bears little morphological similarity to *Eragrostis,* and this relationship had never been suggested before.

Although these studies have raised the important issue of whether *Eragrostis* is a natural group, they suffered from some limitations. The Van den Borre and Watson (1997) study used composite terminals. These groups are far from uniform in many of the characters used in the phylogenetic analysis, which necessitates coding a number of polymorphisms in the matrix. In addition, the monophyly of these two subgenera has not been confirmed by phylogenetic analyses of independent data sets (Ingram and Doyle 2003). Both of these factors could have seriously affected the outcome of the phylogenetic analysis of the subfamily. The Hilu and Alice (2001) study suffered from poor sampling of *Eragrostis* species and the probable misidentification of some plant materials (see Results).

Another complicating factor in determining whether *Eragrostis* is a monophyletic group is the profusion of segregate genera. *Acamptoclados,* a monotypic genus (= *E. sessilispica*) from the plains and prairies of North America, was separated from *Eragrostis* by Nash on the basis of its free pericarp and lack of secondary inflorescence branches (Small 1903). Phillips (1982) treated *Acamptoclados* as a synonym of *Eragrostis* in her analysis of Eragrostideae. *Cladoraphis* is a genus of two species distributed in southern Africa. The plants are halophytic or glycophytic and have free pericarps and a unique ''armed'' growth form. Panicle branches in *Eragrostis* species terminate with spikelets, but the terminal spikelets in *Cladoraphis* are lacking. This in combination with hardened panicle branches and tough, inrolled leaves produces thorny appendages. *Diandrochloa,* a genus of seven widely distributed species, was separated from *Eragrostis* by De Winter (1960) on the basis of membranous ligules (other *Eragrostis* species have a ligule that is a fringe of hairs; Watson and Dallwitz 1992). Phillips (1982) rejected this treatment, however, and included these species in *Eragrostis. Eragrostiella* comprises five species in southern Asia and Australia and was separated from *Eragrostis* by Bor (1940) based on the spicate inflorescences and tufted habit found in these species. *Neeragrostis* was described by Bush (1903) based on a single dioecious species (= *N. reptans*). Two additional species of *Eragrostis,* one dioecious and another with hermaphrodite flowers (*E. hypnoides*), have been positioned in *Neeragrostis* by some authors. All three species are prostrate annuals distributed in the Americas. *Stiburus,* a small genus of two species from southern Africa, was first described by Stapf (1900). These species were separated from *Eragrostis* on the basis of their villous spikelets, but Phillips (1982) pointed out that a number of Old World *Eragrostis* species have pubescent spikelets and submerged *Stiburus* into *Eragrostis.* The monotypic genus *Thellungia* (= *E. advena*) is native to Australia and was first described in 1920 by Stapf from alien material found growing among wool refuse near a worsted mill in Switzerland (Stapf 1920). *Thellungia advena* was originally thought to have close affinities to *Sporobolus* R. Br. due to its similar floral structure and free pericarps, but the author noted that *T. advena* differed from *Sporobolus* in having multiple florets per spikelet (as is found in *Eragrostis*). Phillips (1982) placed this species in *Eragrostis,* pointing out that the floral structure of *T. advena* fit her concept of *Eragrostis* and that free pericarps are found elsewhere in the genus. The Van den Borre and Watson (1997) analyses suggested that *Acamptoclados, Diandrochloa, Neeragrostis,* and *Thellungia* are closely related to *Eragrostis,* but because composite terminals were used, it is impossible to determine whether these genera are separate lineages or if they are better included within *Eragrostis.* Hilu and Alice (2001) sampled *Eragrostis advena* (= *Thellungia advena*) and found in their analysis that it did not group with the clade containing most of the other *Eragrostis* species and *Eragrostiella.*

Here we assess the monophyly of *Eragrostis* and the relationships of the segregate genera by conducting phylogenetic analyses of a broad sample of *Eragrostis* species, including taxa from both subgenera identified by Van den Borre and Watson (1994) and representatives of the major spikelet disarticulation types and other infrageneric taxa that have been recognized in the genus. We include all available segregate genera and other genera identified by previous phylogenetic analyses to be potentially closely related to *Eragrostis.* The fit of the relationships uncovered by these molecular data to existing infrageneric classification systems is also examined. We use sequence data from the plastid locus

*rps*16 and a portion of the nuclear gene *waxy* (granule-bound starch synthase I; GBSSI) in our phylogenetic analyses.

MATERIALS AND METHODS

Sampling

Thirty-seven *Eragrostis* species were sampled, representing all of the major morphological groups in the genus, based primarily on spikelet disarticulation type (Table 1). Representatives from both subgenera as delimited by Van den Borre and Watson (1994) were included. Species representing six of the seven segregate genera were also included in the taxon sample (material of *Eragrostiella* was not available). Additionally, 18 species from other chloridoid genera that are potentially close relatives of *Eragrostis* were included to test the monophyly of *Eragrostis.* Plant materials were obtained from the USDA National Plant Germplasm System (NPGS), herbarium specimens, or from personal collections (Table 1). Voucher specimens are deposited at Cornell University (BH) except where noted in Table 1.

DNA Extraction, Sequencing, and Data Analysis

DNA was isolated from fresh greenhouse-grown, field-collected and silica-dried, or herbarium material using the DNeasy kit (QIAGEN, Valencia, California, USA) according to manufacturer's instructions. PCR amplification, sequencing, and sequence alignment of *waxy* and *rps*16 were performed as described in Ingram and Doyle (2003). Some of the sequences used in this study were previously published (Ingram and Doyle 2003: GenBank accessions AY136828–AY136942); new sequences were deposited in GenBank (accession numbers AY508649–AY508691, AY509525, and AY509526). All sequences were aligned with Clustal_X (Thompson et al. 1997) using a variety of gap opening and extension penalties to test for the sensitivity of the phylogenetic analyses to different alignments. The aligned sequences were read into WinClada vers. 1.00.08 (Nixon 1999*a*) for manual alignment adjustment, phylogenetic analysis, and tree manipulation. Unequivocal gaps (excluding autapomorphies) were coded as presence/absence characters following the simple gap coding method of Simmons and Ochoterena (2000). *Coelachyrum piercei,* a chloridoid grass not thought to be included in *Eragrostis,* was used to root the phylogenetic trees. This species was chosen on the basis of its early divergence in pilot analyses that included *rps*16 and *waxy* sequences from *Zea mays* L., a panicoid grass.

The data sets were initially analyzed separately due to the presence of allopolyploid species in the taxon sample. To assess the fit of existing infrageneric classification systems to the relationships defined by these molecular data, the polyploid sequences were culled from both the *waxy* and the *rps*16 data sets. These smaller sets of sequences were then combined into a single matrix and analyzed simultaneously. All parsimony analyses were conducted with WinClada and NONA (Goloboff 1993), using traditional heuristic search strategies and the ratchet (Nixon 1999*b*). Strict consensus trees were calculated in WinClada. Bootstrap analyses were performed in WinClada to assign support values to the clades.

RESULTS

Some accessions obtained from the USDA were misidentified in the NPGS database (Germplasm Resources Information Network). These accessions are marked with an asterisk in Table 1. Also, the accession identified as *Pappophorum bicolor* turned out to be a mixed collection. This is the same accession whose *mat*K sequence in the Hilu and Alice (2001) analysis of Chloridoideae was embedded within a clade of *Eragrostis* sequences and suggested that *Pappophorum* was nested within *Eragrostis.* When grown in the Cornell University greenhouse, this seed lot was found to contain a mixture of seeds from *E. botryodes* and *P. bicolor,* so it appears that the *mat*K sequence obtained from this accession was actually from an *E. botryodes* individual. Sequences from *rps*16 and *waxy* for *P. bicolor* resolve as sister to *P. mucronulatum* in our analyses, and both of these species fall well outside *Eragrostis* (Fig. 1, 2).

Hilu and Alice (2001) also showed that *Eragrostiella brachyphylla* (Stapf) Bor was sister to a core *Eragrostis* clade. Sequences for *waxy* and *rps*16 that we obtained from the same DNA used in that study are identical to sequences obtained from *Eragrostis tef,* suggesting that this material may also have been misidentified. Voucher specimens were not made available to confirm this suspicion, but given that *Eragrostiella brachyphylla* bears little morphological similarity to *Eragrostis tef,* it seems unlikely that their DNA sequences would be identical. Additionally, seed identified as *Eragrostiella brachyphylla* that we requested from the USDA turned out to be tef. The Hilu and Alice (2001) accession was reported to be from a different source, but the coincidence is striking.

A number of species (including *Cladoraphis spinosa, Neeragrostis reptans, Stiburus conrathii,* and *Uniola pittieri*) were included in the *rps*16 data set but not in the *waxy* data set. The DNAs for these taxa were extracted from old, poorly preserved herbarium material and were so degraded that it was impossible to amplify the nuclear gene.

The *rps*16 sequences were easily aligned, and the phylogenetic analysis recovered 351 equally parsimonious trees (length [L] = 230, consistency index [CI] = 72, retention index [RI] = 88). The strict consensus tree (Fig. 1) was poorly resolved within *Eragrostis,* but this data set was able to resolve relationships among the other chloridoid genera included in the analysis. The bootstrap values (Fig. 1) were generally quite high and showed high levels of support for many of the major clades. However, support for clades within *Eragrostis* was generally low.

The *waxy* sequences were difficult to align, particularly in the rapidly evolving introns in taxa outside *Eragrostis.* The exons, however, were simple to align across all of the taxa. Three equally parsimonious trees (L = 4995, CI = 40, RI = 62) were recovered from the phylogenetic analysis of the complete data set. The strict consensus tree (Fig. 2) was much better resolved within *Eragrostis* than with the *rps*16 data set. Bootstrap values were generally lower for this data set (Fig. 2), but the *Eragrostis* clade was very strongly supported.

The *rps*16 and *waxy* data sets were analyzed both with and without (data not shown) the gap characters. The topology of the trees resulting from both types of analyses were

Table 1. Collection data for taxa included in this study. USDA accessions (indicated by ''PI'' or ''NSL'') marked with an asterisk (*) were misidentified in the Germplasm Resources Information Network. Vouchers not located at Cornell University (BH) are indicated with the herbarium abbreviation in parentheses next to the voucher number.

Taxon	Collection	Locality	Voucher
Acamptoclados sessilispicus (Buckley) Nash		Texas, USA	*Kruse 256* (TAES)
Calamovilfa gigantea (Nutt.) Scribn. & Merr.	NSL 22960	New Mexico, USA	*Ingram 01-02*
C. longifolia (Hook.) Hack. ex Scribn. & Southw.	PI 433949	Missississpi, USA	*Ingram 02-02*
Cladoraphis spinosa (L. f.) S. M. Phillips	*Braun 5732*	South Africa	*Ingram 15-02*
Coelachyrum piercei (Benth.) Bor	PI 197534	Ethiopia	*Ingram 04-02*
Dactyloctenium aegyptium (L.) Willd.	PI 215592	Punjab, India	*Ingram 05-02*
D. australe Steud.	PI 299588	Cape Province, South Africa	*Ingram 06-02*
D. giganteum B. S. Fisher & Schweick.	PI 364504	Natal, South Africa	*Ingram 07-02*
D. radulans (R. Br.) P. Beauv.	PI 238276	Queensland, Australia	*Ingram 08-02*
Diandrochloa japonica (Thunb.) A. N. Henry	PI 213410	India	*Ingram 33-01*
Eleusine coracana (L.) Gaertn.	PI 462423	Bihar State, India	*Ingram 12-02*
Enneapogon scoparius Stapf	PI 208126	Transvaal, South Africa	*Ingram 03-02*
Eragrostis airoides Nees	PI 309995	Brazil	*Ingram 14-02*
E. aspera (Jacq.) Nees	PI 368248	Zimbabwe	*Ingram 01-99*
E. bahiensis Schrad. ex Schult.	PI 204185	Uruguay	*Ingram 01-01*
E. barrelieri Daveau		Arizona, USA	*Reeder 9835*
E. bicolor Nees	PI 165732	South Africa	*Ingram 02-99*
E. botryodes Clayton	2000-09	Chafanna, Ethiopia	*Ingram 02-01*
	2000-13	Debre Birhan, Ethiopia	*Ingram 03-01*
E. chapelieri (Kunth) Nees	2000-17	Ziha, Ethiopia	*Ingram 06-01*
E. cilianensis (Bellardi) Vignolo ex Janch.	PI 299912	South Africa	*Ingram 03-99*
	2000-24	Tis Abay, Ethiopia	*Ingram 07-01*
E. ciliaris (L.) R. Br.		Florida, USA	*Lewis 050-01*
E. curvula (Schrad.) Nees	PI 226071	Kenya	*Ingram 04-99*
E. dielsii Pilg. ex Diels & Pritz.	PI 238301	Australia	*Ingram 08-01*
E. echinochloidea Stapf	PI 184741	South Africa	*Ingram 09-01*
E. elegantissima Chiov.	2000-16	Ziha, Ethiopia	*Ingram 10-01*
E. heteromera Stapf	PI 208129	South Africa	*Ingram 12-01*
E. hypnoides (Lam.) Britton, Sterns & Poggenb.		Mississippi, USA	*Alford 2829*
E. intermedia Hitchc.	PI 216400	Mexico	*Ingram 13-01*
E. lehmanniana Nees	PI 226073	Kenya	*Ingram 15-01*
E. lugens Nees	PI 203862	Brazil	*Ingram 16-01*
E. macilenta (A. Rich.) Steud.	PI 194929	Ethiopia	*Ingram 05-99*
E. mexicana (Hornem.) Link	PI 203652	Brazil	*Ingram 06-99*
E. minor Host	PI 223367	Iran	*Ingram 16-99*
E. neesii Trin.	PI 203650	Brazil	*Ingram 18-01*
E. nutans (Retz.) Nees ex Steud.	PI 217616	India	*Ingram 19-01*
E. paniciformis (A. Braun) Steud.	2000-03	Wolaita Sodo, Ethiopia	*Ingram 20-01*
E. papposa (Roem. & Schult.) Steud.	2000-01	Awasa, Ethiopia	*Ingram 22-01*
E. patenti-pilosa Hack.	2000-26	Tis Abay, Ethiopia	*Ingram 23-01*
E. pilosa (L.) P. Beauv.	PI 213255*	India	*Ingram 07-99*
	PI 219588	Pakistan	*Ingram 08-99*
	PI 221926	Afghanistan	*Ingram 09-99*
	PI 222988	Iran	*Ingram 10-99*
E. polytricha Nees	PI 202443	Chile	*Ingram 24-01*
E. rigidior Pilg.	2000-07	Gidole, Ethiopia	*Ingram 26-01*
E. schweinfurthii Chiov.	2000-12	Ametsegna Ager, Ethiopia	*Ingram 28-01*
E. secundiflora J. Presl	PI 216405	Texas, USA	*Ingram 27-01*
E. tef (Zucc.) Trotter 'Red Dabi'	PI 557457	Ethiopia	*Ingram 12-99*
E. tenella (L.) P. Beauv. ex Roem. & Schult.	PI 320980*	Sierra Leone	*Ingram 32-01*
E. tremula Hochst. ex Steud.	PI 220220	Liberia	*Ingram 30-01*
E. trichophora Coss. & Durieu	PI 364802	South Africa	*Ingram 17-99*
E. truncata Hack.	PI 299962	South Africa	*Ingram 31-01*
E. unioloides (Retz.) Nees ex Steud.	PI 213254	India	*Ingram 16-02*
Fingerhuthia sesleriiformis Nees	PI 299968	South Africa	*Ingram 11-02*
Leptochloa dubia (Kunth) Nees	PI 216460	Mexico	*Ingram 14-99*
Neeragrostis reptans (Michx.) Nicora		Texas, USA	*Kruse 284*
Pappophorum bicolor E. Fourn.	PI 216526	Mexico	*Ingram 09-02*
P. mucronulatum Nees	PI 477097	Uruguay	*Ingram 10-02*
Pogonarthria squarrosa (Roem. & Schult.) Pilg.		Mpumalanga, South Africa	*Snow 7023* (MO)
Schmidtia pappophoroides Steud. ex J. A. Schmidt	PI 209163	South Africa	*Ingram 17-02*

Table 1. Continued.

Taxon	Collection	Locality	Voucher
Spartina pectinata Link	PI 599561	USA	*Ingram 19-02*
Sporobolus indicus (L.) R. Br.	PI 310313	Brazil	*Ingram 15-99*
Stiburus conrathii Hack.	PI 11456	South Africa	*Adams 11456*
Tetrachne dregei Nees	PI 209829	South Africa	*Ingram 16-02*
Thellungia advena Stapf	CANB 468782	Australia	*Ingram 18-02*
Uniola paniculata L.	J. I Davis	Florida, USA	No voucher
U. pittieri Hack.		Jalisco, Mexico	*Columbus 4083* (RSA)

identical, but the bootstrap values were generally higher for the data sets that included gap characters. As they did not affect the tree topologies, the gap characters were included in the final analyses reported here.

The trees from the plastid and nuclear data sets were largely congruent. This sample of *Eragrostis* species (with the addition of a few segregate genera) is strongly supported as a monophyletic group. This is in striking contrast to results from previous phylogenetic analyses. It seems clear that *Acamptoclados, Diandrochloa, Neeragrostis,* and *Pogonarthria* are embedded within *Eragrostis. Thellungia advena,* however, shares a clear affinity with *Sporobolus,* an association that was highlighted in the original species description (Stapf 1920). *Cladoraphis* and *Stiburus* are found outside *Eragrostis* in the *rps*16 data set, but *waxy* sequences were impossible to obtain, so further data will be necessary before they can be accurately placed.

The simultaneous analysis of the *waxy* and *rps*16 sequences for diploid taxa yielded a single most parsimonious tree (Fig. 3; L = 1750, CI = 56, RI = 59). The infrageneric groups (sensu Clayton and Renvoize 1986; Lazarides 1997; Cope 1998) to which the various species are assigned are indicated by the letters and numbers adjacent to the taxon names. The cladogram was well resolved, but it is clear that the infrageneric groups circumscribed by mode of spikelet disarticulation and other morphological characters do not represent monophyletic groups. Few of these species have been anatomically typed, but those that have were assigned to the subgenera of Van den Borre and Watson (1994) and mapped on the tree (Fig. 3). According to the limited sample neither subgenus is monophyletic.

DISCUSSION

Grass taxonomists have struggled with the classification of *Eragrostis* for many years. Its placement within Chloridoideae has been controversial, though recent analyses of several molecular data sets, including both plastid and nuclear loci, have all suggested it is sister to Uniolinae (Hilu and Alice 2001; Columbus et al. 2007), which is consistent with our analysis of *waxy* (Fig. 2) (relationship unresolved in our analysis of *rps*16; Fig. 1). Relationships within the genus have been difficult to assess. It is a large group with an extensive geographic distribution, and other factors such as polyploidy and phenotypic plasticity have confounded attempts to delimit infrageneric groups. The focus of this study has not been on devising a new infrageneric classification, but rather to answer basic questions about whether the genus is monophyletic, how to place its segregate genera, and whether existing infrageneric classifications are consistent with the results of phylogenetic analyses of molecular data.

Recent phylogenetic studies have suggested that *Eragrostis* may need to be split into at least two distantly related groups (Van den Borre and Watson 1997; Hilu and Alice 2001). However, both the plastid and nuclear sequence data in our study support *Eragrostis* as a monophyletic group when a few of the segregate genera are included in it. Genera that almost certainly belong within *Eragrostis* according to these data sets include *Acamptoclados, Diandrochloa, Neeragrostis,* and *Pogonarthria.* This placement of *Acamptoclados* is not surprising. This species was originally described as *Eragrostis,* and the characters used to segregate it were weak: both the absence of secondary inflorescence branching and free pericarps appear elsewhere in the genus. It was also to be expected that *Neeragrostis* should be returned to *Eragrostis.* The major feature distinguishing *N. reptans* from the rest of *Eragrostis* is its dioecy, but its other morphological characters clearly ally it with *Eragrostis.* Its other distinguishing feature is its prostrate habit, but *N. reptans* shares this and a number of other morphological features with *E. hypnoides.* These species were resolved as sister taxa in the *rps*16 analysis (Fig. 1).

Diandrochloa, as represented by *D. japonica,* is placed firmly within *Eragrostis* by both data sets. Species assigned to this genus display a number of unique characteristics, including a membranous ligule and a distinctive panicle form. On a gross morphological scale, these differences have seemed significant enough to some agrostologists to suggest that these species should be placed in their own genus. However, these molecular data suggest that this genus is in fact nested within *Eragrostis.* Further sampling of *Diandrochloa* species will be necessary to confirm its placement within the genus.

Pogonarthria is firmly placed within *Eragrostis* in both the plastid and nuclear data sets. This corroborates results obtained from phylogenetic analyses of sequence data from the nuclear ITS and plastid *trn*L–F regions (Columbus et al. 2007). Morphological characters linking this genus to *Eragrostis* include three-nerved lemmas and fringed ligules. Like *Acamptoclados sessilispicus,* this genus is characterized by a lack of secondary inflorescence branching, which has been thought to be rather important in higher-level classification in chloridoid grasses. However, the *waxy* data set shows that these species are not closely related, suggesting that this character evolved multiple times within the *Eragrostis* clade. At least in this group, a lack of secondary

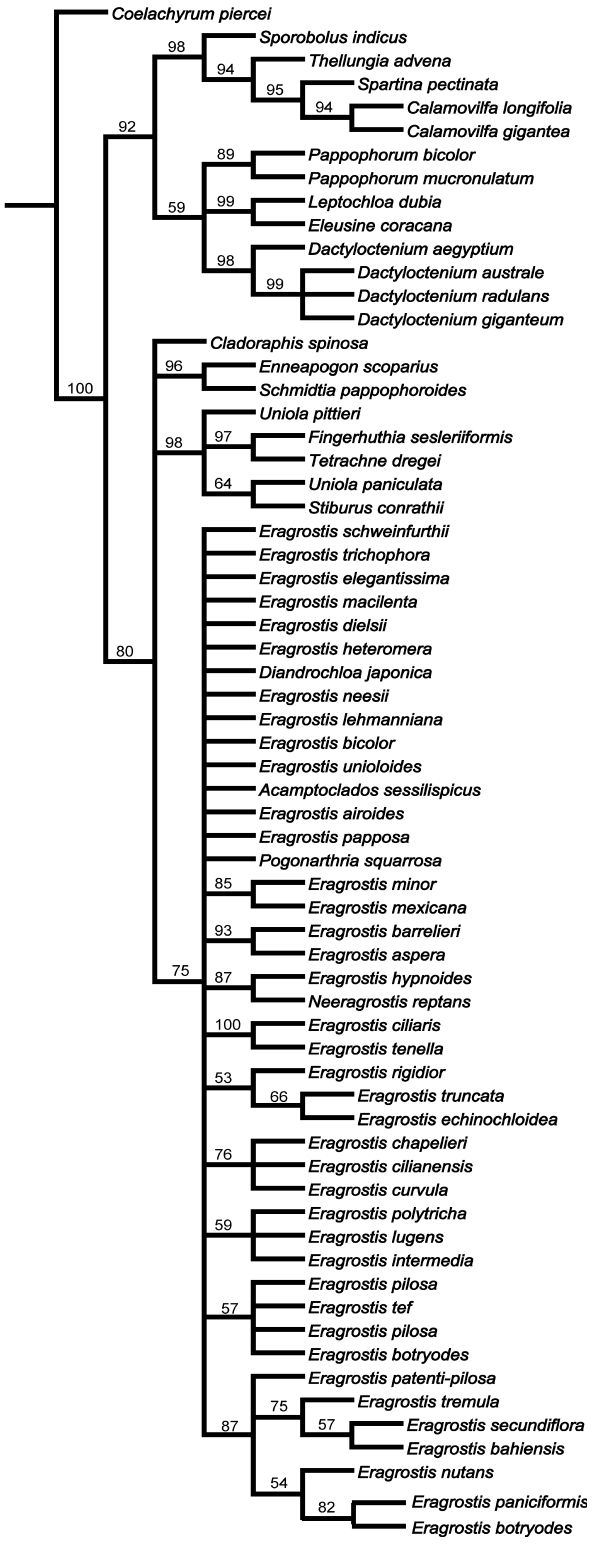

Fig. 1.—Strict consensus of 351 most parsimonious trees (L = 230, CI = 72, RI = 88) from the phylogenetic analyses of *rps*16 sequence data. Bootstrap values are above the branches.

inflorescence branching appears not to be an indicator of close relationship.

The original description of the monotypic genus *Thellungia* cited an affinity with *Sporobolus,* which has been confirmed by these analyses. *Thellungia advena* is placed well away from *Eragrostis* in a clade with *Sporobolus, Spartina* Schreb., and *Calamovilfa* Hack. in both the *rps*16 and *waxy* analyses. This result mirrors that found by Ortiz-Diaz and Culham (2000) in an analysis of *Sporobolus.* These authors found *Thellungia advena* to be nested well within *Sporobolus.*

The placement of *Cladoraphis* and *Stiburus* is not yet certain. As mentioned in the Results, *waxy* sequences could not be obtained for the species in these genera. Additionally, the plastid sequences were of relatively poor quality and included some uncertain base calls. Neither of these taxa is placed within the *Eragrostis* clade in the *rps*16 analysis, so they may indeed stand as separate genera, but further data will be necessary to confirm this result. The placement of *Stiburus* seems more certain than that of *Cladoraphis,* however. There are three uncontradicted molecular synapomorphies that support the *Stiburus–Uniola* L.–*Fingerhuthia* Nees ex Lehm.–*Tetrachne* Nees clade, but there are no characters that unequivocally support the placement of *Cladoraphis.*

In general, the molecular data suggest that many of the species that have at times been segregated from *Eragrostis* based on morphological differences do not actually merit generic status. This is a situation that has been observed in other large genera as molecular data have become increasingly important in phylogenetic studies. A well-known example of this phenomenon is *Solanum* L. According to D'Arcy (1991), ''Many of the 62 sections [of *Solanum*] now recognized are of such distinctive appearance that in other plant groups they would be recognized as separate genera.'' Phylogenetic analyses of molecular data have repeatedly shown that these groups, most notably *Lycopersicon* Mill., which includes the cultivated tomato and its wild relatives, are best included within a broad *Solanum* (e.g., Spooner et al. 1993; Bohs and Olmstead 1997; Olmstead and Palmer 1997). A similar situation can be seen in *Eragrostis.* Many of the species that have been segregated into small genera possess minor but conspicuous morphological differences. To recognize these as genera would require splitting *Eragrostis* into numerous smaller genera, which would generate both nomenclatural instability and the loss of an easily recognized genus that is generally useful for communication.

Congruence with Previous Cladistic Analyses

The results from the *waxy* and *rps*16 analyses are largely congruent with the results from the *mat*K data set collected by Hilu and Alice (2001) when the misidentified taxa are excluded. Some of the genera are placed differently in their analysis, including *Dactyloctenium* Willd. and *Eleusine* Gaertn., but this may be due to their broader sampling within Chloridoideae. However, most of the other major groupings found in our analyses are also present in the *mat*K analysis albeit with some minor rearrangements. These results also agree with the cladograms in Columbus et al. (2007), particularly in terms of the placement of *Neeragrostis reptans* and *Pogonarthria squarrosa.* An interesting result from this

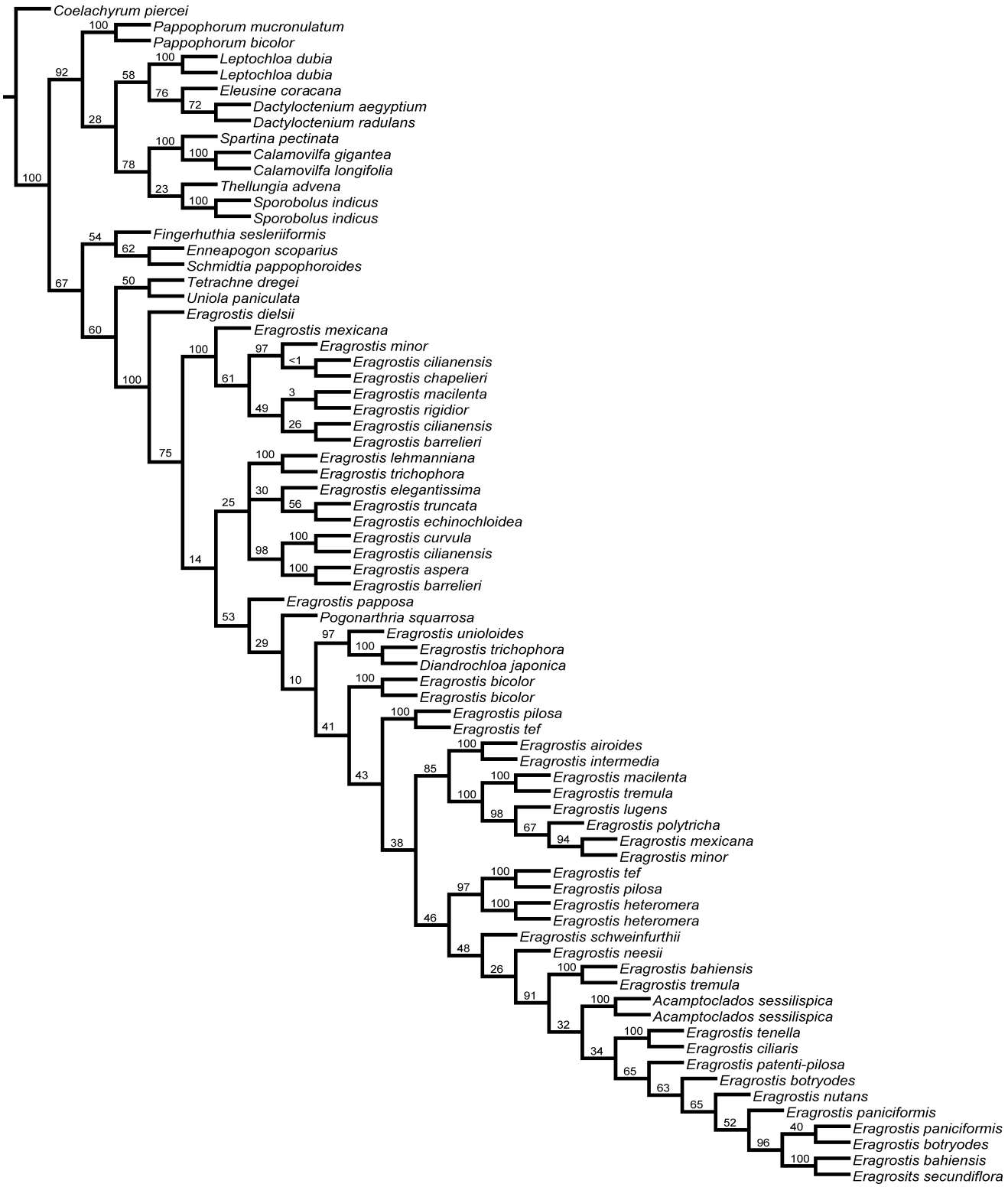

Fig. 2.—Strict consensus of three most parsimonious trees (L = 4995, CI = 40, RI = 62) from the phylogenetic analyses of *waxy* sequence data. Bootstrap values are above the branches.

analysis was that *Ectrosia leporina* R. Br. was firmly nested within the clade containing all sampled *Eragrostis* species. Clearly this is another genus that must be considered in future analyses of *Eragrostis.*

The Van den Borre and Watson (1997) analysis of morphological and anatomical characters shows much less congruence with the *rps*16 and *waxy* data sets. One of the major problems is that the two subgenera of *Eragrostis* that the authors used as terminals in their analyses do not correspond to any clades found in our analyses of the molecular data,

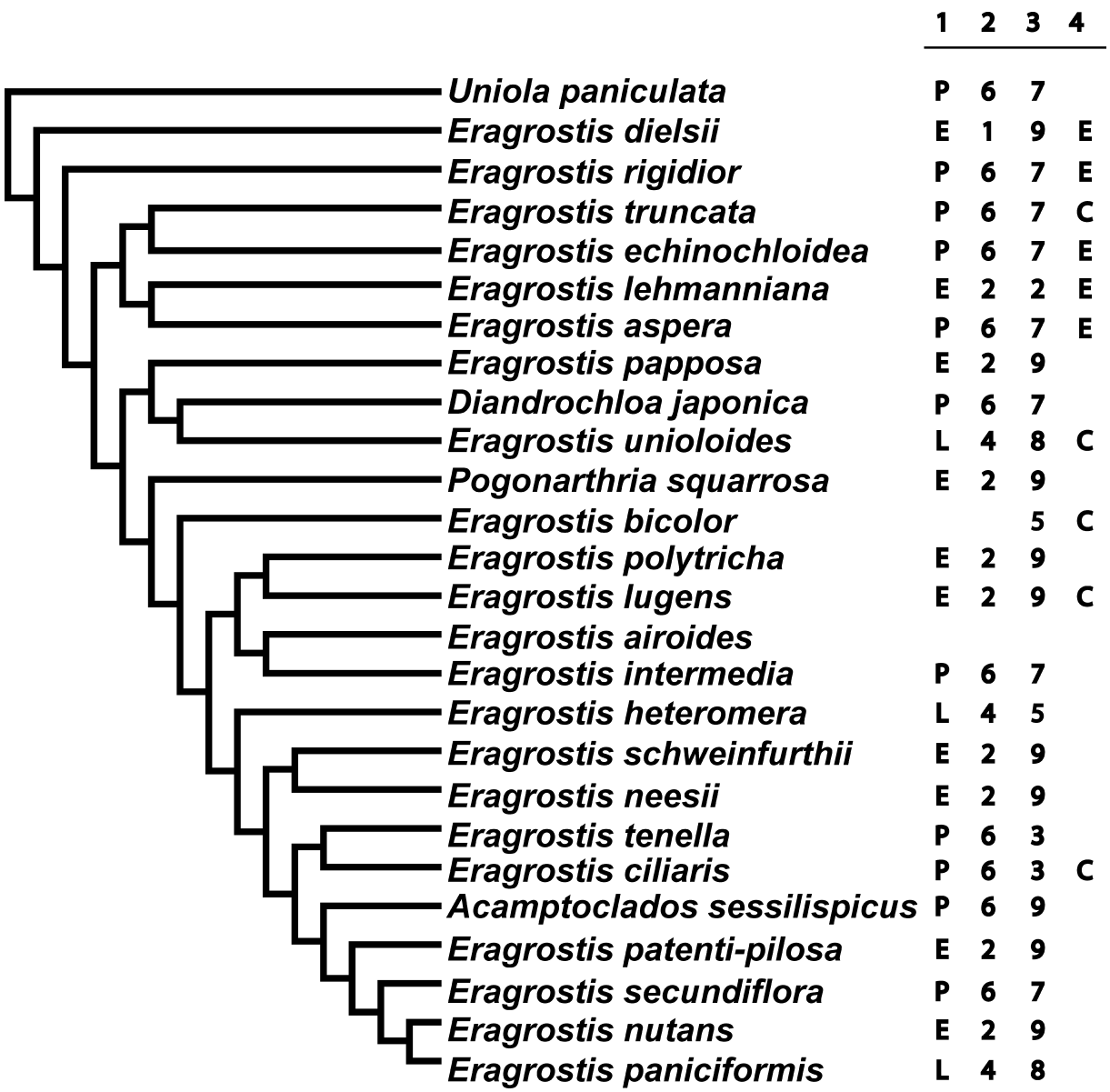

Fig. 3.—Single most parsimonious tree (L = 1750, CI = 56, RI = 59) from the simultaneous analysis of *rps*16 and *waxy* sequences from diploid species. The columns adjacent to the taxon names show the distribution of various classification systems. Column 1 represents Clayton and Renvoize (1986; E = sect. *Eragrostis,* L = sect. *Lappula,* P = sect. *Psilantha*). Column 2 shows to which group of Lazarides (1997) the taxa belong. Column 3 indicates the groups of Cope (1998). Column 4 represents the subgenera of Van den Borre and Watson (1994; E = subgen. *Eragrostis* and C = subgen. *Caesiae*).

precluding direct comparisons between the cladograms. However, it is clear that few of the major clades of other chloridoid genera recovered in the analyses of our molecular data were also found in the Van den Borre and Watson analysis. This suggests that there may be little correspondence between the phylogenetic signals present in the molecular data examined so far and the morphological and anatomical characters used in that data set.

Congruence with Existing Infrageneric Classifications

The combined analysis of the sequence data for the nuclear and plastid loci from the diploid taxa produced a single most parsimonious tree, and the infrageneric groups for three of the existing morphology-based classifications to which the taxa we sampled belong are labeled next to the species names (Fig. 3). The four sections of *Eragrostis* recognized by Clayton and Renvoize (1986) are sect. *Platystachya* Benth., in which the spikelets fall entire; sect. *Psilantha* (K. Koch) Tzvelev, which is characterized by the florets disarticulating from the top; sect. *Eragrostis,* whose spikelets disarticulate from the bottom with persistent paleas; and sect. *Lappula* Stapf, whose spikelets disarticulate from the bottom with the paleas falling with the lemmas. Our taxon sample did not contain any diploid representatives of sect. *Platystachya,* but the other three sections are indicated in the first column next to the taxon names in Fig. 3. In the Lazarides (1997) classification (second column in Fig. 3), Group 5 cor-

responds to sect. *Platystachya,* Group 6 corresponds to sect. *Psilantha,* and Group 4 corresponds to sect. *Lappula.* In Group 3 spikelets disarticulate from the bottom with the rachilla breaking up, while Groups 1 and 2 disarticulate from the bottom up with a persistent rachilla. In Group 1 the spikelets are terete or biconvex, whereas they are strongly compressed laterally in Group 2. In the Cope (1998) classification (third column in Fig. 3), Group 6 corresponds to sect. *Platystachya,* Group 7 is roughly equivalent to sect. *Psilantha,* Group 9 roughly corresponds to sect. *Eragrostis,* and Group 8 roughly corresponds to sect. *Lappula.* Cope also included a number of new groups in his classification. Group 1 consists of species with slender panicle branches that are widely divaricate and have a purple, pilose pulvinus in each axil. Group 2 corresponds to *Cladoraphis* and has stiff panicle branches that terminate in a naked bristle or abortive spikelet. In Group 3, the species have tuberculate-ciliate palea keels, and Group 4 consists of species with three to five sterile lemmas at the base of the spikelet. In Group 5 the lemmas are semi-ovate in profile. The taxon sample was limited for this combined analysis, but it is apparent that none of these classifications represents monophyletic groups.

Many of the taxa included in this sample have not been examined anatomically, but enough data are available to suggest that the classification proposed by Van den Borre and Watson (1994) may have some value. Subgenus *Caesiae* (panicoid-type microhairs, PCK-like leaf anatomy) forms a largely monophyletic group (with the exception of *E. truncata*), and subgen. *Eragrostis* (chloridoid-type microhairs, NAD-ME-like leaf anatomy) forms a grade at the base of the tree (Fig. 3). The combination of morphological and anatomical characters suggested by Van den Borre and Watson (1994) may be more useful than the classifications based on morphology only, but neither subgenus as currently delimited is monophyletic, and more data will be necessary to fully evaluate this classification. With further refinement and exploration of these character systems and increased sampling for the molecular data set, these features may be shown to be useful in constructing a functional infrageneric classification.

Towards a Classification of Eragrostis

Based on these preliminary analyses of a broad sample of *Eragrostis* species and some generic segregates, it appears that a number of taxonomic changes will probably be required in this group. The strongest cases for return to *Eragrostis* include *Acamptoclados* and *Neeragrostis. Pogonarthria* should also almost certainly be submerged within an expanded *Eragrostis. Thellungia,* however, does not appear to be closely related to these taxa and should be removed from *Eragrostis.* It will be necessary to sample more species of *Diandrochloa* before making any strong recommendations on its taxonomic status. More data will also be required for *Cladoraphis* and *Stiburus* before it will be possible to determine whether these genera should continue to be recognized. It will also be important in the future to sample more widely within *Eragrostis,* with particular emphasis on South African and Australian taxa. *Eragrostis* and some of its putative close relatives are particularly diverse in these regions, and a number of unusual *Eragrostis* species occur in these areas that may well belong outside the genus and that were not available for these analyses. Including these taxa will be crucial for making informed taxonomic decisions about the delimitation of *Eragrostis* and its segregates. However, it does appear that some of the large-scale modifications to *Eragrostis* s.s. suggested by the results of some previous phylogenetic analyses (Van den Borre and Watson 1997; Hilu and Alice 2001) will not be required.

After determining which species belong in *Eragrostis,* it will be important to construct a functional infrageneric classification, preferably based in part on morphological synapomorphies that can be readily recognized in the field and on herbarium specimens. Such a resource would provide valuable clues for identifying plants in this genus, which is a task that can be extremely daunting in the geographical regions where it is species-rich. One of the most serious hindrances with previous attempts at identifying morphological characters that may be used to circumscribe infrageneric taxa has been the inclusion of a large number of allopolyploids in the study groups. Allopolyploids are difficult for many reasons, most notably the irregular pattern of morphological characters that they exhibit. Development and gene expression are extremely complex and unpredictable in these taxa (Wendel 2000), and the aberrant behavior of allopolyploids in phylogenetic analyses of morphological data preclude the use of more rigorous phylogenetic methods to identify morphological synapomorphies for infrageneric taxa. In fact, only sequence data from low-copy nuclear genes can provide useful information for determining relationships of allopolyploids given that plastid sequences can only elucidate the history of the maternal progenitor of these taxa.

A possible solution to this problem is to use only diploid species in reconstructing the evolutionary relationships within the genus and to use the resulting cladograms to identify morphological characters that mark the infrageneric groups. With diploids, it is possible to combine characters from a number of sources, including morphology, anatomy, and both plastid and nuclear sequence data to construct robust phylogenies. Unfortunately this solution will be difficult to apply in *Eragrostis.* The proportion of the species in the genus that are thought to be polyploids is large (Hunziker and Stebbins 1986), but the ploidy level is not known for all species. For those species that have been subjected to cytological studies, the question of ploidy is not always straightforward. For species where there have been multiple chromosome counts, there have been a number of cases where a single morphological species is known to have individuals with a range of ploidy levels (e.g., *E. curvula* has documented chromosome counts ranging from $2n = 40$ to $2n = 80$, including euploid and aneuploid numbers; de Wet 1954; Spies and Jonker 1987; Bir and Sahni 1988). A great deal of cytological work will be necessary to fully understand the chromosomal complexity in this group and to facilitate the identification of diploids for use in reconstructing evolutionary relationships.

Even if these difficulties are overcome and a phylogeny of *Eragrostis* diploids can be used to delimit infrageneric groups, it will still be extremely complicated to incorporate the allopolyploids into the taxonomic framework. The results from the analysis of the *waxy* data show that several poly-

ploids derive their homoeologous genomes from widely divergent diploid progenitors (Fig. 2), suggesting that it may be impossible to assign such taxa to an infrageneric group. Despite these difficulties, however, this may prove to be the most appropriate strategy for this genus and should provide a great deal of useful information to guide further research in *Eragrostis*.

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