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Natural hybridization in the *Andropogon lateralis* complex (Andropogoneae, Poaceae) and its impact on taxonomic literature

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In north-eastern Argentina, Paraguay and south-eastern Brazil, morphologically intermediate plants involving *Andropogon lateralis*, *A. bicornis*, *A. glaziovii*, *A. arenarius* and *A. hypogynus* were found. The possibility that they were natural hybrids was tested in two ways: (1) where they were sterile, their morphology was compared with that of the putative parents, and their meiosis and reproductive behaviour were studied; (2) where they were fertile, studies of artificial hybrids were also made. Most of the hybrids were sterile. The only fully fertile combination, generating recombination and hybrid swarms, was *A. lateralis* × *A. hypogynus*. In spite of apparently normal chromosome pairing, fertility was low in all other combinations on both the male and female sides. Sterility is probably a result of 'cryptic' or 'gametic sterility', which produces complete sterility of the gametes. Many of the hybrids survive and compete successfully with the parental species in natural populations, but their sterility maintains the genetic isolation of the majority of the taxa involved. Meiotic chromosome behaviour in all the hybrids indicates that the group of species shares slightly different forms of three basic genomes. Several specimens of natural hybrids were found in historical herbarium collections. In the past, they were given the status of type specimens of at least five taxonomic entities (*A. lindmanii*, *A. coloratus*, *A. lateralis* var. *subtilior*, *A. multiflorus* and *A. lateralis var. bogotensis*). The taxonomic consequences of these findings are discussed.

ADDITIONAL KEYWORDS: bluestems – genomes – Gramineae – polyploidy – reproduction – South America.

INTRODUCTION

Andropogon L. is a pantropical genus of grasses estimated to contain 100 (Clayton & Renvoize, 1986) to 120 (Campbell & Windisch, 1986) species, distributed mainly in the grasslands of Africa and the Americas. Considered in the strict sense, that is excluding allied genera, such as *Bothriochloa* Kuntze, *Dichanthium* Willem. and *Schizachyrium* Nees, the genus in America remains somewhat heterogeneous (Clayton, 1964; Gould, 1967; Norrmann, 1985; Kellogg & Campbell, 1987a,b). Although most African species are diploids or tetraploids ($2n = 2x = 20$; $4x = 40$) (Campbell, 1983b; Norrmann, 1999), American *Andropogon* species are usually diploid or hexaploid ($2n = 2x = 20$ or $6x = 60$) (Gould, 1967; Norrmann, 1985; Campbell & Windisch, 1986; Galdeano & Norrmann, 2000; Norrmann & Scarel, 2000), with only a few exceptions (see Boe *et al.*, 2004). Recently, it has been shown that the genomic architecture of two South American hexaploids includes the S genome present in South American diploids (Norrmann *et al.*, 2004).

American hexaploid species of *Andropogon* belong to three taxonomic sections, delineated for Africa by Stapf (1919): (1) section *Andropogon*, which includes the ecologically important large bluestem *A. gerardii* Vitman from North America; (2) section *Leptopogon* Stapf, characterized by the presence of a concave nerveless first glume of the sessile spikelet (Clayton, 1964) and comprising the *A. virginicus* L. complex (Campbell, 1983b) and the *A. lateralis* Nees complex; and (3) section *Notosolen* Stapf, represented by *A. exaratus* Hack., *A. pohlianus* Hack., *A. glaucophyllus* Roseng. B.R., Arrill. & Izag. and *A. barretoi*

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Norrmann & Quarín (Norrmann & Quarin, 2001). Based on morphological traits (Norrmann, 1985), more than 20 additional putative hexaploid entities exist, but they lack precise chromosome counts.

Andropogon species are sexually reproducing (Campbell, 1982; Norrmann & Quarin, 1991; Norrmann & Scarel, 2000), and the variation in breeding systems reflects the disposition and function of the spikelets in the pair (Fig. 1): one sessile (ss, hermaphrodite or female) and the other pedicellate (ps, usually male or neuter). Pairs of spikelets aggregate in racemes, racemes attach in false panicles, and false panicles in flowering stalks, creating all sorts of combinations. *Andropogon bicornis* L. racemes always have two pedicellate male spikelets on top (Fig. 1A) and the rest of the pedicellate spikelets are neuter (Fig. 1B). Cross-pollination is required in sections *Andropogon* and *Notosolen* because of genic self-incompatibility (Norrmann & Scarel, 2000), but self-incompatibility has been lost in most species of section *Leptopogon*, opening up the way to self-pollination and even cleistogamy (Campbell, 1982). In a group of species in this section, defined as the *A. lateralis* complex (*A. lateralis* Nees, *A. hypogynus* Hack., *A. glaziovii* Hack., *A. bicornis* L., *A. arenarius* Hack., *A. lindmanii* Hack., *A. coloratus* Hack. and *A. multiflorus* Renvoize, among others), anther size and the number of pollen grains in fertile sessile spikelets are strongly reduced compared with those of pedicellate spikelets (Fig. 1). This synapomorphy of dimorphic anthers defines this complex (Campbell, 1983b; Campbell & Windisch, 1986), which is composed entirely of American species. Anthers in sessile spikelets are so reduced in *A. lateralis* and its sister species *A. hypogynus* that the stamens look like staminodes (Fig. 1C, in black), carry almost no pollen grains and do not dehisce, making the spikelet functionally female and the plant monoecious (Norrmann & Quarin, 1991). The physical separation of the two floral types, enhanced by the temporal barrier of protogyny, makes the species objectively cross-pollinated, and this is presumed to be a derived condition in an otherwise self-pollinating group (Norrmann & Quarin, 1991; Norrmann & Scarel, 2000).

In a series of field studies in north-eastern Argentina, Uruguay, Paraguay and Brazil over a period of more than 20 years, I have discovered multiple *Andropogon* individuals with morphological characteristics intermediate between those of well-characterized species. Representative specimens were collected and kept at the experimental garden to enable detailed reproductive and cytogenetic analyses to be made.

In this article identify and characterize natural hybrids between hexaploid species within the *A. lateralis* complex. The parental species involved are as follows: (1) *A. lateralis* and (2) *A. hypogynus*, the two most important climax species of native grasslands in southern South America; (3) *A. bicornis* and (4) *A. arenarius*, representing ruderal, colonizing, self-pollinating species; and (5) *A. glaziovii*, restricted to the wet hot marshes of Paraguay, Bolivia and Brazil (pantanal). The only thorough report of natural hybridization in this group of species was that of Campbell & Windisch (1987), dealing with *A. arenarius* × *A. lateralis* hybrids and other 'intermediate' individuals.

To investigate the basis of sterility in most hybrids, selected specimens were characterized by studying their meiotic chromosome behaviour and reproductive biology. In the only fertile combination, *A. lateralis* × *A. hypogynus*, hybrids were re-synthesized under controlled conditions. In order to assess the relevance of natural hybrids for the taxonomy of the genus, historical herbaria were screened for their presence as types in hisorical botanical collections.

MATERIAL AND METHODS

To identify a specimen as a natural hybrid, the following criteria were used: (1) putative parents should be present at the field collection sites; and (2) cytological, embryological and flowering characteristics typical of hybrids should be observed. The sterility of most hybrids and the lack of variation between them also contributed greatly in the final decision.

Plant material

Intermediate plants

Collection trips were undertaken from April 1982 to 2006, covering north-eastern Argentina (Misiones, Formosa, Corrientes, Entre Ríos, Santa Fe), Brazil (Rio Grande do Sul, Santa Catarina, Paraná), Paraguay and Uruguay. Material from Bolivia was kindly provided by Dr Timothy Killeen and material from Brazil by Dr Francisco Valls.

Living plants from naturally occurring populations were transplanted to clay pots at the experimental garden of the Instituto de Botanica del Nordeste (IBONE), Corrientes, Argentina, where different individuals from each hybrid combination were cultivated. Vouchers of Norrmann's collections (N) were deposited at IBONE Herbarium (CTES), among others. A full list of the accessions is given in Appendix 1 (under 'living material').

Herbaria and taxonomy

Specimens from the following herbaria were screened for natural hybrids: BAA, CEN, CORD, CTES, FI, G, GH, HB, ICN, K, L, LE, LIL, M, MEXU, NY, P, R, RB, S, SI, US, W. Exsiccatae of selected vouchers are

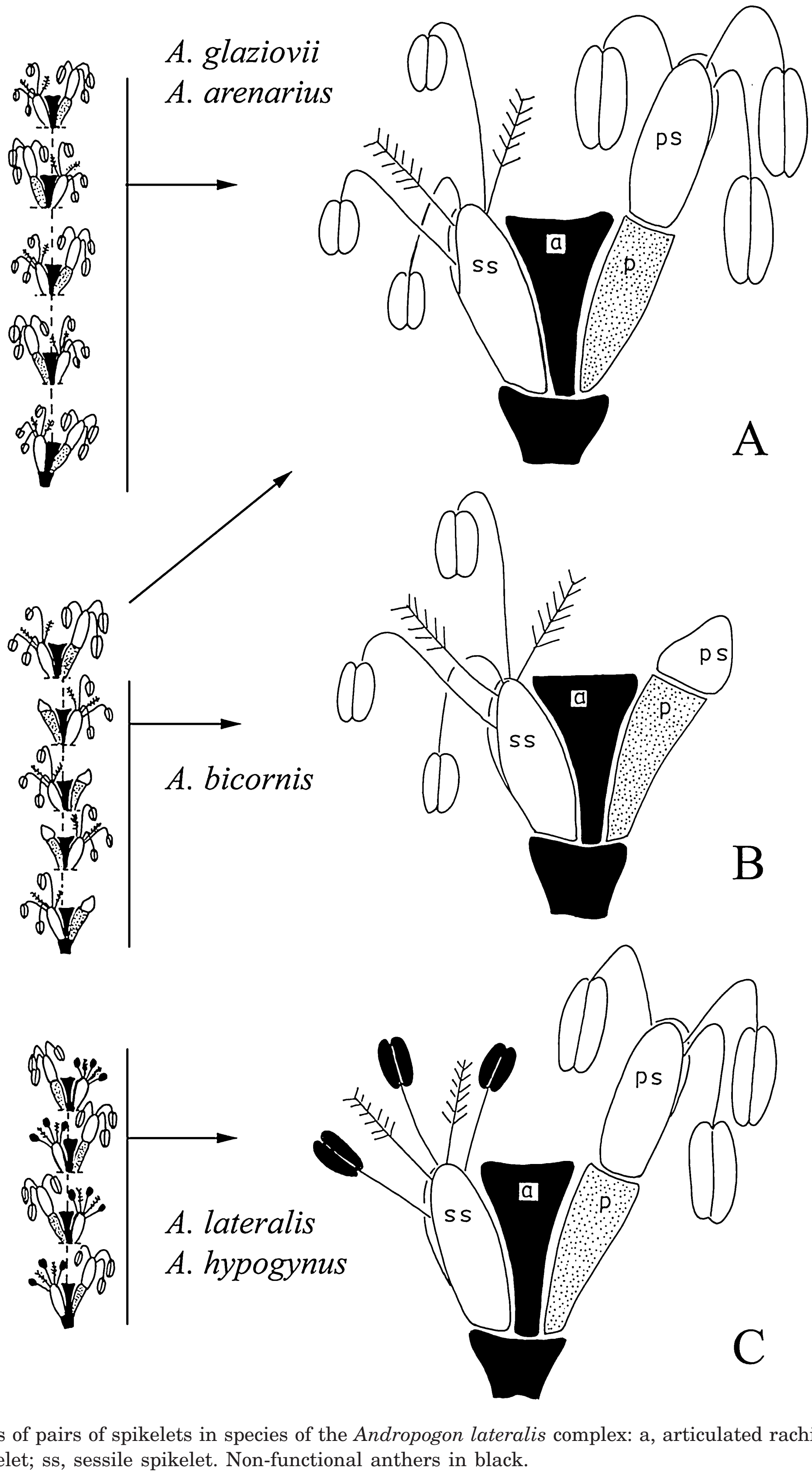


Figure 1. Types of pairs of spikelets in species of the *Andropogon lateralis* complex: a, articulated rachis; p, pedicel; ps, pedicellate spikelet; ss, sessile spikelet. Non-functional anthers in black.

given in Appendix 1, under 'specimens in herbaria'. A full list of specimens will be provided on request to gnorrmann@hotmail.com.

Artificial crosses

In only one combination (*A. lateralis* and *A. hypogynus*) are both parents monoecious, protogynous, cross-pollinating species that flower at sunset (Norrmann & Quarin, 1991); therefore, whole inflorescences of Norrmann 36 were emasculated by cutting pedicellate male spikelets a few days before anthesis and keeping sessile female flowers isolated until pollination. The procedure for hybridization was as follows: the female parent was placed in a pollen-proof chamber on the night before the cross was made, and stigmas were dusted with pollen of the male parent (Norrmann 72) at the next sunset.

Analysis of morphological traits

Morphological studies of natural hybrids and accessions of putative parental species were made (see Appendix 1). Several qualitative characters typical of and/or restricted to each species were assessed, mostly related to the sessile spikelet, which is considered to be the most informative for the taxonomy of the genus (Clayton & Renvoize, 1986; Wipff, 1996).

Analysis of cytological and reproductive characters

Chromosomes and meiotic behaviour

Chromosome numbers were obtained from mitotic squashes of root tips collected from potted plants and pretreated for 2 h with α-bromonaphthalene at room temperature (20/25 °C). Subsequently, material was hydrolysed with 1 M HCl at 60 °C for 10 min and stained with fuchsin. Squashes were made in a drop of aceto-orcein. For the study of meiosis, young inflorescences were fixed in Carnoy's solution and kept refrigerated in 70% ethanol. Pollen mother cells (PMCs) were stained with aceto-carmine. Preparations were made permanent with Venetian turpentine.

Reproduction and fertility

Embryo sac development was determined by the clearing-squash technique (Herr, 1971). Seed set was determined in test garden-grown plants and wild plants in the field by counting the number of pistillate flowers that developed fruit. Pollen fertility was estimated by determining the percentage of stainable pollen in Lugol: 2% iodine–potassium iodide (I_2–KI) solution. To observe pollen germination on the stigma surface and tube growth following pollination, ovaries were fixed in formaldehyde–acetic acid–70% alcohol (FAA, 5 : 5 : 90 v/v ratio), placed in 1 M NaOH for 15 min, transferred into 0.1% aniline blue solution for 15–30 min, mounted on a glass slide with a drop of aniline blue, and covered with a cover glass for examination by fluorescence microscopy. The percentage of pollen germination was determined by counting germinated and non-germinated pollen grains on the stigmas, 2 h after pollination. Penetration of the tube up to the micropylar zone was also recorded. All described tests were performed mainly on test garden-grown plants, but seed set and pollen fertility were also applied to plants in the field and even on herbarium specimens. In these, the formation of fruits was inferred by the transparency of illuminated spikelets, thus using a non-destructive technique.

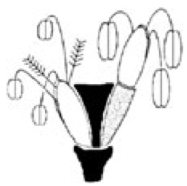
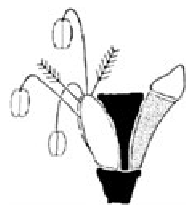
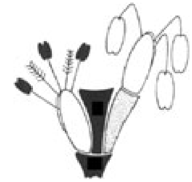
RESULTS

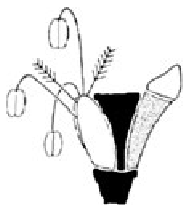
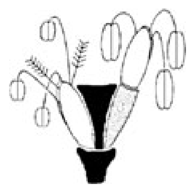
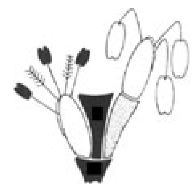
The following five hybrid combinations within section *Leptopogon* are described, arranged according to their relative weight in field collections: (1) *A. lateralis* × *A. bicornis*; (2) *A. lateralis* × *A. arenarius*; (3) *A. lateralis* × *A. hypogynus*; (4) *A. bicornis* × *A. glaziovii*; and (5) *A. bicornis* × *A. arenarius*. A sixth combination (*A. hypogynus* × *A. bicornis*) was not found in the field, but its existence is discussed on the basis of herbarium specimens. The standard practice of listing the seed parent first is not followed here, as putative seed parents are not known in all cases (see below). The distribution and morphological characters for each species and hybrids are summarized in Table 1. Chromosome data and reproductive issues are presented in Table 2.

Distribution and habitat of parental species and hybrids

Ecologically, the parental species involved in these hybridization events may be divided broadly into either invasive self-pollinating species or climax outcrossing species. The most invasive parental species is *A. bicornis* (Fig. 2B, E), widespread from Argentina to North America (Campbell, 1983a). *Andropogon bicornis* is a prolific producer of both pollen and seeds, because of the large numbers of flowers generated in the flowering period (Fig. 3B). In Argentina, old rice fields and wet roadsides provide ideal habitats for *A. bicornis*, enabling it to establish huge populations and hence to generate large quantities of pollen and seeds that can invade neighbouring grasslands. These are mostly occupied by *A. lateralis* (Figs 2A, 3A), an open-pollinated forage (*paja colorada*) which ranges from Argentina to Brazil and Peru, with scattered populations in Central America and Cuba. *Andropogon lateralis* × *A. bicornis* hybrids (Figs 2G, 3D) occur wherever parental species live together (i.e.

Table 1. Ecological and morphological data of parents and hybrids in the *Andropogon lateralis* complex

Pivotal species *A. bicornis*	Species				
Character	*A. glaziovii*	*A. glaziovii* × *A. bicornis*	*A. bicornis*	*A. bicornis* × *A. lateralis*	*A. lateralis*
Distribution and habitat	Marshes of Paraguay, Bolivia and Brazil	Borders of marshes, at the habitat hybrid	Wetlands of tropical and subtropical America	Zones of hybrid habitat	Open wet grasslands in subtropical South America
Foliar hairiness	Velvety	Intermediate	Glabrous	Glabrous	Glabrous
Stigma colour	Purple to white	Purple to white	White	Purple to white	Purple to white
Floral arrangement (anthers in white, functional; anthers in black, non-functional)					
Sessile spikelet	Awned, hermaphrodite	Awned, hermaphrodite	Awnless, hermaphrodite	Awned, hermaphrodite	Awned, female
Pedicellate spikelet	Developed, usually male	Developed, usually male	Reduced, except for upper pair which is male	Developed, male	Developed, male
Anthesis	Strictly at dawn	Strictly at dawn	Strictly at dawn	Strictly at dawn	Sunset and late evening

Distribution and habitat	Wetlands of tropical and subtropical America	Hybrid habitat in sand dunes and wet prairies of Brazil	Sand near Atlantic Ocean in south-east Brazil and Uruguay	Zones of hybrid habitat in Brazil and Uruguay	Open wet grasslands in subtropical South America
Sheath	Long, conduplicate	Intermediate	Cuneiform, short	Intermediate	Conduplicate
Stigma colour	White	White	White	Purple to white	Purple to white
Floral arrangement					
Sessile spikelets	Awnless, hermaphrodite	Inconspicuous awn to awnless, hermaphrodite	Inconspicuous awn to awnless, hermaphrodite	Awned, hermaphrodite	Awned, female
Pedicellate spikelets	Reduced, except for upper pair which is male	Developed, usually male	Developed, usually male	Developed, male	Developed, male
Anthesis	Strictly at dawn	Strictly at dawn	Strictly at dawn	Strictly at dawn	Sunset and late evening

A. lateralis and *A. hypogynus* have reduced functionless stamens but regular meiotic behaviour (Norrmann & Quarin, 1991).

Table 2. Chromosomes and reproductive issues in the *Andropogon* species and hybrids used in this investigation

Species and hybrids	2*n*	Meiotic chromosome associations		Pollen fertility (%)	Mature embryo sacs (%)	Breeding system	Seed set (%)	
		I	II				Self	Open
A. lateralis	60		30	> 95	> 95	Cross-pollinated	35	67
A. hypogynus	60		30	> 95	> 95	Cross-pollinated	34	70
A. bicornis	60		30	> 95	> 95	Self-pollinated	> 90	> 90
A. arenarius	60		30	> 95	> 95	Self-pollinated	> 90	> 90
A. glaziovii						Self-pollinated	> 90	> 90
A. lateralis × *A. arenarius*	60	0.8 (0–2)	29.6 (29–30)	0	5	Largely sterile	0	1
A. lateralis × *A. bicornis*	60	0.6 (0–4)	29.4 (28–30)	0	0	Sterile	0	0
A. lateralis × *A. hypogyus*	60	0.02 (0–2)	29.99 (29–30)	88	98	Fertile	23	45
Natural hybrids								
A. lateralis × *A. hypogyus*	60		30	78	100	Fertile	14	56
Controlled hybrids								
A. bicornis × *A. arenarius*	60	1.6 (0–4)	29.2 (28–30)	0	0	Sterile	0	0
A. bicornis × *A. glaziovii*	60	0.4 (0–6)	29.8 (27–30)	0	0	Sterile	0	0

Argentina, Paraguay, Brazil), and are found in the hybridization habitat, i.e. on the border of the grasslands/marshes (Table 1, Fig. 4). In this combination, the direction of the cross can be assessed: *A. lateralis* flowers at sunset and stigmas stay receptive all night long. Therefore, at dawn of the following day, pollen of *A. bicornis* is released and is able to pollinate *A. lateralis*. The converse is unlikely to occur, as the stigmas of *A. bicornis* do not remain receptive for more than a few hours under the summer sun conditions waiting to receive *A. lateralis* pollen at sunset (unpubl. data). This hybrid combination is the most common of those described here, with more than 100 hybrids being found in less than 300 km along the road from Corrientes to Posadas (Argentina).

Andropogon glaziovii (Figs 2I, 3C) occurs in swamps of Paraguay, Bolivia and Brazil. Its hybrids with *A. bicornis* (Figs 2J, 3E) have been recorded at two sites in Paraguay and one in Brazil (Fig. 4). The number of hybrids at both sites in Paraguay was high (i.e. more than 25 plants per site) and they clearly occupied the hybridization habitat zone.

Andropogon hypogynus (Fig. 2D) is similar to *A. lateralis*, in both floral biology and morphology (Normann & Quarin, 1991). The main differences between these two species are the number of racemes, size of the spikelets, presence of awns and ecological preference (*A. hypogynus* prefers heavy, humid soils of sedimentary origin). *Andropogon hypogynus* hybridizes readily with *A. lateralis* in Paraguay, northern Corrientes, Chaco and north-eastern Santa Fe in Argentina, producing fertile progeny (Figs 2H, 4). As hybrids are fertile, hybrid swarms could presumably occur with all possible combinations (parents, F1, F2, backcrosses). In contrast, *A. hypogynus* does not seem to hybridize easily with other species that might be sympatric, such as *A. bicornis*. Herbarium specimens that might represent this hybrid combination were collected in Bolivia and Colombia.

Another important parental species is *A. arenarius* (Fig. 2C), an aggressive sand colonizer from the Atlantic coasts of Uruguay and Brazil. It forms huge and dense communities similar to those of *A. bicornis*. It produces large amounts of pollen and hybridizes with its sympatric relatives *A. bicornis* (Fig. 2K) and *A. lateralis* (Fig. 2G). Thus, the two colonizing species, *A. bicornis* and *A. arenarius*, appear to be pivotal in hybridization (Table 1).

From all the surveyed areas, three sites are worthy of comment (Fig. 4). (1) The Corrientes–Chaco–Misiones (Argentina) area includes combinations involving *A. lateralis*, *A. hypogynus* and *A. bicornis*. This region contains the boundaries of Chaco and Amazonic phytogeographical domains. The Parana River divides the two, leaving the heavy sedimentary soils to the west (*A. hypogynus*), whereas eastwards is

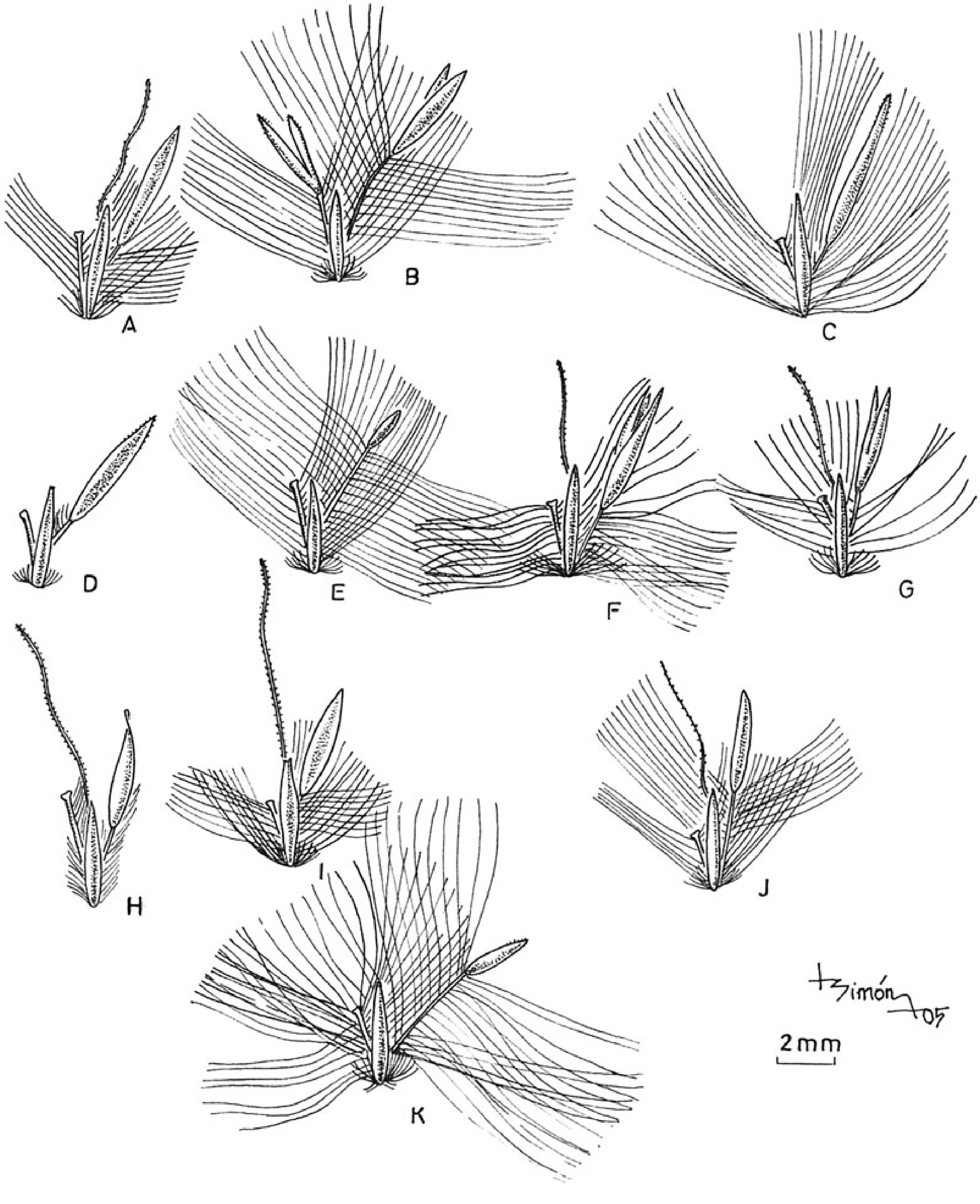


Figure 2. Spikelets in *Andropogon* species and hybrids: A, *A. lateralis*; B, *A. bicornis* upper spikelet; C, *A. arenarius*; D, *A. hypogynus*; E, *A. bicornis* intermediate spikelet; F, *A. lateralis* × *A. arenarius*; G, *A. lateralis* × *A. bicornis*; H, *A. lateralis* × *A. hypogynus*; I, *A. glaziovii*; J, *A. glaziovii* × *A. bicornis*; K, *A. arenarius* × *A. bicornis*.

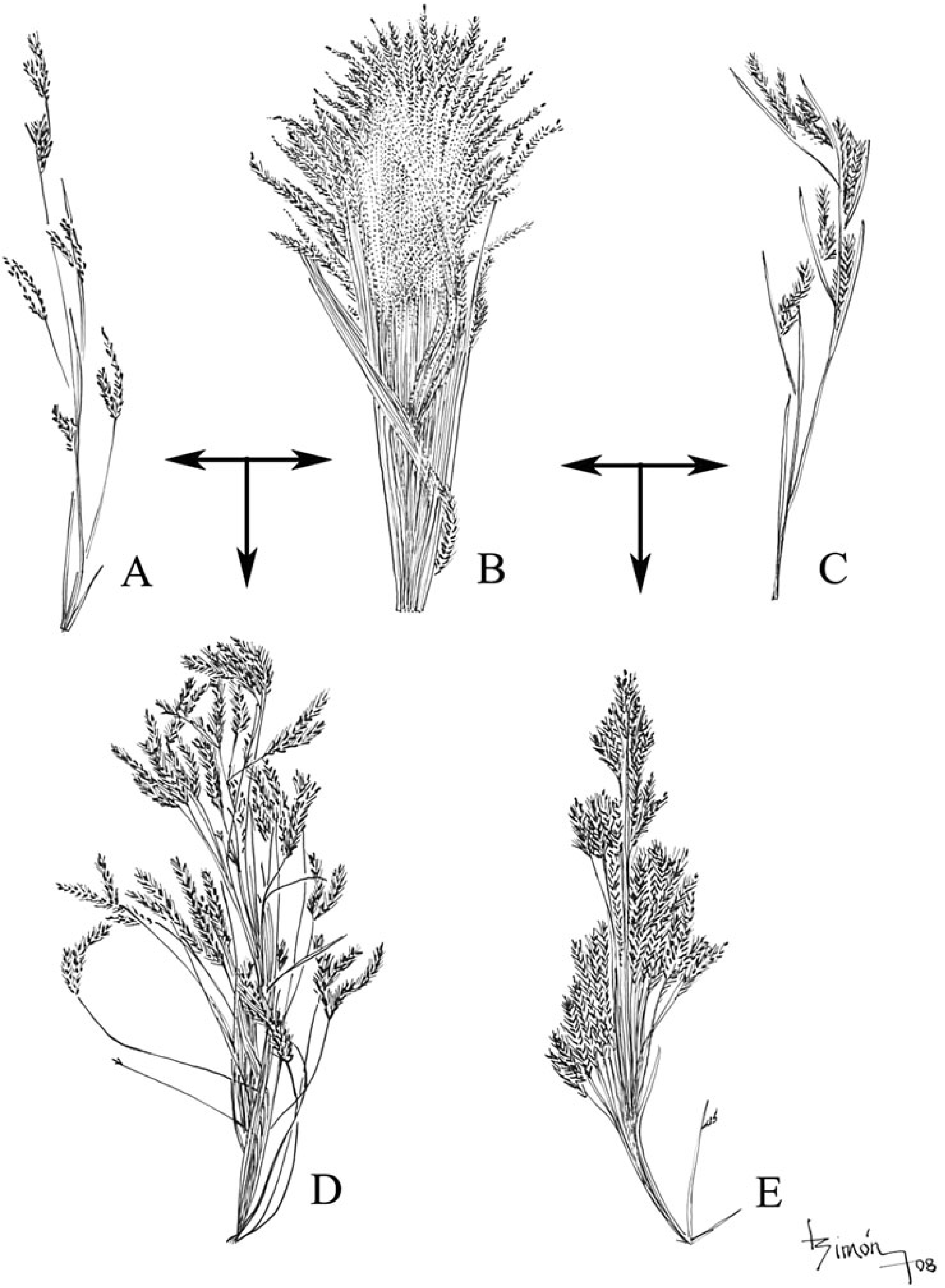


Figure 3. Synflorescences in *Andropogon* species and hybrids: A, *A. lateralis*; B, *A. bicornis*; C, *A. glaziovii*; D, natural hybrid *A. lateralis* × *A. bicornis*; E, natural hybrid *A. bicornis* × *A. glaziovii*.

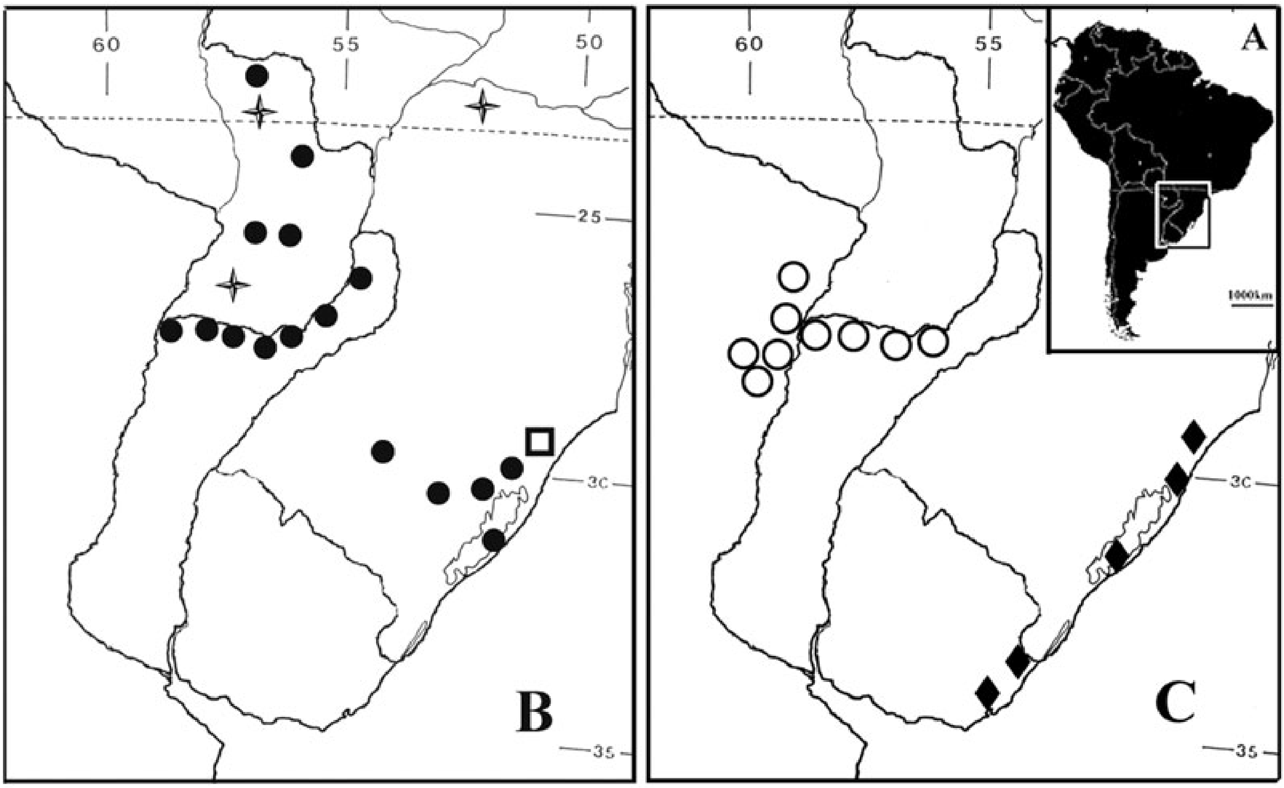


Figure 4. Geographical distribution of natural hybrids in *Andropogon*: A, location of the surveyed area; B, *A. lateralis* × *A. bicornis* (filled circles), *A. bicornis* × *A. glaziovii* (stars), *A. bicornis* × *A. arenarius* (open square); C, *A. lateralis* × *A. hypogynus* (open circles), *A. lateralis* × *A. arenarius* (filled diamonds).

the most suitable habitat for *A. lateralis*. (2) The Itapirubá site (Brazil, Santa Catarina State) contains combinations involving *A. arenarius*, *A. lateralis* and *A. bicornis*. This hybridization site is found 3 km eastward from highway BR101 to the village. All necessary ecotones for each species and the hybrids are present there: moving dune (*A. arenarius*); fertile, damp soil, generally used for forage (*A. lateralis*); and wet roadsides and swamps (*A. bicornis*). (3) The Paraguayan area possesses the restricted combination *A. bicornis* × *A. glaziovii*; Paraguay is the south-east limit of *A. glaziovii*. It is predicted that further sites containing this hybrid will be found in the warmer regions of Brazil, Paraguay and Bolivia, where these species live in sympatry.

Finally, the ability of pieces of rhizomes to regrow following collection was observed to be greater in hybrid plants than in their putative parents, perhaps because of hybrid vigour. This was especially evident in the hybrids *A. lateralis* × *A. bicornis* and *A. bicornis* × *A. arenarius*. For example, although collections of both putative parent and hybrid rhizomes of *A. lateralis* × *A. bicornis* and *A. bicornis* × *A. arenarius* were made at the Itapirubá site, only the hybrid rhizomes survived.

Analysis of morphological traits

The discrete morphological characters chosen for the analysis of parents and natural hybrids are described in Table 1. From parallel research (Norrmann, 1999; G. A. Norrmann, unpubl. data), it is known that several traits show dominant/recessive characteristics. For instance, in more than 20 hybrid combinations, the trait 'awned spikelets' behaves as dominant (⇒) over 'awnless'; morning anthesis ⇒ sunset anthesis; pedicellate spikelet developed ⇒ reduced; purple stigma ⇒ white stigma; normal stamens ⇒ staminodes; among others (Norrmann, 1999). These data were of great predictive value for the analysis of each combination (see also Figs 2, 3): for example, *A. bicornis* has awnless spikelets plus undeveloped pedicellate spikelets (Fig. 2E), whereas both *A. lateralis* (Fig. 2A) and *A. glaziovii* (Fig. 2I) have awned and developed pedicellate spikelets. When these species cross, the hybrids possess awned spikelets and developed (male) pedicellate spikelets (Fig. 2F, J).

In other features, such as synflorescence ramification, clearly defined genic action is not apparent and the phenotypes are intermediate; that is, the synflorescence shares characteristics from both parents. For example, *A. lateralis* has few branches along the floral axis, with internodes regularly separated (Fig. 3A), whereas *A. bicornis* has multiple branches on the top of the floral axis, with internodes closely aggregated towards the top (Fig. 3B). Hybrids are multibranched, with internodes gradually decreasing towards the apex (Fig. 3D). The same basic situation applies even more dramatically when *A. bicornis* hybridizes with *A. glaziovii* (Fig. 3C), producing an exuberant synflorescence with thousands of spikelets (Fig. 3E).

In the only combination in which control hybrids were made, *A. lateralis* × *A. hypogynus*, these were morphologically indistinguishable from natural ones (data not shown). These two species are considered to be the most closely related, with differentiation occurring mainly at the ecological level. Many morphological differences are quantitative, for example hairiness and size of spikelets, number of racemes. Fortunately, awns are absent or minuscule in *A. hypogynus* (Fig. 2D) and well developed in *A. lateralis* (Fig. 2A, Table 1). All hybrids had awned lemmas (Fig. 2H), a useful distinction that allowed them to be distinguished from *A. hypogynus*.

Analysis of reproductive and cytological characters

Chromosomes and meiotic behaviour

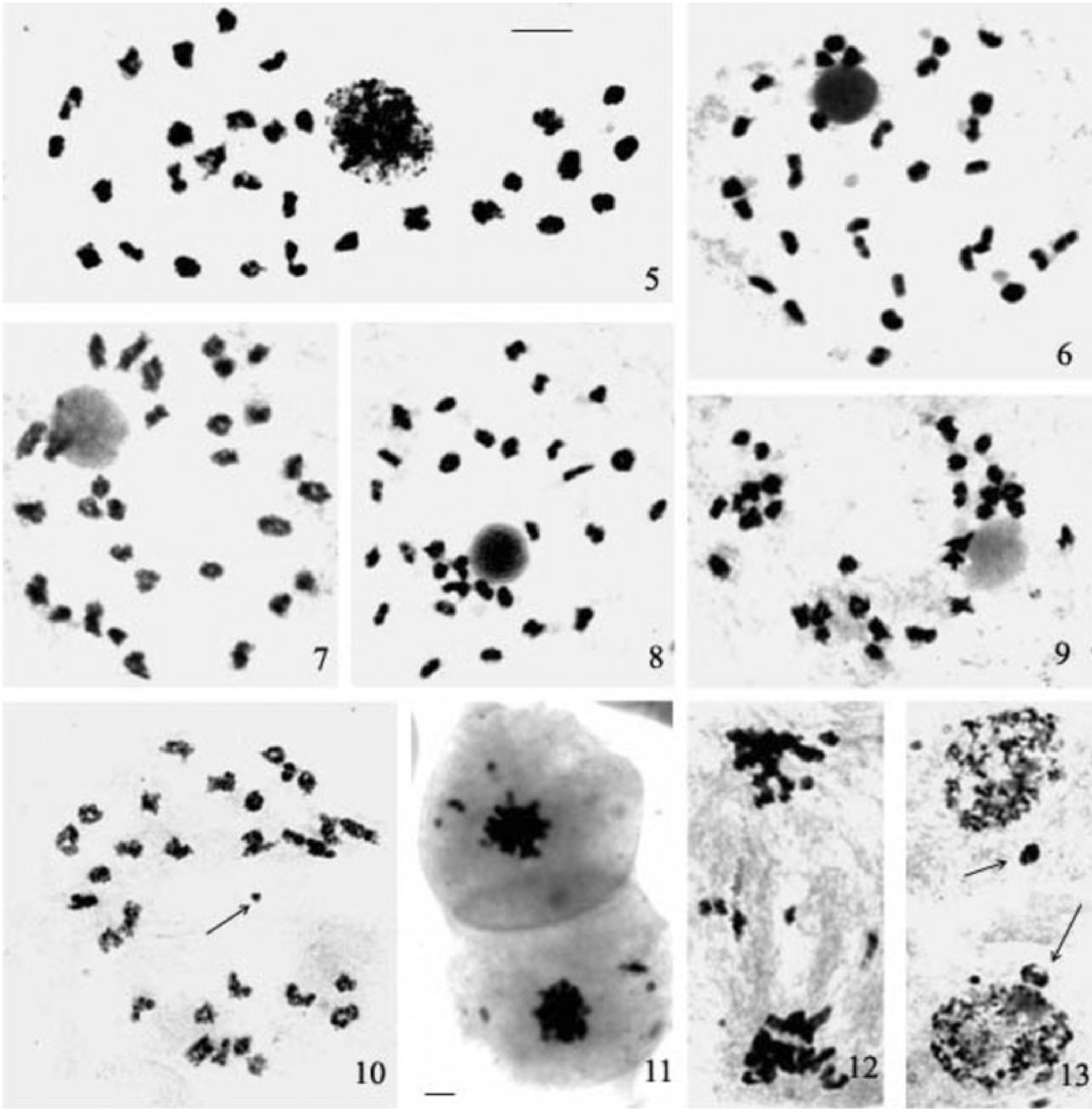
All parental species have $2n = 6x = 60$ chromosomes and exhibit normal meiosis (Table 2), characterized by the formation of 30 bivalents (II) and regular segregation (Norrmann, 1985, 1999: Norrmann & Scarel, 2000). All studied hybrids also have $2n = 60$ chromosomes which pair to form up to 30 bivalents per PMC (Table 2, Figs 5–10). B chromosomes are often present in *A. lateralis* and its hybrids (Fig. 10), but they are not considered here. The most frequent configurations observed in diakinesis-prometaphase I of hybrids was 30 II (Table 2). Other figures, such as 29 II + 2 I, 28 II + 4 I or 27 II + 6 I, were also observed in the remaining cells. In spite of relatively high pairing, irregular segregation at anaphase I and lagging chromosomes were observed in most PMCs of *A. lateralis* × *A. bicornis* (Fig. 12), *A. bicornis* × *A. arenarius* and *A. glaziovii* × *A. bicornis* hybrids, leading to the production of micronuclei (Fig. 13) and the inability to form viable gametes (Table 2). *Andropogon arenarius* × *A. lateralis* hybrids presented fewer irregularities than those described above, but were also incapable of producing good quality pollen. Several authors (Leitch & Bennett, 1997; Soltis & Soltis, 1999; Wendel, 2000; Ozkan, Levy & Feldman, 2001; Liu & Wendel, 2003) have emphasized the importance of rapidly establishing intra- and intergenomic rearrangements after polyploidization. The occurrence of small 'rearrangements' in the origin of the *A. lateralis* complex would explain the high level of pairing in hybrids, but it also provokes meiotic segregation disharmony and hence causes sterility. Additional post-zygotic barriers, such as genic incompatibilities, developed during speciation (see Soltis & Soltis, 2000; Coyne & Orr, 2004), may also play a part in the origin of different rates of sterility among different hybrids (see also 'Discussion'). Whatever the reasons for this 'cryptic sterility', the high degree of bivalent formation during meiosis in the hybrids points to the existence of ancient homology between the genomes of all species.

In the only fertile combination, *A. lateralis* × *A. hypogynus*, both artificial and naturally occurring individuals had completely regular meiosis with 30 bivalents formed at metaphase I (Figs 9, 10), followed by normal segregation and viable gamete formation. One of these hybrids was also used as the female parent in several interspecific crosses (Norrmann, Quarin & Keeler, 1997; Norrmann & Keeler, 2003).

Reproduction and fertility

Irregularities in floral development were observed in all hybrids, except for the *A. hypogynus* × *A. lateralis* combination. The flowering events were as follows. At anthesis, stigmas protruded whereas stamens extended from the spikelets through filament elongation, but no anther dehiscence was observed. When anthers were gently squashed and the pollen grains were released, these grains could not be stained with Lugol (I_2–KI), indicating that they were not viable. Pollen from putative parents was then dusted on to the stigmas of the hybrids and its development was scored through fluorescence microscopy: pollen grains germinated and penetrated the stigmas, the ovaries and arrived at the micropyles, but none of the flowers produced caryopses. These tests were performed in hybrids for five consecutive flowering periods.

Embryological studies revealed that megasporogenesis occurred in most ovaries, leading to the formation of four megaspores, but from this point onwards there was a high level of abortion. Consequently, ovaries at anthesis lacked embryo sacs, probably as a result of the same meiotic irregularities that affected PMCs. Complete failure to form embryo sacs was universal in *A. lateralis* × *A. bicornis*, *A. bicornis* × *A. arenarius* and *A. bicornis* × *A. glaziovii* (Table 2). However, up to 5% mature embryo sacs were observed in *A. lateralis* × *A. arenarius* hybrids and a few (average 0.87%) 'mature' seeds were recovered, although the seeds were mostly shrunken and underdeveloped. The apparent capability of *A. lateralis* × *A. arenarius* hybrids to 'start developing seeds', mentioned by Campbell & Windisch (1987), was observed in material growing at the experimental garden only when



Figures 5–13. Meiotic chromosome behaviour in hybrids within the *Andropogon lateralis* complex. Figs 5–10. Pollen mother cells (PMCs) showing 30 bivalents. Fig. 5. *A. lateralis* × *A. bicornis*. Fig. 6. *A. lateralis* × *A. arenarius*. Fig. 7. *A. bicornis* × *A. glaziovii*. Fig. 8. *A. bicornis* × *A. arenarius*. Fig. 9. *A. lateralis* × *A. hypogynus* (natural hybrid). Fig. 10. *A. lateralis* × *A. hypogynus* (controlled hybrid) plus one B chromosome (arrow). Figs 11–13. Irregular meiotic behaviour. Fig. 11. Metaphase I in two cells with univalents outside the plate in *A. bicornis* × *A. arenarius*. Fig. 12. Five lagging chromosomes in anaphase I in *A. lateralis* × *A. bicornis*. Fig. 13. Prophase II with two micronuclei (arrows) in *A. bicornis* × *A. glaziovii*. Scale bar, 10 μm.

parental pollen was supplied. An embryological survey in these developing ovaries indicated that fertilization is occasionally accomplished and endosperm even starts to develop, filling the grain completely, but most apparently healthy fruits will shrink less than four days after pollination, producing dried underdeveloped kernels. A few well-developed fruits were finally recovered, but have been incapable of germination so far. These phenomena are similar to those observed in $2x \times 4x$ crosses of *Paspalum rufum*

Nees ex Steud. (Norrmann, Bovo & Quarin, 1994), where breakdown of the 3*x* embryos occurred as a result of imbalance in the endosperm/embryo chromosome ratio.

These results thus point to a strong sterility barrier between these two species (although we do not know the reason for the frequent seed abortion), and despite the fact that a few seeds sometimes develop, especially in the presence of backcross pollen. This hybrid combination presents an intermediate level of sterility compared with the complete sterility obtained in most other hybrid combinations.

As meiotic abnormalities were observed on the male side of sterile crosses, I am keen to address female sterility, also reasoned to be a result of small intra- and intergenomic rearrangements. As discussed above (see 'Chromosomes and meiotic behaviour'), small internal genomic rearrangements might explain the breakdown of gametes in the hybrids. Different sterility levels between species might also reflect genic incompatibilities which have evolved during the speciation time of each taxon (Soltis & Soltis, 2000; Coyne & Orr, 2004).

Flowering in the fertile *A. lateralis* × *A. hypogynus* hybrids was normal from anthesis to maturity, and was similar to that of the parental species. Thus, reproductive isolation does not seem to cause these species to remain distinct, as discussed below. Instead, other factors, such as geographical separation coupled with ecological preferences, might explain why both species are separated outside their points of contact.

Impact on taxonomy

Once the external morphology of verified hybrids had been characterized, a search in many key herbaria was made. Many natural hybrids were identified in the collections, some of which had been described as new taxa. The hybrids located were as follows (locality information of specimens given in Appendix 1).

1. *A. lateralis* × *A. bicornis*. In spite of the abundance of this combination in the field, only three collections were found in herbaria: *Balansa 228* and *Fiebrig 770* from southern Brazil, *Regnell 1091* from south-eastern Brazil. *Balansa 228* was collected in 1887 and was cited by Hackel (1889) among the syntypes for *A. incanus* var. *subtilior* (Hack.) Hack. Henrard (1921) synonymized *A. incanus* under *A. lateralis* and the variety was transferred accordingly; hence, he placed the voucher as the type of *A. lateralis* var. *subtilior* (Hack.) Henrard.
2. *A. lateralis* × *A. arenarius*. One of the oldest collections of this combination, *Regnell 855* from south-eastern Brazil, was described as a new species: *A. lindmanii* Hack. These hybrids are easily discriminated and therefore this taxon has been recognized for Brazil (Hervé & Valls, 1980; Zanin & Longhi-Wagner, 2006) and Uruguay (Rosengurtt, Arrillaga & Izaguirre, 1970). J.F. Valls, CENARGEN (Centro Nacional de Recursos Geneticos), Brazil (pers. comm.) was the first to realize the hybrid origin of *A. lindmanii*, an insight published formally by Campbell & Windisch (1987).
3. *A. lateralis* × *A. hypogynus*. Several collections were found from the same area as that of the current research (see Appendix 1). One of these specimens (*Stuckert 20275*), collected in Argentine Chaco, was sent to Hackel, who described it as *A. coloratus* Hack., a legitimate species according to Zuloaga *et al.* (1994). As this is a fertile combination, further generations of crosses and backcrosses could generate plants showing a wide range of intermediate characters, making the differentiation between *A. hypogynus*, *A. lateralis* and hybrids quite difficult to determine. Therefore, the name *A. coloratus* may actually refer to a hybrid swarm.
4. *A. bicornis* × *A. glaziovii*. Three specimens were collected by *Campbell* (*4977*, *4898*, and *4589*) in Guzolandia, São Paulo, Brazil, and were analysed thoroughly by Zanin (2001a, b). In the west of São Paulo and close to Matto Grosso do Sul (Fig. 4), the only hexaploid species living in sympatry are *A. bicornis* and *A. glaziovii*, and the description and the drawings presented by Zanin (2001a) match this hybrid combination perfectly, especially in relation to the presence of awns and developed pedicellate spikelets and the precisely described differences in foliar hairiness. In the Flora of São Paulo, Zanin (2001b) referred to these specimens with the provisional name *Andropogon* sp. 1, as she was not sure about the new taxon.

The hybrid combination *A. hypogynus* × *A. bicornis* has not been reported in the wild, and no detailed cytogenetic and fertility analyses have been conducted. However, two herbarium specimens have been located that could represent this hybrid combination. (1) *Haase 1* was collected together with *Haase 653* (*A. hypogynus*) in a poorly collected area of Amazonian Bolivia near Beni. Based on *Haase 1*, Renvoize (1998) erected the new taxon *A. multiflorus*, stating that 'the new species was close to *A. hypogynus* but with many branches and spikes'. These characteristics and others, such as the development of pedicellate spikelets, match precisely with what would be expected from this hybrid. (2) At the Llano de San Martin, Colombia, Karsten collected what would be the type for *A. incanus* var. *bogotensis* Hack. (1889), as well as the putative parents *A. hypogynus* and *A. bicornis* (see Appendix 1). Henrard (1921) transferred

Table 3. Hybrid combinations and specific names used in type collections

Parental species	*A. lateralis*	*A. bicornis*	*A. hypogynus*
A. bicornis	*A. incanus* var. *subtilior* *A. lateralis* var. *subtilior*		
A. hypogynus	*A. coloratus*	*A. incanus* var. *bogotensis* *A. lateralis* var. *bogotensis* *A. multiflorus* *A. bogotensis*	
A. arenarius	*A. lindmanii*	Found in nature. Not found in herbaria	No contact
A. glaziovii	Not found yet	*Andropogon* sp. 1	Not found yet

this variety and its type specimen to *A. lateralis* var. *bogotensis* (Hack.) Henrard. Zanin & Longhi-Wagner (2003) upgraded the taxon to species rank as *A. bogotensis* (Hack.) A.Zanin & Longhi-Wagner, keeping the Karsten collection as the type. Zanin & Longhi-Wagner also agreed that the two collections (*Haase 1* and *Karsten*) were quite similar, and thus synonymized *A. multiflorus* under *A. bogotensis* (2003). My analysis of the specimens suggests that the two plants might well be natural hybrids between *A. hypogynus* and *A. bicornis*, but this needs to be confirmed by comparison with living hybrid plants, which are currently unavailable.

No specimens that might represent offspring of *A. bicornis* × *A. glaziovii* or *A. bicornis* × *A. arenarius* have been found in herbaria to date. The combination *A. arenarius* × *A. hypogynus* is not considered here, as these species are not sympatric (Table 3). Hybrids of *A. lateralis* × *A. glaziovii* have not been located in natural populations or in herbaria, despite occurring sympatric ally and hybridizing with other species.

DISCUSSION

Evolutionary considerations

Andropogon has diversified into a larger number of species in America and Africa than in Asia or Europe (Clayton & Renvoize, 1986; Norrmann, 1999). Genetic differences between American and African species are poorly, however, understood. Chromosomal evolution, such as polyploidy, appears to be more extensive in America, as hexaploids are almost entirely restricted to this continent (and especially to South America). Section *Leptopogon*, to which all species treated here belong, has been considered to be the most advanced of the genus (Campbell & Windisch, 1986; Clayton & Renvoize, 1986; Norrmann, 1999).

This study provides extensive new data on the *A. lateralis* complex, which is composed of a group of related South American species. The high frequency of bivalents observed in all crosses (30 observed, of 30 maximum) points to the existence of ancient chromosomal homology or homoeology in all species treated here, with small differences among the 'three' basic genomes (Table 2, see Norrmann *et al.*, 2004).

Assumptions about genome relatedness based entirely on chromosome pairing must be treated with caution, as pairing can be controlled by factors other than the degree of homology alone. In particular, genic control of pairing favours truly homologous pairing and prevents homoeologous chromosomes from pairing (see Moore, 2002). When this control of pairing is broken, for instance in hybridization, the amount of pairing changes. In this sense, it is likely that the three supposed genomes in *Andropogon*, shown by classic meiotic analysis (Norrmann, 1985, 1999; Norrmann *et al.*, 1997), might well be fewer than three, but obscured by preferential pairing. At least one of the genomes present in *A. lateralis* and *A. bicornis* is unique and has been identified as the S genome, which constitutes diploids of the *A. virginicus* group in North America and *A. selloanus* in South America (Norrmann *et al.*, 2004), but the origins of the other genome or genomes are still unknown.

The strong sterility barrier observed in most of the hybrids studied can be classified as 'intrinsic post-zygotic isolation' (Coyne & Orr, 2004), being caused by reorganization of polyploid genomes and/or genetic incompatibilities. This type of isolation is considered to be difficult to reverse, as genetic incompatibilities accumulate rapidly as divergence proceeds (Orr, 1995). The high fertility of *A. lateralis* × *A. hypogynus* hybrids suggests that they have the same genomes, with specific differences at the gene level.

At least two different scenarios for the origin of the *A. lateralis* complex can be hypothesized: (1) several ancient hybridization–polyploidization events involving different races or species took place giving rise to different species (recurrent polyploidy); or (2) no matter how many recurrent episodes occurred, a single hybridization–polyploidization event succeeded, giving rise to a common allohexaploid ancestor that subsequently evolved and diversified into the species observed today. Therefore, specific differences arising prior to polyploidization would account for variation in the group [scenario (1), polyphyly], or variation arose post-polyploidization [scenario (2) monophyly]. Recurrent polyploidy among different races, scenario (1), has been described in at least 45 genera (see Soltis & Soltis, 1999), and its occurrence is common in grass genera. Scenario (2) remains the only hypothesis for a monophyletic origin for the complex: if the ancestor became widespread, simple divergence of geographically isolated genomes would account for the gradual breakdown of genomic homology thereafter.

These hybridization events can be considered as experimental tests of the 'biological species concept', detecting reproductive isolation in sympatry. It is true that the biological species concept fails to apply in the case of uniparentalism (Solbrig, 1970; Grant, 1981), but a certain rate of cross-pollination occurs in *A. bicornis*, *A. arenarius* and *A. glaziovii*, as shown by their natural hybrids. A fair amount of population genetic theory suggests that even a small amount of gene flow is sufficient to maintain Hardy–Weinberg equilibrium (see Hartl, 2000), and so these experiments might suggest that these parental species really are 'biological species'.

Unlike the other hybrids studied here, *A. lateralis* × *A. hypogynus* hybrids are fully fertile. Homoploid or recombinational speciation is discarded, as hybrids backcross with both parents. More probably, hybridization is occurring only in certain areas, making a syngameon (a hybridizing group of species) in the sense of Grant (1981), but without leading to the formation of a new species. The broad concept of biological species presented by Coyne & Orr (2004) reflects effectively what seems to occur among these taxa. Both are well separated geographically, but they interbreed where they co-occur, hybrids are created, they breed and backcross, and form a syngameon. This does not happen outside the hybrid habitats, where species remain well separated because of their ecological preferences. This phenomenon is similar to that described in North America with *A. gerardii* (fertile prairie soils) and *A. hallii* (moving sands). Both species cross in habitat hybridizing zones (e.g. Nebraska sand hills) and hybrid swarms are formed (see Wipff, 1996; Boe *et al.*, 2004). Hybridization in the *A. gerardii*–*A. hallii* complex was recorded as early as 1891, when an individual was collected in Kansas and described as *A. chrysocomus* Nash (Wipff, 1996). Hybridization between the two taxa has also been used for breeding to improve big bluestem hardiness (Peters & Newell, 1961). Although hybrids in this combination are fertile, they disappear outside the hybridization habitat, indicating that the species are ecologically distinct (see Boe *et al.*, 2004).

Taxonomic implications

Several natural hybrids similar to those collected personally have been collected in the last two centuries and, in most cases, have caught the attention of taxonomists, who described them formally as new taxa. Therefore, taxonomic entities based on them have been validly published (Table 3).

Natural hybridization has increased taxonomic complexity and led to additional splitting in *Andropogon* species. From less than 12 taxonomic entities in the *A. lateralis* complex, at least five legitimate names correspond to natural hybrids (i.e. *A. coloratus*, *A. lindmanii*, *A. lateralis* var. *subtilior*, *A. lateralis* var. *bogotensis* and *A. multiflorus*). A taxonomic group such as this, with nearly half of its entities based on natural hybrids, is remarkable.

As observed by Solbrig (1970), naming species requires two steps: the first is to discover discrete characters and the second is to name them. In most *Andropogon* hybrids, both requirements are fulfilled. Therefore, these hybrids could be considered as 'good species' in the classical topological taxonomic sense (Cronquist, 1981): the specimens are easily recognizable by classical means (Hackel did not miss a single one), they are morphologically homogeneous, and generally flower together and live together. Certainly most of them do not breed true, but, in most combinations, they are formed year after year and persist for many more.

A central species in hybridization within the complex is *A. bicornis*. Wherever *A. bicornis* is found, it readily hybridizes with *A. lateralis*, *A. glaziovii*, *A. arenarius* and, possibly, *A. hypogynus*. Looking at old collections in northern South America, it is also possible that hybridization involving *A. bicornis* has produced such rare specimens as *Burchell 808* (K! LE! W!) or *Ule 7747* (L! K! G!), although I am not sure of the other putative parent in these cases. A second species that is apparently central in hybridization in the complex is *A. lateralis* itself. Its particular reproductive system, largely outbreeding, makes it a good maternal parent, and it is abundant in native grasslands. *Andropogon lateralis* not only hybridizes with other members of the complex, but also with species of sections *Notosolen* (Norrmann, 1999) and *Andropogon* (Norrmann & Keeler, 2003). Future research is clearly needed to test the ability of *A. bicornis* and *A. lateralis* to hybridize with other hexaploid species in central and northern South America.

Finally, it is worth considering whether giving formal taxonomic names to natural hybrid combinations clarifies the taxonomic picture of the group. Again, this aim has also been of concern to other systematists dealing with natural hybridization (see Funk, 1985). However, data on hybridization and fertility characteristics in the group still remain incomplete. Moreover, at least for these species, the hybrids are morphologically distinct, so that names for them are useful for identification. Therefore, it is proposed that, until a complete picture of the hybridization phenomena in America is available, the original names should be retained (*pro. sp.*). According to this, the hybrid swarm formed by *A. lateralis* × *A. hypogynus* fits well into *A.* × *coloratus* Hack. (*pro. sp.*). *Andropogon* × *lindmanii* Hack. (*pro. sp.*) remains a legitimate name for *A. arenarius* × *A. lateralis* hybrids. *Andropogon* sp. 1 is the only available name for *A. glaziovii* × *A. bicornis* hybrids, until A. Zanin decides on a name for the taxon. If my hypothesis about *A. hypogynus* × *A. bicornis* is supported, the names *A. multiflorus* and *A. lateralis* var. *bogotensis* apply, of which *A.* × *multiflorus* Renvoize (*pro. sp.*) has priority at species rank. The widespread combination of hybrids between *A. lateralis* × *A. bicornis* could continue to be recognized as *A. lateralis* var. *subtilior*, but it makes sense to give this combination a species rank, as the others deserved. Hence, I propose the following combination:

Andropogon subtilior (Hack.) Norrmann **comb. nov.** Basionym: *Andropogon incanus* var. *subtilior* (Hack.) Hack. A.DC & C.DC. Monogr. Phan. 6:432.1889. Lectotype here designated: Paraguay: Jenaius in fiche a Costa Pucu, entre le Pirayu et Paraguarí. *Balansa 228*, II 1877 (lectotype: L, isotypes: K, G, P, LE).

Gaining knowledge about natural hybridization, or 'the most important single cause of a species problem in plants' (Grant, 1981), can match perfectly with classical taxonomy, by helping us understand the genetic mechanisms underlying the origin of taxonomic entities and providing sound data to explain their variation. This is exactly the objective of biosystematics as described by Solbrig (1970): to understand the 'why' and 'how' of the diversity of organisms.

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APPENDIX 1

Origin and identification of the *Andropogon* material[1] cited in this investigation.

A. BICORNIS L.

Living material: Argentina: Provincia de Corrientes, 15 km N de Bella Vista, *Norrmann & Quarín 89*, 16.v.1983 (CTES, HPR). 18 km ESE de Corrientes,

[1]Cited herbaria specimens were studied personally by the author and data are minimized, but a complete list of vouchers is available on request.

Ruta 5, *Norrmann 51*, 25.iii.1982 (CTES, LIL). Ruta 14 y Río Aguapey, *Norrmann & Quarín 91*, 17.v.1983 (CTES, BAA, US).

Specimens in herbaria: Types: Brasilia, ad Corcovado, prope Rio de Janeiro, *Schott A. 4816* (W L, as var. *absconditus*). Brasilia, *Gaudichaud 260* (G as var. *hybridus*), *Glaziou 16584* (K, W, P). In siccis apricis ad Sorocaba pr. Santos prov. S. Paulo, *Mosén* (L S, as var. *gracillimus*). s/loc *Glaziou 2736* (K), 4302 (K, P). Paraguay: Lamboré pr. Assumpcion 1874, *Balansa 271* (K, S, G, W, LE), *Balansa 2977* (K, G, W, P, L, LE). Colombia, *Karsten* (W, LE).

Incertae sedis Brazil: Prope Río de Janeiro *Burchell 808* (K, L, US, as var. *burchellii*). Amazonas, Rio Branco, Auf fuechten Campo bei S. Marcos, *Ule 7747* i.1909 (K, L, G, as *A. ulensis* Henrard ined.).

A. GLAZIOVII HACK.

Living material: Brazil: Estado de Matto Grosso do Sul, 5 km W de Ribas ao Rio Pardo, *Valls et al. 11765*, 14.iv.1988 (CTES, ICN). Campo Grande, *Norrmann 311* (CTES). Paraguay: Dep. Amambay, Parque Nacional Cerro Corá, ruta 5, *Norrmann et al. 163*, 18.iv.1995 (CTES, K, LIL, US, BAA). Dep. Concepción, ruta 5, 32 km noreste de Concepción, *Norrmann et al. 196*, 20.iv.1995 (CTES, K, US, LIL, BAA). Dep. San Pedro, Ayo. Ipané y ruta 3, *Norrmann et al. 203*, 20.iv.1995 (CTES). Dep Misiones, 2 km E de San Juan Bautista por ruta 1, *Norrmann et al. 217*, 21.iv.1995 (CTES).

Specimens in herbaria: Types: Brasilia, pr. Rio Janeiro, *Glaziou 11672* (W, K, L, LE, S). Other: Brazil: Estado de São Paulo, Guzolandia, Route SP 310, km 574, *Zanin 793* 12.vi.1999 (SPF).

A. GLAZIOVII × *A.* BICORNIS = *A*NDROPOGON × SP. 1 A.ZANIN

Living material: Paraguay: Dpto Concepción, 38 km E de Concepción por ruta 5, *Norrmann et al. 199*, 20.iv.1995 (CTES, US). Dpto Misiones, 2 km E de San Juan Bautista por ruta 1, *Norrmann et al.* 218, 219, 222 a, b, c, d, 21.iv.1995 (CTES, US).

Specimens in herbaria: Brazil: Estado de São Paulo, Guzolandia, Route SP 310, km 574, *Campbell 4705 & 4706*, 27.xii.1984 (SP).

A. LATERALIS NEES

Living material: Argentina: Provincia de Corrientes, Corrientes city, *Norrmann 111* (CTES, HUEFS, WIS, ALCB, CUVC). 20 km NO de Virasoro, ruta 38, *Norrmann 71*, 3.iii.1982 (CTES, ANSM). 17 km S de Santo Tomé, ruta 40, *Norrmann 72*, 3.iii.1982 (CTES, MICH, BAA, BAB). Bolivia, Dep. Sta Cruz, 1 km E of Intern. Airport Viru Viru, *Killeen 1550*, 1.i.1986 (CTES, MO).

Specimens in herbaria: Types: Brazil: Habitat in Brasilia australi, *Sellow 107* (US, K). Brasiliae, in campis siccis fejuco, *Lansgsdorff s/n* (LE, as var. *brevis*). Paraguay: Caaguazú, sur les collines incultes, *Balansa 226*, 19.xi.1874 (P, K, G, L, SI, as var. *trichocoleus*). Assumption, sur les collines incultes, *Balansa 227*, 5.iii.1875 (K, G, L, P, as *A. incanus*). Idem, *Balansa 229*, 16.ii.1877 (K, G, L, as var. *ramosissimus*). Uruguay: Montevideo, *Sellow* (W) as *A. incanus.*

A. LATERALIS × *A.* BICORNIS = *A.* × SUBTILIOR (HACK.) NORRMANN (*PRO. SP.*)

Living material: Argentina: Provincia de Corrientes, Ciudad de Corrientes, *Norrmann 142*, 1.vi.1996 (CTES, MEXU, BAB, US, SI). 36 km E de Ituzaingó, *Norrmann 34*, 29.iii.1982 (CTES, US, BAA, ICN). Provincia de Misiones, 12 km W de Posadas, *Norrmann 108*, 19.ii.1991 (CTES, US). Brazil: Estado de Rio Grande do Sul, entre Porto Alegre y Guaiba, *Norrmann et al. 88*, 28.i.1983 (CTES, US); Estación Experimental Guaiba, *Norrmann et al. 87*, 28.i.1983 (CTES, US); 60 km E de Santa María, *Norrmann et al. 313*, i.1992 (CTES). Paraguay: Dpto Amambay, 5 km N del río Aquidabán, *Norrmann et al. 176* (CTES). Dep. Concepción: 13 km NW de Horqueta a Loreto, *Norrmann et al. 177*, iv.1995 (CTES).

Specimens in herbaria: Types: Paraguay: Jenaius in fiche a Costa Pucu, entre le Pirayu et Paraguarí. *Balansa 228*, ii.1877 (K, G, P, L, LE, as *A. lateralis* var. *subtilior*). Other: Dpto. Cordillera, Cerro Tobatí, *Fiebrig 770*, 16.i.1903 (K, G, BAA, P). Brazil: Rio Grande do Sul, *Regnell 1091* (S).

A. HYPOGYNUS HACK.

Living material: Argentina: Provincia de Chaco, Colonia Benitez, *Norrmann 342* (CTES), Provincia de Corrientes, 40 km E de Ituzaingó, *Norrmann 117* (CTES US); 36 km E de Ituzaingó, por ruta 12, *Norrmann 36*, 29.iii.1982 (CTES, US, BAA). Paraguay: Dpto. Itapua, Ruta 1, 6 km E de Gral Delgado, *Norrmann et al.* 223, 20.iv.1995 (CTES, K, G, US).

Specimens in herbaria: Types: Brazil: in paludosis ad Rio Tamanduaté et prope Aracoara, *Riedel 1655* (K). Idem, prope Aracoara, *Riedel 2199* (LE). Idem, in prov. Minarum, *Weddell 1858* (G); Pr. Lagoa

Santa in litore lacus, *Warming 1865* (LE, S, W), in campis Provinciae Piauhiensis, *Martius* (M). Columbia pr. Apiai, *Karsten* (W). Other: Argentina, Provincia del Chaco: Colonia Florencia, SW de Basail, *Schulz 14837*, 23.iii.1965 (CTES). Bolivia: Dpto. La Paz, Prov. Iturralde, Lousita, *Haase 653,* 28.viii.1985 (K).

A. BICORNIS × *A.* HYPOGYNUS = *A.* × MULTIFLORUS RENVOIZE (*PRO. SP.*)

Specimens in herbaria: Types: Bolivia: Dpto. La Paz, Prov. Iturralde, Lousita, *Haase 1*, 28.viii.1985 (K, as *A. multiflorus*). Colombia, prope Apiai, *Karsten* (W LE, as *A. incanus* var. *bogotensis*).

A. HYPOGYNUS × *A.* LATERALIS = *A.* × COLORATUS HACK. (*PRO. SP.*)

Living material: Argentina: Provincia de Santa Fe, Florencia, *Norrmann & Scarel 333*, 25.iv.2001 (CTES). Chaco: Colonia Benitez, *Norrmann 340* (CTES) Corrientes, controlled hybrid between *A. hypogynus* N36 × *A. lateralis* N72, *Norrmann 109*, ii.1991 (CTES). Idem, plant 2, *Norrmann 110*, ii.1991 (US, SI, BAB, LIL).

Specimens in herbaria: Types: Argentina, Provincia de Chaco, Colonia Benitez, leg. Nic. Rojas Acosta 2.ix.1909 *Stuckert 20275* (K, W, CORD, L, as *A. coloratus*). Other: Argentina Provincia de Corrientes: Ea. Las Tres Marías, flooded land by the Paraná, *Pedersen 8095*, 15.iii.1967 (CTES). Rincón de Sta. María, Ea. Abelenda, *Carnevali 506*, 9.vii.1955 (CTES). Paso de la Patria, Costa Toledo. *Meyer 9032*, 25.iii.1945 (LIL); Prov. del Chaco: Resistencia, *Parodi 8262*, 21.i.1928 (BAA). Provincia de Santa Fe: Villa Guillermina, *Meyer 2890*, 25.iii.1939 (LIL, BAA). Villa Ocampo, *Pire 739*, 17.xii.1980 (CTES). San Justo, *Ragonese* 2447, 2.i.1937 (K). Reconquista, Colonia Vanguardia, *Calot 126*, v.1876 (P). In between Fives Lille and Desvio Km 167, *Castellanos 18458*, 4.i.1937 (M).

A. ARENARIUS HACK.

Living material: Brazil: Estado de Rio Grande do Sul, Capão da Canoa, *Norrmann 104*, iii.1992 (CTES, CEN, US, BAA). Estado de Santa Catarina, Laguna, *Norrmann 224*, 2.i.1994 (CTES, MERL, MBM, US). Itapirubá, *Norrmann 139*, 2.i.1994 (K, CTES, MBM, SI).

Specimens in herbaria: Types: Brazil: Río Grande do Sul, Ilha dos Marinheiros prope oppidum Río Grande, *Regnell A. 699* (W S). Uruguay: Montevideo, in arenosis, *Arechavaleta 204* (K W) Other: Brazil: Rio Grande do Sul, Yunnccao ad opp Rio Grande do Sul, *Regnell 1589* (S). Osorio, praia de Atlántida, *Valls 1468*, 2.ii.1974 (CTES, ICN). Uruguay: Dpto. Rocha, médanos de Santa Teresa, *Burkart 21530*, 20.ii.1960 (K). Dpto. Canelones, Ayo Sarandi, cerca Costa Azul, *Rosengurtt B-6518*, 26.ii.1956 (K). Playa Sta. Rosa, *Berro 7951*, 3.iii.1915 (K).

A. LATERALIS × *A.* ARENARIUS = *A.* × LINDMANII HACK. (*PRO. SP.*)

Living material: Brazil: Estado de Santa Catarina, Itapiruba, *Norrmann 327, 328, 329, 330* (CTES).

Specimens in herbaria: Types: Brazil: Brasilia australi, Rio Grande, Exp. I. *Regnell, A. 855* (S W, as *A. lindmanii*). Other: Brazil: Rio Grande do Sul, Osório, Balneario Arroio do Sal, *Valls 3296* (ICN). Balneario Xangri-la, *Valls 3286* (ICN). Uruguay: Dpto. Canelones, Playa de Sta. Rosa, *Berro 7796*, 12.xi.1915 (K). Dpto. Rocha, Parque Nacional S. Teresa, *Rosengurtt 10835*, 6.ii.1967 (K, BAA). Dpto. Canelones, Ayo Sarandi, cerca de Costa Azul, *Rosengurtt 6518 b*, 26.ii.1956 (K).

A. BICORNIS × *A.* ARENARIUS

Living material: Brazil, Estado de Santa Catarina: Itapiruba, *Norrmann 331, 332* (CTES).