Cross-Ability in the genus *Lachenalia*

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Abstract

***Lachenalia* is a bulbous genus endemic to southern Africa. The genus has been utilized in a breeding program with the aim to develop a new pot plant product for the international floriculture market. The genus has approximately 120 described species and is unusually variable. The variation in terms of flower-form, -colour, -length and -posture opens up a range of possibilities in terms of pot plant types as well as cut flower potential. The extent of the variation, however, also causes several natural crossing barriers influencing the cross-ability among species. The genus is just as varied in chromosome number as in phenotype. The basic chromosome numbers present in the genus are x=5, 6, 7, 8, 9, 10, 11 and 13. Ploidy levels range from diploid to octoploid and polyploidy is present in several species. A large number of interspecies crosses have been made. The results of these and the implication on the cross-ability of different species are discussed. Cross-ability between species with the same basic chromosome number is fairly successful. Cross-ability between species with different basic chromosome numbers is, however, low. The crossing data are compared to results from studies on the phylogeny of the genus as determined using sequencing of the transfer RNA intergenic spacer (trnL-F) sequencing and chromosome numbers. The same tendency can be observed if the inter-species crosses are linked to the phylogenetic groups identified within the genus. Within groups cross-ability is fairly successful, whilst between groups cross-ability is low. The crossing data thus, in most cases, correlate with the phylogenetic data. Where discrepancies occur further phylogenetic analysis is required.**

INTRODUCTION

Lachenalia J.Jacq. ex Murray is a bulbous genus endemic to southern Africa. The genus has been utilized in a breeding program at the Agricultural Research Council's Vegetable and Ornamental Plant Institute (ARC-VOPI) with the aim to develop a new pot plant product for the international floriculture market. The genus has approximately 120 described species (Duncan, 2005) and is unusually variable (Kleynhans, 2006). The extent of the variation, however, also causes several natural crossing barriers influencing cross-ability among species.

External and internal crossing barriers exist (Lubbinge, 1980 and Kleynhans, 2006). The external barriers can be easily overcome by growing the plants in controlled conditions and the successful storage of pollen for a 12-month period (Kleynhans, 2006). Stored pollen is used to overcome the diverse flowering times (April – Nov) of the species in the genus. The internal barriers have not been studied in detail.

Closely linked to the internal barriers is the remarkable variability of chromosome numbers found in the genus. Somatic chromosome numbers ranging from 2n=10 to 2n=56 have been reported in the literature (Moffett, 1936; Sato, 1942; Therman, 1956; De Wet, 1957; Fernandes and Neves, 1962; Riley, 1962; Mogford, 1978; Ornduff and Watters, 1978; Nordenstam, 1982; Crosby, 1986; Hancke and Liebenberg, 1990; Hancke, 1991; Johnson and Brandham, 1997; Kleynhans, 1997; Hamatani et al., 1998; Hancke and Liebenberg, 1998; Kleynhans and Spies, 1999; Spies et al., 2000; Du Preez et al., 2002; Spies et al., 2002; Van Rooyen et al., 2002; Hamatami et al., 2004). The basic

chromosome numbers of x=7 or 8 are the most frequent, but x=5, 9, 10, 11, 12, 13 and 15 have also been reported (Ornduff and Watters, 1978; Johnson and Brandham, 1997; Hancke et al., 2001). Polyploidy is also fairly common in the genus (Johnson and Brandham, 1997; Kleynhans and Spies, 1999; Spies et al., 2000; Spies et al., 2002). Polyploidy seems to be more common in species with x=7 as basic chromosome number (Spies et al., 2002).

To assess the genomic variability, studies were initiated to determine the phylogeny of the genus (Minnaar, 2004 and Spies, 2004). The trnL-F region was sequenced and phylogenetic analysis was done (Spies et al., 2002). From the initial results it was clear that none of the sub-generic taxonomic systems applied (Barker, 1897, Crosby, 1986; Duncan, 1988) conformed to the natural phylogeny of the genus.

The first inter-species crosses of the genus, in the ARC-VOPI breeding programme, were made in 1968. Since then approximately 25 cultivars have been released and hundreds of crossing combinations have been made. The results of the inter-species crosses are used to indicate the cross-ability among species. The cross-ability is linked to the phylogeny of the genus (Spies, 2004) as determined using transfer RNA intergenic spacer (trnL-F) sequencing and chromosome numbers.

MATERIALS AND METHODS

Crosses are made via hand pollinations after emasculation of flowers (Lubbinge, 1980). Emasculation takes place one or two days before anther dehiscence. Anther dehiscence varies from species to species. The correct time of emasculation, thus has to be determined for each species. Even ecotypes within a species might differ with regard to time of dehiscence (Kleynhans, 2006).

Anthers are collected in gelatine capsules and left in a desiccator overnight to dehisce. Capsules are then closed and stored in tightly sealed glass bottles in the refrigerator at -4^0C. Storage of pollen is important because of the diverse flowering times (April-November) among the species (Duncan, 1988). Pollen germination tests are done by the hanging drop technique with 10% sucrose and 0.01% boric acid (Hancke and Liebenberg, 1998).

Stigmas are receptive from one to seven days after anthesis (defined as anther dehiscence for this discussion). In trials performed on six species (*L. aloides* (L.f.) Engl., *L. bulbifera* (Cirillo) Engl., *L. liliflora* Jacq., *L. mutabilis* Sweet, *L. rubida* Jacq., and *L. unicolor* Jacq.) the optimum period of receptiveness varied greatly (Kleynhans, 2006). According to these results the optimal time for pollination was three to five days after emasculation.

RESULTS AND DISCUSSION

More than 1498 inter-species crosses were made at ARC-VOPI from 1974 to 2005 (Table 1). The chromosome numbers of all the accessions used in the crosses are not available. The basic chromosome numbers reported in the literature, and as used by Spies (2004), were used to assess the cross-ability between groups with similar or different basic chromosome numbers. Cross-ability was determined as the percentage of successful crosses made. Seventy-seven percent of all inter-species crosses made at ARC-VOPI were unsuccessful, either because no seed (possible pre-fertilisation barrier) or non-viable seed (possible post-fertilisation barrier) were formed (Table 1). The processes causing these failures need further investigation.

The success rate is higher when crosses are made between species with a similar basic chromosome number (Table 1). Few crosses were made between species with a basic chromosome number of 11 and 13, thus making it difficult to determine the cross-ability between these species. The cross-ability between species with a basic chromosome number of 8 was more than 50% (Table 1). The cross-ability between species with different basic chromosome numbers were all below 25%, except for the x=11 crossed with the x=10 group. Only two crosses were made within this group, which is too small a sample to assess on the cross-ability.

Within the basic chromosome group of x=7 the cross-ability was also fairly low (31%). The highest ploidy is however, found within the basic x=7 group (Spies, 2004). Approximately 15 tetraploid taxa, as well as hexaploids and octoploids, are found in this group (Kleynhans and Spies, 1999 and Spies; 2004). This fact complicates the understanding of crosses within the basic x=7 group. Most inter-specific crosses (66%) where polyploid *L. bulbifera* plants were used as the pollen parent were unsuccessful because of the production of abnormal or non-viable seed. The crossing barrier is, thus at the post fertilisation stage, and reduced hybrid viability is most probably caused by disharmony either between the parental sets of chromosomes or between the developing embryo and endosperm. The specific chromosome numbers and ploidy level of many of the specific accessions used in these crosses are unknown, making it difficult to equate ploidy level with compatibility.

Spies (2004) identified four distinct groups within the Adams consensus cladogram determined from the trnL-F sequencing data. These groups were provisionally named the *L. juncifolia* group, the *Lachenalia* 1 group, the *Lachenalia* 2 group and the *L. zebrina* group (Fig. 1). The data from the interspecies crosses were divided between the four groups to see what the success rate between these groups was (Table 2). The numbers in Table 2 do not correlate with those in Table 1, since not all of the species were included in the phylogenetic analysis. The success rate is higher when species within a provisional group are crossed with each other (Table 2). The success rate dropped from above 25% to below 15% when crosses between species from different groups were attempted. Most of the inter-species crosses made were between species within the *Lachenalia* 1 group.

Within the *Lachenalia* 1 group, six sub-groups could be identified (Spies, 2004). These are the *L. pallida*, *L. elegans, L. violaceae, L. rubida, L. bulbifera* and *L. mathewsii* sub-groups (Fig. 2). The crossing data of species in the group was linked to these sub-groups to indicate the cross-ability. For this purpose the two sub-groups (sister groups in the cladogram) *L. bulbifera* and *L. rubida* (consisting of a single species) were combined. Sixty one percent of all crosses made between these two species succeeded despite the fact that in 98% of these crosses, a polyploidy *L. bulbifera* accession was used.

The within group cross-ability was again higher than the between group cross-ability, with success rates above 50%. The only exception was the *L. mathewsii* within group success rate. The *L. bulbifera* x *L. mathewsii* (sister groups) between group success rate was high, indicating a stronger relationship between the species of these two groups. This stresses the fact that an additional gene should be sequenced to increase the resolution of the different groupings and species. The lower success rate when the *L. mathewsii* within group cross-ability is viewed can be ascribed to the presence of polyploids as well as to the external crossing barrier of flower size. Lubbinge (1980) was first to describe this mechanical isolation. Large flowered species of *Lachenalia* have flowers of over 25mm long, whilst in smaller flowered species the flower length can be less than 10mm. Pollen tubes from small flowered species is, thus not adapted to traverse the long distance from the stigma to the ovary of large flowered species (Stebbins, 1950).

CONCLUSIONS

This study indicates a correlation between the cross-ability of *Lachenalia* species and their basic chromosome numbers, as well as their phylogenetic relationships. In general, the inter-species cross-ability, as determined by crossing successes, confirms the phylogenetic groupings identified by Spies (2004). Sequencing another gene to obtain better resolution in the cladogram and making more crosses with species not included in the *Lachenalia* 1 group is needed to confirm these links. A study on crossing barriers may assist breeders to understand the low cross-ability.

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Tables

Table 1. Inter-species crosses made between different *Lachenalia* species. Results are linked to the basic chromosome complement of the species.

Inter-specific cross type – related to basic chromosome numbers	Successful crosses	Unsuccessful – no seed set [a]	Unsuccessful - abnormal seed [b]	Total per crossing type
7 x 7	172 (31%)	227 (41%)	157 (28%)	556
8 x 8	79 (53%)	32 (21%)	38 (26%)	149
7 x 8 / 8 x 7	67 (14%)	249 (50%)	178 (36%)	494
7 x 11 / 11 x 7	13 (24%)	30 (56%)	11 (20%)	54
8 x 11/ 11 x 8	4 (17%)	10 (43%)	9 (40%)	23
11 x 11	1 (50%)	1 (50%)	0	2
11 x 13	0	5 (50%)	5 (50%)	10
7 x 13 / 13 x 7	1 (1%)	46 (50%)	45 (49%)	92
8 x 13 / 13 x 8	1 (3%)	15 (38%)	23 (59%)	39
13 x 13	3 (75%)	0	1 (25%)	4
10 x 7 / 7 x 10	1 (2%)	23 (49%)	23 (49%)	47
10 x 8 / 8 x 10	1 (4%)	13 (50%)	12 (46%)	26
11 x 10	1 (50%)	1 (50%)	0	2
Total per success rate	344 (23%)	652 (44%)	502 (33%)	1498

[a] – possible pre-fertilization barriers
[b] – possible post-fertilization barriers

Table 2. Inter-species crosses made between different *Lachenalia* species. Results are linked to the four phylogenetic groups in the genus as previously identified. Source: Spies (2004).

Inter-specific cross type – related to phylogenetic grouping	Successful crosses	Unsuccessful – no seed set [a]	Unsuccessful - abnormal seed [b]	Total per crossing type
L. juncifolia group X *Lachenalia* 1 group	3 (4%)	41 (52%)	34 (44%)	78
L. juncifolia group X *Lachenalia* 2 group	1 (14%)	4 (57%)	2 (29%)	7
Lachenalia group 1 X *Lachenalia* 1 group	303 (27%)	475 (41%)	369 (32%)	1147
Lachenalia 1 group X *Lachenalia* 2 group	8 (4%)	86 (46%)	95 (50%)	189
Lachenalia 2 group X *Lachenalia* 2 group	3 (33%)	2 (22%)	4 (45%)	9
Total	318	608	504	1430

Table 3. Interspecies crosses made between different *Lachenalia* species. Results are linked to the phylogenetic sub-groups within the *Lachenalia* 1 group in the genus as previously identified. Source: Spies (2004).

Inter-specific cross type – related to phylogenetic grouping: sub groups within the *Lachenalia* 1 group	Successful crosses	Unsuccessful – no seed set [a]	Unsuccessful - abnormal seed [b]	Total per crossing type
L. pallida sub group X *L. pallida* sub group	78 (53%)	32 (22%)	38 (25%)	148
L. pallida sub group X *L. elegans* sub group	1 (10%)	6 (60%)	3 (30%)	10
L. pallida sub group X *L. bulbifera* sub group	16 (11%)	83 (56%)	50 (33%)	149
L. pallida sub group X *L. mathewsii* sub group	47 (15%)	112 (36%)	155 (49%)	314
L. elegans sub group X *L. bulbifera* sub group	0	9 (75%)	3 (25%)	12
L. elegans sub group X *L. mathewsii* sub group	0	27 (87%)	4 (13%)	31
L. bulbifera sub group X *L. bulbifera* sub group	37 (61%)	13 (22%)	10 (17%)	60
L. bulbifera sub group X *L. mathewsii* sub group	83 (31%)	84 (32%)	99 (37%)	266
L. mathewsii sub group X *L. mathewsii* sub group	47 (30%)	76 (48%)	34 (22%)	157
Total	309	442	396	1147

Figures

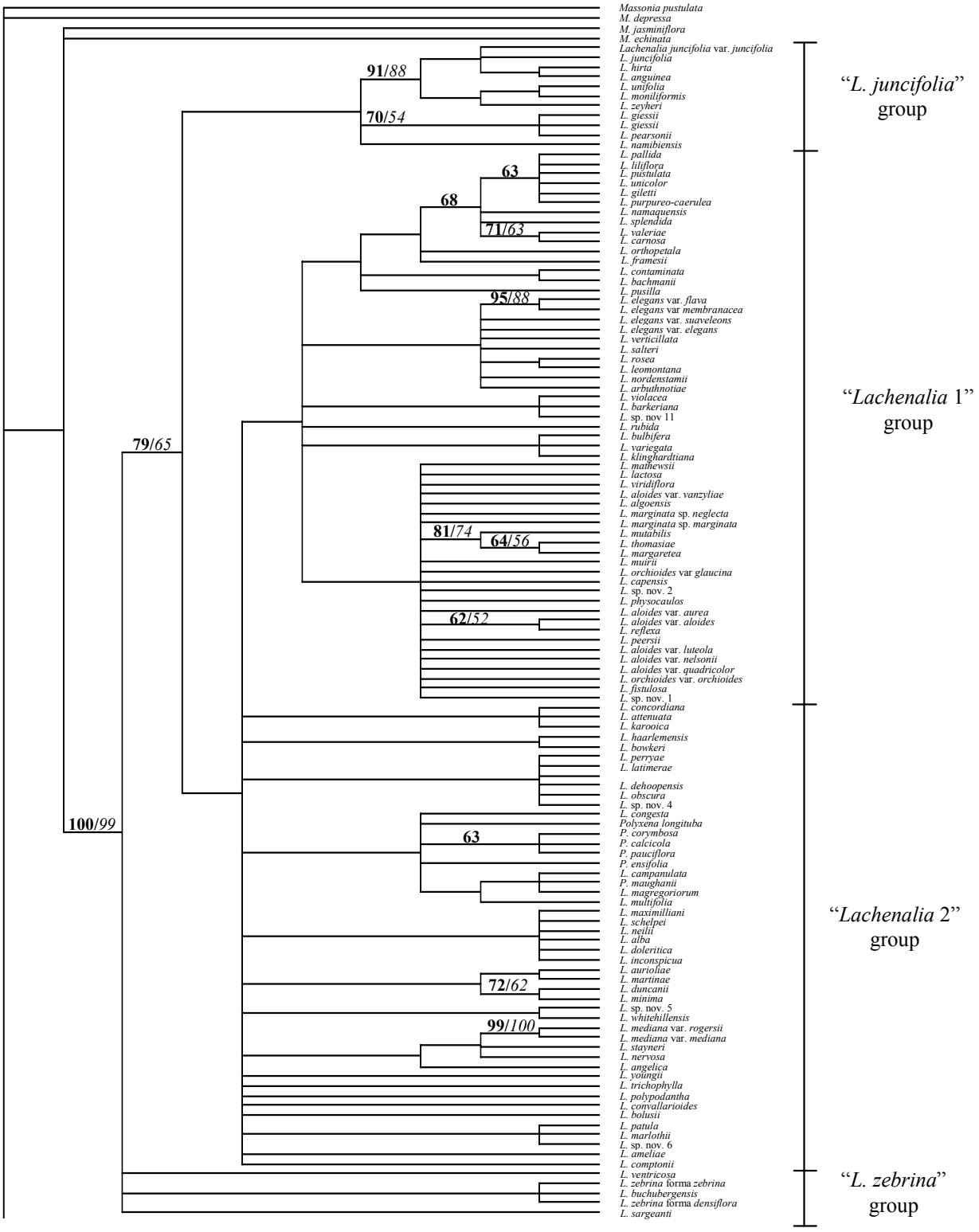

Fig. 1. The Adams consensus cladogram of the genera *Lachenalia, Massonia* and *Polyxena*. Bootstrap (first value in bold) and Jackknife confidence values (second value in italic) greater than 50% are shown. Source: Spies (2004).

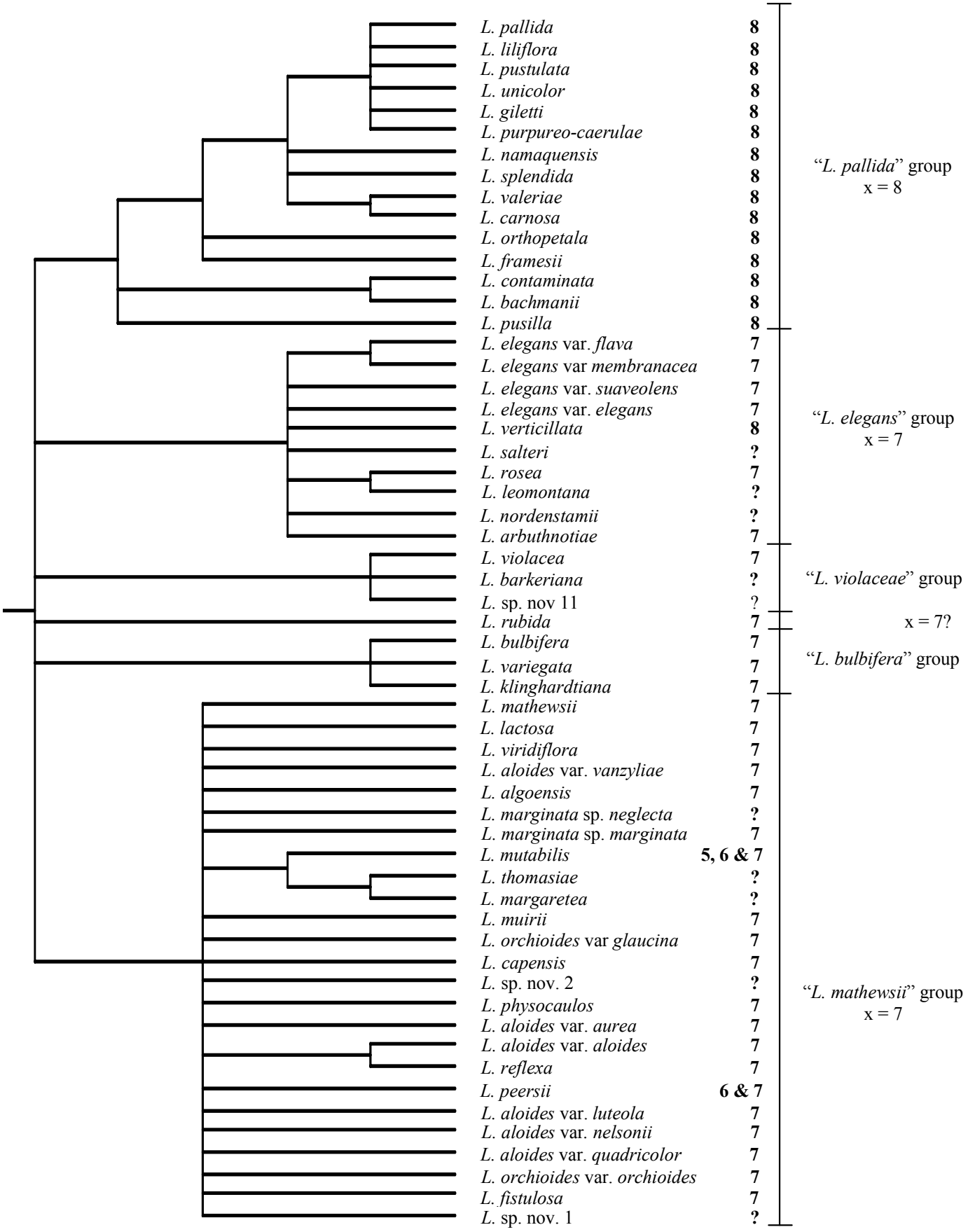

Fig. 2. The *Lachenalia* 1 group from the Adams consensus cladogram with the six sub-groups indicated, as well as their basic chromosome number. Source: Spies (2004).