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Cytogenetic and Phylogenetic Review of the Genus *Lachenalia*

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ABSTRACT

The genus *Lachenalia* (family Asparagaceae), endemic to southern Africa, is a horticultural diverse genus, with many species featuring in the red data list of southern Africa. The extensive morphological variation within some species complicates species delimitation and has led to taxonomic confusion. The genus is utilised in a breeding programme where cytogenetic and phylogenetic information is important for the development of breeding strategies. Chromosome numbers of 89 species have been recorded in literature, with $2n$ = 10 to 56 and n = 5 to 28. B-chromosomes have been described in some species. Basic chromosome numbers include x = 5, 6, 7, 8, 9, (probably 10), 11, (probably 12), 13 and (probably 15). Polyploidy was reported in 19 taxa (23%), and is most common in the x = 7 group. Molecular cytogenetic studies using 5S rDNA, 18S rDNA probes and DAPI staining, as well as molecular systematic studies using *trnL-F* and *ITS1-2* were used to assess the phylogeny of the genus. All these studies indicated that species with the same basic chromosome number are closely related. The one deviation is that it appears as if there are two separate groups within the x = 7 group. The cytogenetic and molecular studies are further supported by breeding studies, where improved results are generally obtained from crosses within a phylogenetic group or between closely related groups. This review of the literature reveals how different studies obtain similar results regarding the phylogenetic relationships within the genus and how these results can be utilized to improve breeding strategies. It also accentuates that further multidisciplinary studies are needed to solve the evolutionary history of the complex genus *Lachenalia*.

Keywords: chromosome numbers, cladograms, cross-ability, phylogeny, polyploidy
Abbreviations: **APG**, Angiosperm Phylogeny Group; ***atpB***, ATPase beta chain; **DAPI**, 4',6-diamidino-2-phenylindole; **FISH**, Fluorescent *in situ* hybridization; ***ITS1-2***, Internal transcribed spacer 1 and 2; **MEGA**, Molecular Evolutionary Genetics Analysis; ***n***, gametic chromosome number; **RAPD**, Random amplified polymorphic DNA; ***rbcL*** ribulose bisophosphate carboxylase (large); **SANBI**, South African National Biodiversity Institute; ***trnL***, leucyl-transfer RNA intron; ***trnF***, phenylalanine-transfer RNA; **VOPI**, Vegetable and Ornamental Plant Institute; ***x***, basic chromosome number; **2*n***, somatic chromosome number; **5S rDNA** and **18S rDNA**, 5S and 18S ribosomal DNA

CONTENTS

INTRODUCTION

The genus *Lachenalia* Jacq. *f.* ex Murray, previously a member of the family Hyacinthaceae (Manning *et al*. 2004; Duncan and Edwards 2006, 2007), but since 2009 reclassified under the family Asparagaceae Juss. (APG III group 2009), is endemic to southern Africa. The genus now also includes the former genus *Polyxena* (Manning *et al*. 2004). *Lachenalia* is a horticultural diverse genus, with a distribution range extending from the south-western coast of Namibia, southward throughout the Northern, Western and Eastern Cape provinces of South Africa (Duncan 1998). One species extends as far inland as the south western part of the Free State Province (Duncan 1996). Of the 126 species and

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Fig. 1 Morphological variation in *Lachenalia* in the greenhouse.

subspecies described, 10% are endangered, 17% are vulnerable, 2% are considered to be near threatened, 6% are critically rare, 9% are rare and 2% are declining (SANBI 2009).

The genus is geophytic, deciduous and is usually winter growing. The centre of diversity is in the Worcester grid (3319) in the Western Cape province of South Africa, with species diversity decreasing toward the eastern and northern parts of its range (Duncan 2005). Although *Lachenalia* species like *L. bulbifera* and *L. obscura* are widely distributed, a substantial number of species (e.g. *L. moniliformis*, *L. mathewsii*) have a restricted distribution, contributing to the vulnerability of these species (Duncan 1998).

Lachenalia occurs in a wide range of habitats, ranging from arid to high rainfall areas. *Lachenalia rubida* for example always grows in deep, pure sand often very close to the sea, whilst a species like *L. campanulata* on the other hand is found in heavy soil at altitudes exceeding 2000 metres (Duncan 1998). Between these two extremes, there is a multitude of other habitats, including humus-rich soil on granite, mineral rich soil, barren stony flats, limestone outcrops and seasonally inundated, heavy clays (Duncan 1998).

The morphological diversity within the genus is well known (**Fig. 1**). Variation occur in several morphological characters, such as plant size, leaf number and posture, flower-size, -colour and -orientation and flowering period (**Fig. 2**). The extensive morphological variation within some species complicates species delimitation and has led to considerable taxonomic confusion (Duncan 1992). Several attempts have thus been made to establish some sub-generic classification within this complex genus, starting with the work by Baker (1897), who divided the genus into five sub-genera based on morphology. The first cytogenetic work by Moffett (1936), however, already indicated that true relationships cut across the groups of Baker and this has been confirmed by various studies (Crosby 1986; Spies 2004; Hamatani *et al*. 2009; amongst others).

Due to the extensive morphological diversity in colour and appearance, collectors have recognized the horticultural potential of the genus for centuries (Duncan 1988; Du Plessis and Duncan 1989; Kleynhans 2009, 2011; Reinten *et al*. 2012). The huge phenotypic variation was also the most important reason for the initiation of a breeding programme at the Agricultural Research Council in South Africa. This led to the production of various hybrids and the introduction of new products to the international pot plant market (**Fig. 3**) (Kleynhans 2006).

The variability of the genus in terms of morphology and cytogenetics, however, lead to specific challenges for the breeding of new cultivars. Both incompatibility and other isolation barriers exists (Kleynhans and Hancke 2002). A large number of inter-species crosses are unsuccessful (Kleynhans *et al*. 2009) and future breeding progress is dependent on information about the genetic variation in the genus. Results generated from cytogenetic and phylogenetic research has value for the breeding programme (Kleynhans *et al*. 2009) and can furthermore assist in the classification and delimitation of species (Crosby 1986; Spies *et al*. 2002).

This paper reviews the current information available on cytogenetics and phylogeny for the genus *Lachenalia* and correlates this information to breeding results on cross-ability with the aim to draw some conclusions on relationships among the different species within the genus.

CYTOGENETIC STUDIES

Chromosome counts

Lachenalia is unusually variable in chromosome number with the presence of different basic chromosome numbers (Moffett 1936; Crosby 1986; Johnson and Brandham 1997), polyploidy (Kleynhans and Spies 1999) and B-chromosomes (Hancke and Liebenberg 1990; Johnson and Brandham 1997). The first cytogenetic studies on the genus came from Moffett (1936). Chromosome numbers steadily increased over many years with information coming from various authors (**Table 1**). Currently the chromosome num-

Fig. 2 Morphological variation in different *Lachenalia* species. (**A**) *L. aloides*; (**B**) *L. carnosa*; (**C**) *L. splendida*; (**D**) *L. bulbifera*; (**E**) *L. longibracteata*; (**F**) *L. violacea*; (**G**) *L. contaminata*; (**H**) *L. pustulata*.

bers of 89 species have been recorded in literature. Somatic chromosome numbers vary from 10 to 56 and gametic numbers from 5 to 28.

The cytogenetics is further complicated by varying chromosome number reports for a number of species (**Table 1**). Deviating chromosome counts can first of all be explained by suspected wrong identification of species. In the species *L. orchioides* the variation could most probably be ascribed to accessions being wrongly identified. Crosby (1986) reported that he received both *L. fistulosa* and *L. pustulata* under the name of *L. orchioides*. Schlechter also identified an accession of *L. pallida* as *L. orchioides* (Barker 1983). Both *L. pallida* and *L. pustulata* have chromosome numbers of $2n = 16$ which could explain some of the variation reported for *L. orchioides*. *Lachenalia contaminata* similarly has both $2n = 14$ and $2n = 16$ reported in literature (**Table 1**). Gouws (1965) was the first to report both these numbers. The author, however, described these two numbers in one specific bulb of *L. contaminata* exhibiting cells with both $2n = 14$ and $2n = 16$. In this case the $2n = 16$ could be B-chromosomes that was not identified. Most other chromosome counts of this species, except two by Spies *et al*. (2008, 2009), are $2n = 16$. In this species the variation is not a case of mistaken identity and further investigation is needed to explain the variation.

The small size of the chromosomes (Hancke and Liebenberg 1990; Spies *et al*. 2000) in the genus can furthermore contribute to miscounts and possible miss-identification of B-chromosomes. The presence of B-chromosomes in *Lachenalia* was described by Hancke and Liebenberg (1990). According to the authors, B-chromosomes in *Lachenalia* do not have a specific staining pattern and are similar in size to the smallest chromosome in the normal complement. This behaviour makes them difficult to identify and therefore could explain some erroneous counts, reported in literature. B-chromosomes in *Lachenalia* do not occur in all cells of a specific individual and also not in all plants of a specific accession (Hancke and Liebenberg 1990). It is thus important to investigate the chromosome number of several individuals from a specific population to have accurate chromosome counts and correctly identify the presence of B-chromosomes. Counting insufficient number of cells can similarly lead to miscounts due to chromosome damage occurring during slide preparation.

B-chromosomes have been reported in eight species, namely *L. aloides*, *L. anguinea*, *L. bulbifera*, *L. carnosa*, *L. contaminata*, *L. obscura*, *L. reflexa* and *L. splendida* (Crosby 1986; Hancke and Liebenberg 1990; Johnson and Brandham 1997; Kleynhans and Spies 1999; Spies *et al*. 2009). Hamatani *et al*. (1998) also reported an expected B-

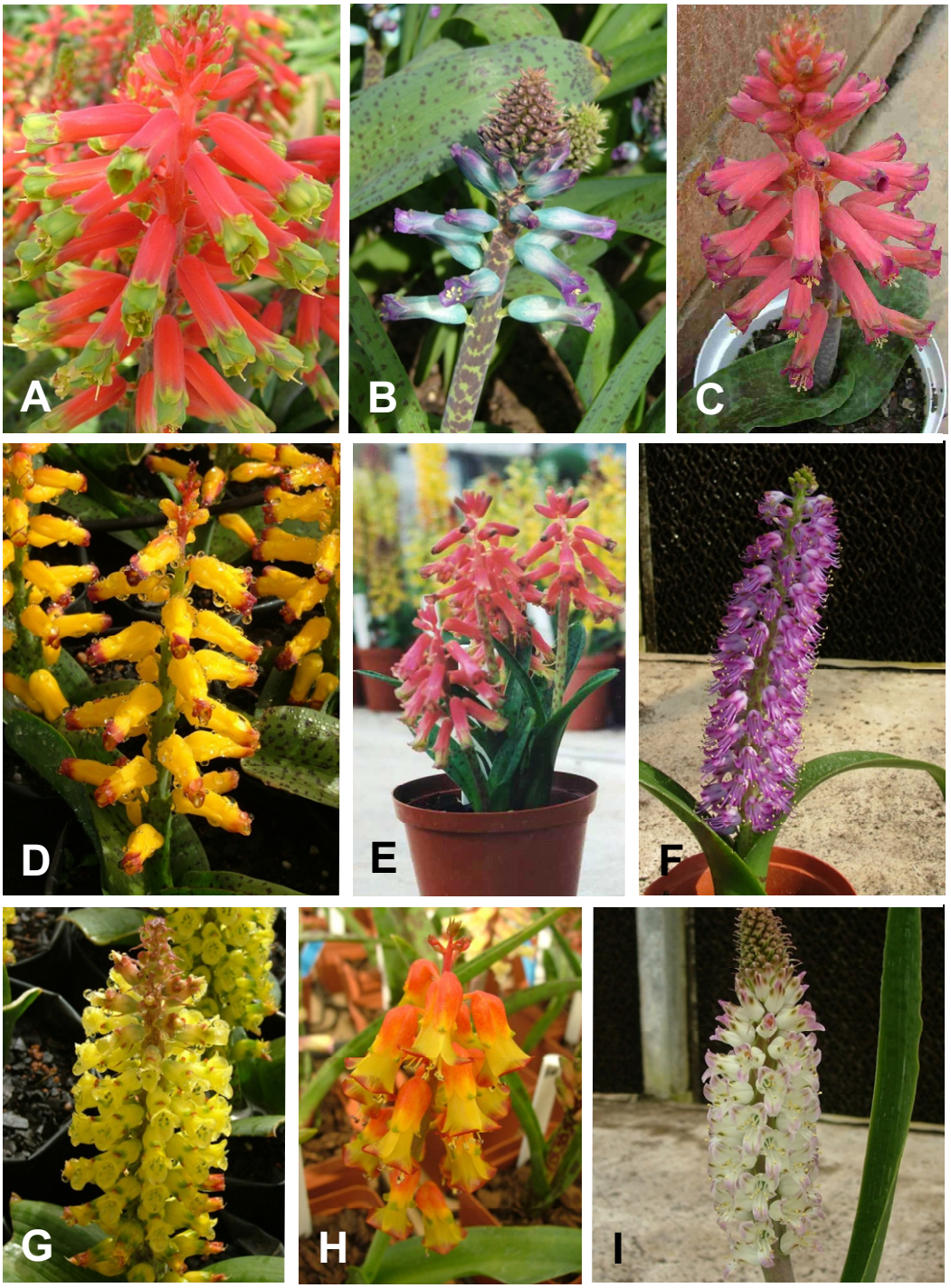

Fig. 3 Different *Lachenalia* cultivars developed at ARC - Roodeplaat VOPI. (**A**) 'Rosabeth'; (**B**) 'Aqua Lady'; (**C**) 'Cherise'; (**D**) 'Namakwa'; (**E**) *L. bulbifera* x *L. rubida*; (**F**) *L. unicolor* x *L. splendida*; (**G**) 'Romaud'; (**H**) 'Rainbow Bells'; (**I**) *L. bachmannii* x *L. carnosa.*

chromosome in a $2n = 23$ accession of *L. zeyheri*. Another example where possible B-chromosomes have not been identified, can be found in *L. barkeriana* where both $2n = 14$ and $2n = 16$ was reported (**Table 1**). The $2n = 16$ was, however, only found in one cell (Müller-Doblies *et al.* 1987) of an otherwise $2n = 14$ accession and could most possibly be ascribed to extra chromosomes.

Chromosome morphology

The chromosome morphology of *Lachenalia* has been described in various reports (Moffett 1936; De Wet 1957; Hamatani *et al.* 1998; Hancke and Liebenberg 1998; Hancke *et al.* 2001; Hamatani *et al.* 2004, 2007, 2009, 2010). Both Moffett (1936) and Hamatani *et al.* (1998, 2004, 2007) attempted to group the species of the genus based on chromosome length and basic chromosome number. The groupings by Moffett (1936) and Hamatani *et al.* (1998) agreed, except for the division of the first group of Moffett into two separate groups by Hamatani *et al.* (1998). Further studies by Hamatani *et al.* (2004, 2007) added four groups based on chromosome numbers and varying numbers of larger chromosomes within specific basic chromosome numbers.

Idiograms presented by De Wet (1957) do not agree with karyograms by Moffett (1936) or Hamatani *et al.* (1998, 2004, 2007). Neither does it agree with idiograms presented by Hancke *et al.* (1998, 2001) and Hamatani *et al.* (2009). The idiogram for *L. aloides* presented by Hancke *et al.* (2001) agrees with Moffet's division, but differs from the karyograms of Hamatani *et al.* (1998, 2004, 2007) in having 6 longer chromosomes and not only 2 long chromo-

Table 1 List of *Lachenalia* species with the somatic- and gametic chromosome numbers reported in literature. Number in brackets (#) indicates number of accessions for which the specific somatic or meiotic number was reported. All numbers were reported in the table under the current accepted botanical name. Aneuploidy and other abnormalities or specific detail around polyploidy are indicated with superscripts.

Species	Somatic no. (#)	Gametic no. (#)	Reference
L. alba W.F. Barker ex G. D. Duncan	18 (1), 20 (3), 20/40 (1)		Johnson and Brandham 1997
L. algoensis Schönland	14 (4)		Crosby 1986; Hamatani *et al.* 2007; Spies *et al.* 2008, 2009
		7 (1)	Ornduff and Watters 1978
	21 (1)		Hancke 1991
L. aloides (L.f.) Engl.	14 (32)+0-1B		Moffett 1936; Therman 1956; De Wet 1957; Mogford 1978; Crosby 1986; Hancke and Liebenberg 1990; Hancke 1991; Johnson and Brandham 1997; Hamatani *et al.* 1998, 2004, 2007; Spies *et al.* 2008; Hamatani *et al.* 2009; Spies *et al.* 2009
		7 (6)	Hancke and Liebenberg 1998; Moffett 1936
	15 (1)[1]		Crosby 1986
	21 (2)[1]		Moffett 1936; Crosby 1986
	28 (7)		Crosby 1986; Hancke and Liebenberg 1990; Hamatani *et al.* 1998; Spies *et al.* 2009; Hamatani *et al.* 2010
		14 (1)	Ornduff and Watters 1978
L. ameliae W.F. Barker	18 (2)		Johnson and Brandham 1997
L. anguinea Sweet	30 (1)+2B		Johnson and Brandham 1997
L. arbuthnothiae W.F. Barker	14 (6)		Crosby 1986; Johnson and Brandham 1997; Hamatani *et al.* 1998; Spies *et al.* 2008, 2009
		7 (1)	Spies *et al.* 2009
L. attenuata W.F. Barker ex G.D. Duncan	14 (1)		Spies *et al.* 2009
L. bachmannii Baker	16 (5)		De Wet 1957; Crosby 1986; Johnson and Brandham 1997; Hamatani *et al.* 2004
L. barkeriana U. Müller-Doblies *et al.*	14 (3)		Müller-Doblies *et al.* 1987
	16 (2)		Nordenstam 1982; Müller-Doblies *et al.* 1987
L. bolusii W.F. Barker	18 (1)		Spies *et al.* 2009
L. bowkeri Baker	16 (1)		Dold and Philipson 1998
L. bulbifera (Cyrillo) Engl.	14 (1)		Crosby 1986
	28 (7)		Kleynhans and Spies 1999; Spies *et al.* 2009
		14 (1)	Ornduff and Watters 1978
	42 (15)+0-1B[1]		Moffett 1936[c]; Crosby 1986; Johnson and Brandham 1997; Hamatani *et al.* 1998; Kleynhans and Spies 1999; Spies *et al.* 2008
	49 (1)		Kleynhans and Spies 1999
	56 (5)		Crosby 1986; Johnson and Brandham 1997; Kleynhans and Spies 1999
L. capensis W.F. Barker	16 (1)		Hamatani *et al.* 1998
	28 (2)		Johnson and Brandham 1997; Spies *et al.* 2008
L. carnosa Baker	16 (26)		Crosby 1986; Johnson and Brandham 1997; Hamatani *et al.* 1998; Du Preez *et al.* 2002; Spies *et al.* 2008; Hamatani *et al.* 2009; Spies *et al.* 2009
		8 (1)+0-2B	Spies *et al.* 2009
L. cernua G.D. Duncan	28 (1)		Spies *et al.* 2008
L. comptonii W.F. Barker	20 (5)		Crosby 1986; Johnson and Brandham 1997; Spies *et al.* 2009
		10 (1)	Spies 2004
	c26 (1)		Crosby 1986
L. concordiana Schltr. Ex W.F. Barker	14 (1)		Spies *et al.* 2008
L. congesta W.F. Barker	26, 28 (1)		Johnson and Brandham 1997
L. contaminata Aiton	14 (3)		Gouws 1965; Spies *et al.* 2008, 2009
	16 (11)+1B		De Wet 1957; Gouws 1965; Crosby 1986; Hancke 1991; Johnson and Brandham 1997; Hamatani *et al.* 2004
		8 (2)	Ornduff and Watters 1978
	32 (1)		Johnson and Brandham 1997
L. convallarioides Baker	30 (1)		Johnson and Brandham 1997
L. doleritica G.D. Duncan	18 (2)		Spies *et al.* 2008, 2009
L. duncanii W.F. Barker	18 (1)		Spies *et al.* 2008
L. elegans W.F. Barker	14 (6)		Moffett 1936; Johnson and Brandham 1997; Spies *et al.* 2009
	28 (12)		Moffett 1936; Crosby 1986; Johnson and Brandham 1997; Spies *et al.* 2009
		14 (9)	Ornduff and Watters 1978; Spies *et al.* 2009
	42 (4)		Johnson and Brandham 1997; Duncan 2001
		21 (2)	Spies *et al.* 2009
	56 (1)		De Wet 1957
		28 (2)	Ornduff and Watters 1978
L. ensifolia (Thunb.) J.C. Manning and Goldblatt	24 (3)		Johnson and Brandham 1997
	26 (2)		Johnson and Brandham 1997; Hamatani *et al.* 2007
L. fistulosa Baker	14 (8)		Johnson and Brandham 1997; Spies *et al.* 2002; Hamatani *et al.* 2004; Spies *et al.* 2009
		7 (2)	Ornduff and Watters 1978
	28 (1)		Spies *et al.* 2008
L. framesii W.F. Barker	16 (3)		Du Preez *et al.* 2002; Spies *et al.* 2008
L. giessii W.F. Barker	32 (1)		Spies *et al.* 2008
L. gillettii W.F. Barker	16 (1)		Spies *et al.* 2008

Table 1 (Cont.)

Species	Somatic no. (#)	Gametic no. (#)	Reference
L. haarlemensis Fourc.	18 (2)		Johnson and Brandham 1997
L. hirta (Thunb.) Thunb.		9 (1)	Ornduff and Watters 1978
	22 (6)		Johnson and Brandham 1997; Van Rooyen *et al.* 2002; Hamatani *et al.* 2004; Spies *et al.* 2009
		11 (2)	Ornduff and Watters 1978
	24 (3)		De Wet 1957; Hancke 1991; Johnson and Brandham 1997
L. inconspicua G.D. Duncan	18 (1)		Spies *et al.* 2008
L. isopetala Jacq.	30 (2)		Johnson and Brandham 1997
	40 (1)		Spies *et al.* 2008
L. juncifolia Baker	22 (9)		Johnson and Brandham 1997; Hamatani *et al.* 2007; Spies *et al.* 2008, 2009; Hamatami *et al*. 2010
		11 (1)	Ornduff and Watters 1978
L. karooica W.F. Barker ex G.D. Duncan	16 (1)		Duncan 1996
L. klinghardtiana Dinter	14 (2)		Spies *et al.* 2008
L. kliprandensis W.F. Barker	16 (1)		Johnson and Brandham 1997
L. lactosa G.D. Duncan	14 (1)		Spies *et al.* 2008
L. latimerae W.F. Barker	14 (1)		Spies *et al*. 2008
	18 (2)		Hamatani *et al*. 2007, 2010
L. leomontana W.F. Barker	14 (1)		Spies *et al.* 2008
L. liliflora Jacq.	16 (7)		Moffett 1936; De Wet 1957; Hancke 1991; Johnson and Brandham 1997; Hamatani *et al.* 1998, 2009; Spies *et al.* 2009
		8 (1)	Moffett 1936
L. longibracteata Phillips	14 (4)		Crosby 1986; Hamatani *et al.* 2007; Spies *et al.* 2008; Hamatani *et al.* 2009
		7 (2)	Ornduff and Watters 1978
L. longituba (A.M. van der Merwe) J.C. Manning and Goldblatt	28 (2)		Hamatani *et al*. 2007, 2010
L. macgregoriorum W.F. Barker	22 (1)		Spies *et al.* 2008
L. margaretae W.F. Barker	14 (1)		Spies *et al.* 2008
L. marginata W.F. Barker	14 (1)		Spies *et al.* 2008
	28 (3)		Johnson and Brandham 1997
	29 (1)		Johnson and Brandham 1997
L. marginata subsp. *neglegta* Schltr. Ex G.D. Duncan	10 (1)		Duncan 1996
L. marlothii W.F. Barker ex G.D. Duncan	14 (1)		Spies *et al.* 2008
L. martinae W.F. Barker	26 (1)		Spies *et al.* 2008
L. mathewsii W.F. Barker	14 (4)		Johnson and Brandham 1997; Hamatani *et al.* 1998; Spies *et al.* 2002, 2008, 2009
L. maximiliani Schltr. Ex W.F. Barker	16 (1)		Spies *et al.* 2009
L. mediana Jacq.	14 (1)		Johnson and Brandham 1997
	18 (2)	9 (2)	Spies *et al.* 2009
	26 (2)		Crosby 1986; Spies *et al.* 2008
		13 (1)	Spies *et al.* 2009
L. minima W.F. Barker	18 (1)		Spies *et al.* 2008
L. moniliformis W.F. Barker	22 (1)		Spies *et al.* 2008
L. muirii W.F. Barker	14 (3)		Johnson and Brandham 1997; Hamatani *et al*. 2007, 2009
L. mutabilis Sweet	10 (6)		Johnson and Brandham 1997
		5 (2)	Ornduff and Watters 1978
	12 (6)		Spies *et al.* 2000, 2009
		6 (2)	Spies *et al.* 2002, 2009
	14 (20)		De Wet 1957; Crosby 1986; Hancke and Liebenberg; 1990; Johnson and Brandham 1997; Hamatani *et al.* 1998; Spies *et al*. 2000, 2009
		7 (5)	Hancke and Liebenberg 1998; Spies *et al,* 2002, 2009
	24 (1)		Spies *et al.* 2000
	56 (1)		De Wet 1957
L. namaquensis Schltr. Ex W.F. Barker	16 (11)		Crosby 1986; Johnson and Brandham 1997; Du Preez *et al.* 2002; Hamatani *et al*. 2007; Spies *et al.* 2008; Hamatani *et al.* 2009; Spies *et al.* 2009
		8 (2)	Spies *et al.* 2009
L. namibiensis W.F. Barker	22 (2)		Spies *et al.* 2008
L. neilii W.F. Barker ex G.D. Duncan	18 (1)		Spies *et al.* 2008
L. nervosa Ker Gawll	16 (2)		Moffett 1936; Spies *et al.* 2008
		8 (1)	Moffett 1936
	24 (2)		Johnson and Brandham 1997; Hamatani *et al.* 2007
L. obscura Schltr. Ex G.D. Duncan	18 (2)+1B,		Johnson and Brandham 1997
	36 (2)		Spies *et al.* 2008
L. orchioides (L.) Aiton	14 (20)		Crosby 1986; Hamatani *et al.* 2007; Spies *et al.* 2008, 2009
		7 (19)	Moffett 1936; Ornduff and Watters 1978; Spies *et al.* 2009
	16 (5)		Moffett 1936; De Wet 1957; Hancke 1991
		8 (1)	Moffett 1936
	17 (1)[1]		Moffett 1936

Table 1 (Cont.)

Species	Somatic no. (#)	Gametic no. (#)	Reference
	18 (1)		Riley 1962
	28 (13)		Moffett 1936; De Wet 1957; Crosby 1986; Johnson and Brandham 1997; Hamatani *et al.* 2007; Spies *et al.* 2008; Hamatami *et al*. 2010
		14 (2)	Moffett 1936; Ornduff and Watters 1978
	24 (1)		Hancke and Liebenberg 1990
	29 (1)		Johnson and Brandham 1997
L. orthopetala Jacq.	16 (5)		Crosby 1986; Johnson and Brandham 1997; Spies *et al*. 2008, 2009
L. pallida Aiton	16 (7)		Moffett 1936; Crosby 1986; Johnson and Brandham 1997; Hamatani *et al.* 1998, 2004; Spies *et al.* 2008, 2009
		8 (3)	Moffett 1936; Ornduff and Watters 1978
L. patula Jacq.	16 (1)		Johnson and Brandham 1997
L. paucifolia (W.F. Barker) J.C. Manning and Goldblatt	26 (3)		Johnson and Brandham 1997; Hamatani *et al.* 2007, 2010
L. peersii Marloth ex W.F. Barker	14 (3)		Johnson and Brandham 1997; Hamatani *et al.* 2004; Spies *et al.* 2009
L. physocaulos W.F. Barker	14 (1)		Spies *et al.* 2008
L. polyphylla Baker	22 (1)		Spies *et al.* 2008
L. purpureo-caerulea Jacq.	16 (4)		Moffett 1936; Johnson and Brandham 1997; Spies *et al.* 2009
		8 (2)	Moffett 1936; Ornduff and Watters 1978
L. pusilla Jacq.	14 (8)		Crosby 1986; Müller-Doblies *et al.* 1987; Johnson and Brandham 1997; Hamatani *et al.* 1998, 2007, 2009
	16 (1)[1]		Nordenstam 1982
	18 (1)		Spies *et al.* 2009
	28 (1)		Hancke 1991
L. pustulata Jacq.	16 (24)		Moffett 1936; Crosby 1986; Johnson and Brandham 1997; Spies *et al.* 2000; Hamatani *et al.* 2004; Spies *et al*. 2008
		8 (2)	Moffett 1936; Ornduff and Watters 1978
	32 (1)[1]		Spies *et al.* 2000
L. reflexa Thunb.	14 (5)+0-2B		Crosby 1986; Hancke and Liebenberg 1990; Johnson and Brandham 1997; Hamatani *et al.* 1998; Spies *et al.* 2009
		7 (1)	Hancke and Liebenberg 1998
	16 (1)		De Wet 1957
L. rosea Andrews	14 (6)		Moffett 1936; Crosby 1986; Hancke 1991; Johnson and Brandham 1997; Hamatani *et al.* 2007; Spies *et al.* 2008
	21 (1)		Crosby 1986
	28 (2)		Spies *et al.* 2009
L. rubida Jacq.	14 (6)		Moffett 1936; Crosby 1986; Hamatani *et al.* 1998, 2009; Spies *et al.* 2009
		7 (1)	Moffett 1936
	28 (1)		Crosby 1986
L. splendida Diels.	16 (8)+2B		Crosby 1986; Johnson and Brandham 1997; Hamatani *et al.* 1998; Du Preez *et al.* 2002; Hamatani *et al*. 2009; Spies *et al.* 2009
		8 (2)	Spies *et al.* 2009
	18 (1)[1]		Crosby 1986
L. stayneri W.F. Barker	24 (1)		Johnson and Brandham 1997
L. thomasiae W.F. Barker ex G. D. Duncan	14 (1)		Spies *et al.* 2008
L. trichophylla Baker	14 (2)		Johnson and Brandham 1997
		7 (1)	Ornduff and Watters 1978
L. undulata Masson ex Bak.	20 (1)		Johnson and Brandham 1997
L. unicolor Jacq.	16 (45)		Moffett 1936; De Wet 1957; Gouws 1965; Crosby 1986; Hancke 1991; Johnson and Brandham 1997; Hamatani *et al.* 1998; Spies *et al*. 2000; Du Preez *et al*. 2002; Hamatani *et al*. 2009
		8 (4)	Moffett 1936; Ornduff and Watters 1978
	32 (1)		Crosby 1986
L. unifolia Jacq.	16 (1)		Hancke 1991
	21 (1)		De Wet 1957
	22 (24)		Moffett 1936; De Wet 1957; Crosby 1986; Johnson and Brandham 1997; Van Rooyen *et al.* 2002; Spies *et al.* 2009
		11 (16)	Moffett 1936; Ornduff and Watters 1978; Spies *et al.* 2009
	24 (2)		De Wet 1957; Hamatani *et al.* 2004
	26 (2)		Moffett 1936; De Wet 1957
	44 (1)		Johnson and Brandham 1997
L. valeriae G.D. Duncan	16 (1)		Spies *et al.* 2008
L. variegata W.F. Barker	14 (2)		Spies *et al* 2008; Hamatani *et al.* 2009
	12 (1)		Hamatani *et al.* 2004
	28 (1)		Spies *et al.* 2002
L. ventricosa Schltr. ex W.F. Barker	14 (1)		Spies *et al.* 2008
L. verticillata W.F. Barker	16 (1)		Crosby 1986
L. violacea Jacq	14 (13)		Hancke 1991; Johnson and Brandham 1997; Hamatani *et al.* 1998
		7 (3)	Ornduff and Watters 1978; Spies *et al.* 2009
	15 (1)		Johnson and Brandham 1997
	16 (1)		Crosby 1986

Table 1 (Cont.)

Species	Somatic no. (#)	Gametic no. (#)	Reference
L. viridiflora W.F. Barker	14 (7)		Nordenstan 1982; Crosby 1986; Hancke and Liebenberg 1990; Hancke 1991; Johnson and Brandham 1997; Spies *et al.* 2002; Hamatani *et al.* 2007, 2009
		7 (1)	Hancke and Liebenberg 1998
L. youngii Baker	16 (1)		Spies *et al.* 2008
L. zebrina W.F. Barker	30 (2)		Johnson and Brandham 1997; Spies *et al* 2008
L. zeyheri Baker	22 (2)		Johnson and Brandham 1997; Spies *et al* 2002
	23 (2)[1]		Hamatani *et al.* 1998, 2010

somes. Idiograms for *L. aloides* and *L. splendida* constructed by Hamatani *et al*. (2009) again correlate with that of Hancke *et al*. (2001).

Spies *et al*. (2000) reported that accessions of *L. mutabilis* contained 4 to 8 very short chromosomes. According to the authors the number of short chromosomes can vary between different localities and even between specimens collected at the same locality. Hamatani *et al*. (2007) furthermore reported on varying karyotypes within the same species for a number of *Lachenalia* species. This reported variation and conflicting results thus indicate that karyomorphological data alone cannot be utilized successfully to construct phylogenetic relationships in the genus *Lachenalia*. Similar conclusions were reached by Hamatani *et al*. (2008), resulting in a movement towards molecular methods to determine phylogenetic relationships in the genus.

Basic chromosome numbers and polyploidy

Moffett (1936) identified four different basic chromosome numbers (x = 7, 8, 11 and 13) and polyploids, including 3x, 4x and 6x, in the x = 7 group. De Wet (1957) added a basic chromosome number of x = 12 and reported on an accession with 2n = 56, a possible 8x. Ornduff and Watters (1978) added x = 6, in an unidentified species as well as x = 5 and x = 9. Johnson and Brandham (1997) added x = 10 and 15.

For the purpose of this review, the 89 species in **Table 1** was grouped according to their basic chromosome numbers. Basic chromosome numbers of x = 5, 10 and 15 were also included as existing basic numbers for the genus and not as polyploid forms of basic group x = 5. Of the 89 species six species (*L. mediana*, *L. latimerae*, *L. isopetala*, *L. nervosa*, *L. congesta* and *L. capensis*) could not be placed into a specific basic chromosome number due to varying reports in literature indicating different basic chromosome numbers within these species. It is possible that *L. mediana* has two different basic chromosome numbers and that x = 9 are present in *L. mediana* var. *mediana* and x = 13 are found in *L. mediana* var. *rogersii* (Spies *et al*. 2008, 2009). More studies are, however, required to accurately place these six species. Other species with varying chromosome number reports were placed into specific groups according to the most commonly reported chromosome number (**Table 1**). These include:

- basic group x = 8 (*L. contaminata* 14 out of 17 reports indicate 2n = 16);
- basic group x = 7 (*L. barkeriana* 3 out of four accessions had 2n = 14, *L. marginata* 4 out 5 reports indicate either 2n = 14 or tetraploids of x = 7, *L. orchioides* – majority of reports indicate x = 7 and 2n = 16 most probably from wrongly identified species, *L. pusilla* as 8 out of 9 reports indicate 2n = 14, *L. reflexa* as 5 out of 6 reports indicate 2n = 14 and the 2n = 16 could most probably be ascribed to the presence of B-chromosomes, *L. variegata* as 3 out 4 reports indicate basic x = 7 and *L. violaceae* as 15 out of 17 reports indicate basic x = 7);
- basic group x = 10 (*L. alba* as 4 out of 5 had 2n = 20 and Johnson and Brandham (1997) concluded that 2n = 20 forms a diploid based on x = 10 rather than a tetraploid based on x = 5);
- basic group x = 11 (*L. hirta* as 8 out of the 12 reports had 2n = 22 and *L. unifolia* as 27 out of 32 reports indicated 2n = 22 as somatic chromosome number);
- basic group x = 12 (*L. ensifolia* as 3 out of 5 reports indicate 2n = 24 but this species can also be a possible x = 13 and *L. stayneri* because it formed a structural diploid based on x = 12 rather than a tetraploid based on x = 6 (Johnson and Brandham 1997);
- three different basic chromosome numbers have been recorded for *L. mutabilis*. This is the only species in basic group x = 5, as well as basic group x = 6. The majority of reports however comes from basic group x = 7 (24 out of 38).

Of the 83 taxa that could be grouped, basic x = 7 (41%) and basic x = 8 (27%) were the most common, followed by basic x = 9 (11%) and x = 11 (10%). Basic x = 10 (4%), x = 12 (2%), x = 13 (2%) and x = 15 (4%) are only present in a small number of taxa (**Table 1**, **Fig. 4**). Basic x = 5 (1%) and x = 6 (1%) were only present in *L. mutabilis.* Johnson and Brandham (1997) stated that x = 5 reported for *L. mutabilis* were derived from plants with 2n = 14 via Robertsonian fusions. Based on their observations of no constant number of long and short chromosomes in *L. mutabilis*, Spies *et al*. (2000) disagreed with Johnson and Brandham's (1997) conclusion that the x = 5 *L. mutabilis* studied by them resulted from Robertsonian fusions. Spies *et al*. (2000) could not find any long chromosomes as a result of Robertsonian fusions linked to specific specimens or a specific basic number supporting the hypothesis of Johnson and Brandham (1997). Spies *et al*. (2000) thus concluded that the variation in *L. mutabilis* is more likely to be the result of an aneuploid series. More studies are needed to determine the actual mode of chromosome evolution in the species *L. mutabilis*. Dysploid series also occurs in other genera such as *Prospero*: x = 4, 5, 6, 7; *Bernardia*: x = 8, 9; *Hyacinthella*: x = 9, 10, 11, 12 and *Stellarioides*: x = 2, 3, 4, 5, 6, 7, 8 and 9. Like in *Lachenalia* these aneuploid/dysploid series is difficult to interpret (Pfosser and Speta 1999). Combining the chromosome counts with molecular and morphological data might aid in the interpretation of the chromosomal evolution in the genus.

The presence of polyploidy was reported in 19 *Lachenalia* taxa (23%), excluding *L. capensis* and *L. congesta* where basic chromosome numbers could not be determined from published results. Conclusions could thus also not be drawn on polyploidy in these species (**Table 1**). Polyploidy are most common in the basic x = 7 group, with 12 of the 34 species (35%) containing polyploid specimens and a few species exhibiting a range of ploidy levels from triploid to octoploid (**Fig. 4; Table 1**). Polyploidy were also reported in basic group x = 6, 8, 9, 10 and 11, but here only tetraploids were observed. Tetraploids (present in 23% of the 83 grouped taxa) are the most common followed by octoploids (4%) hexaploids (2%), triploids (2%) and heptaploids (1%).

Lachenalia bulbifera is the species with the largest number of reported polyploid accessions including 4x, 6x, 7x and 8x accessions (**Table 1**). The heptaploid accession of *L. bulbifera* originated from seed and it is thus possible that the seed could have originated from an intra-species cross between a 6x and an 8x individual (Kleynhans and Spies 1999). Specific ploidy levels in *L. bulbifera* were better correlated to geographic distribution than morphology (Kleynhans and Spies 1999).

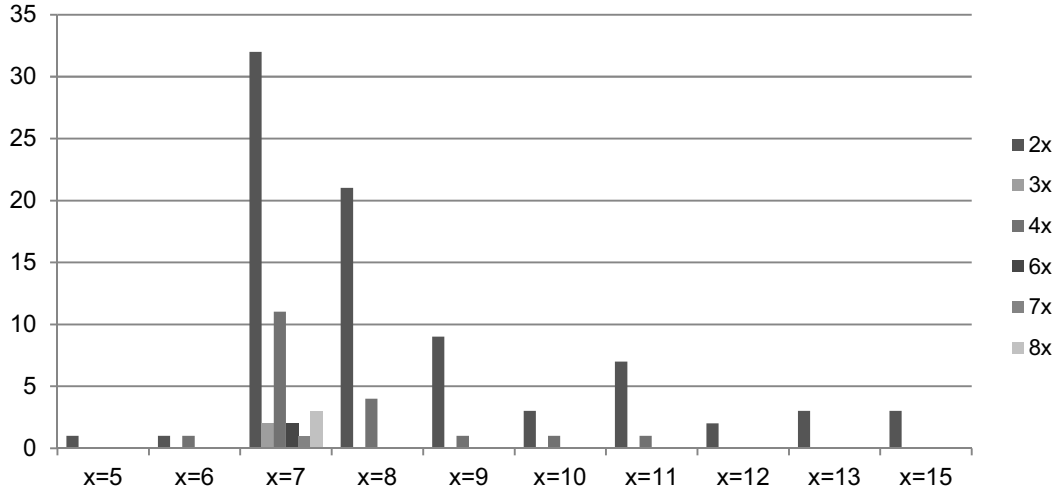

Fig. 4 Basic chromosome numbers in the genus *Lachenalia* indicating the number of taxa for each basic number and the ploidy levels reported for these basic numbers.

The only other species with ploidy levels above tetraploid are *L. elegans* and one report of 8*x* in *L. mutabilis* (**Table 1**). The two triploid accessions in *L. aloides* and *L. rosea* could have resulted from intra-species crosses between diploid and tetraploid individuals in these species followed by vegetative propagation or through an unreduced gamete followed by vegetative propagation as suggested by Moffett (1936).

Meiotic studies

Reports on meiotic studies within the genus are less frequent. Moffett (1936) again presented the first report on meiosis. The author found mostly normal meiosis for 2*n* = 14, 16 and 22 species. The only differences were reported where ploidy was present. Hancke and Liebenberg (1998) reported on the meiosis of several 2*n* = 14 species and hybrids. Species studied displayed normal meiosis with 7 bivalents. Four of the six hybrids studied also displayed normal meiosis with 7 bivalents indicting a close relationship between the species *L. aloides*, *L. orchioides*, *L. viridiflora* and *L. reflex*a. Two hybrids (both between *L. aloides* and *L. mutabilis*) displayed a low percentage of trivalents and quadrivalents. Hancke and Liebenberg (1998) presented evidence of structural chromosomal changes involving three chromosomes of which the acrocentric pair of chromosomes was involved in at least one interchange. This chromosome pair also seemed to be prominent in other abnormalities observed during meiosis (Hancke and Liebenberg 1998).

Hancke *et al*. (2001) studied the chromosome associations of one interspecific dibasic hybrid between *L. splendida* and *L. aloides* and two interspecific dibasic hybrids between *L. unicolor* and *L. aloides*. Results showed that *L. aloides* is more closely related to both *L. splendida* and *L. unicolor* than expected with genome affinity indexes of 0.9 and above. The results of the pairing configurations observed in these hybrids revealed homoeology between two chromosomes of the *x* = 7 karyotype and three chromosomes of the *x* = 8 karyotype. This could indicate that the *x* = 7 plants differ from the *x* = 8 plants by at least two exchanges of chromosome material and involves also the loss of one centromere from the *x* = 8 karyotype. Hancke *et al*. (2001) thus suggested that the change in basic chromosome number of *Lachenalia* involves a reduction in number.

Du Preez *et al*. (2002) reported on normal meiosis with 8 bivalents for the following species, as well as the hybrids between *L. carnosa* and *L. splendida*, *L. splendida* and *L. carnosa*, *L. unicolor* and *L. carnosa* and *L. carnosa* and *L. framesii*. This study indicated that these species are closely related. Hamatani *et al*. (2009) confirmed this relationship.

PHYLOGENETIC STUDIES

Only a few molecular studies have been done on *Lachenalia* and most of these studies concentrated on the phylogenetic position of the genus. The extensive variation in the genus, and even within a species, as indicated by RAPD studies (Kleynhans and Spies 2000), complicates both the phylogeny and taxonomy. In cultivation, a number of species are easily crossed and reproduce by means of offshoots and bulb formation. The existence of possible natural hybrid species thus further complicates the phylogenetics of the genus.

The phylogenetic position of *Lachenalia*

The genus *Lachenalia* was included in several studies to determine the phylogenetic position and classification of the different species, the first being the inclusion of the genus in the family Liliaceae. *Lachenalia* was reclassified in the family Hyacinthaceae (Perry 1985) up to 2009, where after the family Hyacinthaceae was dissolved into other families. *Lachenalia* now belongs to the family Asparagaceae (APG III group 2009).

To find the relative position of *Lachenalia* in the Asparagaceae, Pfosser and Speta (1999) used sequences of the *trnL-F* chloroplast region. From these results the authors were able to group *Lachenalia* in the tribe Massonieae (which consists of all the South African genera investigated, such as *Drimiopsis*, *Ledebouria* and *Polyxena*). This study also presented the first evidence suggesting a close relationship between *Lachenalia* and *Polyxena*, with a bootstrap support of 100%. This was in contrast to that of Müller-Doblies and Müller-Doblies (1997), which placed *Lachenalia* in the subtribe Lachenaliinae and *Polyxena* into Massoniinae. Pfosser and Speta (1999) suggested further studies, since only a few representative species were included in their analysis.

A later study (Pfosser *et al*. 2003) included not only more *Lachenalia* species, but also an additional chloroplast region (*atpB*), as well as data on seed morphology. *Polyxena*, *Lachenalia* and the genus *Periboea* formed a monophyletic clade with a bootstrap support of 100%. This study thus also supported the inclusion of *Polyxena* in the genus *Lachenalia.* Within the monophyletic clade some species of *Lachenalia* and *Polyxena* had low bootstrap support values (66% and 62%, respectively) and it was suggested that the specific delimitation may not be optimal for these clades. Another explanation was that the species are more recently derived, resulting in an insufficient number of base substitutions to resolve the taxa. The authors suggested that seed size and weight is higher in the basal genera such as *Eucomis*, *Merwilla* and *Ledebouria*, with *Veltheimia brac-*

teata having seeds of 0.056 g and with a length of 6.1 mm. The smallest seeds were found in the genus *Lachenalia* (*L. angelica*: 0.0003 g; 0.9 mm long). Analysis on the seed size and weight supports the hypothesis of the authors that *Lachenalia* is a recently derived genus. The seed form and structure of the micropylar swelling of the seed coat in *Lachenalia* suggested that this genus was the most advanced in their study.

The inclusion of *Polyxena* in the genus *Lachenalia* was raised again in three separate studies (Manning *et al*. 2004; Spies 2004; Hamatani *et al*. 2008) using *rbcL*, *trnL-F* and *ITS1-2* sequencing data respectively. In all these studies, *Lachenalia* and *Polyxena* formed a well supported monophyletic group. The two genera were characterised from other genera in the family by their biseriate stamens with the two series inserted at different heights. The two genera can be distinguished from each other by the relative fusion of the perianth (Manning *et al*. 2002). Manning *et al*. (2004) thus included *Polyxena* within *Lachenalia* based on the paraphyletic nature of the two genera.

Phylogeny within the genus

Morphological studies have focused on the entire genus, and many species have, over time, been included and excluded and shifted around from one genus to another. The first of these was when the genus was split into several genera (Salisbury 1866). Later on the species in the genus were sub-divided into smaller groups by Baker (1897), Crosby (1986) and Duncan (1988, 2002). These groupings, except for that of Crosby (1986) were based on different morphological characteristics, and did not correspond with each other.

Duncan *et al*. (2005) used morphological data of all the species in the genus to construct a cladogram. The author included 73 characters which comprised of 57 qualitative and 16 quantitative characters. This study concluded that *Polyxena* is paraphyletic with *Lachenalia* and forms the basal clade. Many of the *Lachenalia* species formed polytomies or unrelated groups, but there were some synapomorphies or taxa sharing some traits.

Spies (2004) produced a cladogram based on chloroplast *trnL*-F sequencing data from 129 taxa, including four *Massonia* taxa as outgroup. Hamatani *et al.* (2008) investigated nuclear *ITS1*-2 sequencing data of 56 taxa, including two *Massonia* and one *Ornithogalum* as outgroup. Both authors identified specific clades within the genus *Lachenalia.* The topologies of the cladograms produced by these authors largely correspond.

CROSS-ABILITY IN *LACHENALIA*

Rev. John Nelson raised the first authenticated *Lachenalia* hybrid in 1878 (Moore 1905). Since then a number of claims of interspecific hybridization were published (Crosby 1978, for review of early work). None of these early hybrids became available commercially. In 1965 the genus was identified as an indigenous genus with potential for development in South Africa. A breeding programme for the development of flowering pot plants was started at the Roodeplaat Vegetable and Ornamental Plant Institute of the Agricultural Research Council and the first hybrids became available commercially in 1997/1998 (Kleynhans 2006).

The extensive morphological and cytological variation in the genus *Lachenalia* resulted in the existence of both internal and external crossing barriers (Lubbinge 1980; Kleynhans and Hancke 2002; Kleynhans 2006). External crossing barriers like geographical separation and varying flowering periods can be overcome through the cultivation of species in controlled environments and the successful storage of pollen for a 12 month period (Kleynhans 2006). Internal crossing barriers include both post- and pre-fertilization barriers. Mechanical isolation (Lubbinge 1980) is one of the first internal pre-fertilization barriers. Flower length in *Lachenalia* species can vary from 5 to 30 mm (Duncan 2005). Pollen 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). The utilization of reciprocal crosses has been successful in overcoming this barrier (Lubbinge 1980; Kleynhans 2006). Other pre- and post-fertilization barriers have not been studied in detail, but the extent of these barriers become clear when the success rate of inter-species crosses are taken into account.

For each crossing combinations at least 10 flowers, within two different inflorescences were pollinated to ensure that wrong conclusions were not drawn, due to specific physiological or developmental problems in the inflorescence or floret. Kleynhans *et al.* (2009) reported that only 33% of the inter-species crosses (1498) made over a 30 year period were successful. With additional crosses (382) made since 2005, this percentage dropped to only 18% (**Table 2**). Of the 82% that did not succeed, 50% was related to the absence of seed, indicating the presence of possible pre-fertilization barriers. A further 31% of the combinations produced abnormal or non-viable seed that could be ascribed to post-fertilization barriers. Lastly, 1% of the crossing combinations did not succeed due to seedling death shortly after germination. The reason for the death of

Table 2 Number of inter-species crosses made among various different *Lachenalia* species over a 35 year period and the results obtained from these crossing combinations. Crosses that did not succeed were linked to three different aspects namely no seed set, abnormal seeds or seedling death. Results are linked to the basic chromosome complement of the species.

Basic chromosome number of parents	No. of successful crosses	No of unsuccessful crosses		
		No. of crosses with no seed set	No. of crosses with abnormal seed	No. of crosses with seedling death
7x7	169 (27%)	274 (44%)	169 (27%)	10 (2%)
8x8	72 (46%)	44 (28%)	40 (45%)	1 (1%)
11x11	2 (67%)		1 (33%)	
7x8	20 (6%)	251 (79%)	44 (14%)	3 (1%)
8x7	59 (18%)	111 (34%)	155 (47%)	6 (2%)
7x10		17 (100%)		
10x7	1 (5%)	5 (25%)	13 (65%)	1 (5%)
7x11	1 (2%)	54 (86%)	8 (13%)	
11x7	4 (6)	23 (33%)	39 (57%)	3 (4%)
9x8			1 (100%)	
8x10		1 (33%)	2 (67%)	
10x8	2 (33%)	1 (17%)	2 (33%)	1 (17%)
8x11	1 (3%)	23 (79%)	5 (17%)	
11x8	1 (3%)	15 (39%)	22 (58%)	
11x10		1 (100%)		
15x7		2 (67%)	1 (33%)	
Unknown basic numbers in one or both of the parents	4 (2%)	117 (59%)	78 (39%)	
Total	336 (18%)	939 (50%)	580 (31%)	25 (1%)

these seedlings can not necessarily be ascribed to hybrid breakdown, as seedlings can also be affected by diseases.

The genetic variability within the genus as described above has a direct influence on the cross-ability. With the additional data presented in this review the comparison between cross-ability and the cytogenetic and molecular data will be discussed in the next section.

COMPARISON BETWEEN CROSS-ABILITY, CYTOGENETIC AND MOLECULAR DATA

The complexity in the genus, in terms of morphology, cytogenetic and genetic variation complicates the determination of the relationship within and between different species. There are questions on the existence and origin of the different basic chromosome numbers, as well as the mode of speciation. Does the different basic chromosome numbers correlate with the phylogeny of the genus? Can the phylogenetic information assist in the taxonomic grouping of some difficult species and, furthermore, can phylogenetic information shed some light on the existence of possible natural hybrids? How does the phylogeny correlate with the cross-ability between species and finally what conclusions can be drawn when the different data sets are compared.

Basic chromosome numbers and cladograms

A comparison between the groupings from Crosby (1986) (based on chromosome numbers), Spies (2004) (chloroplast *trnL-F*), Duncan (2005) (morphology) and Hamatani *et al.* (2008) (nuclear *ITS1-2*) revealed that, with the exception of a few species, there is a good correlation between the basic chromosome numbers and the monophyletic groups identified in the different studies. When chromosome numbers were superimposed on the cladogram of Duncan *et al*. (2005) most of the $x = 7$ and $x = 8$ species fall into exclusive monophyletic groups for each chromosome number. There are only two exceptions where $x = 7$ species (*L. congesta* and *L. mathewsii*) grouped with $x = 8$. Species with $x = 11$ were closely related, even though they did not form a monophyletic group. The rest of the chromosome numbers form a polytomy. Although monophyletic groups linked to basic chromosome numbers were obtained the morphological cladogram is poorly resolved for many of the species.

The study using *trnL-F* chloroplast DNA sequences (Spies 2004) of 129 taxa distinguished several well defined groups. The first group consisted of seven species with a basic number of 11. Species with $x = 7$ and 8 formed a monophyletic clade (the *Lachenalia* 1 group), suggesting a close relationship between these two basic numbers. Within this monophyletic clade, $x = 8$ formed a monophyletic subclade excluding only one species with a basic chromosome number of $x = 8$, *L. verticillata*, and including *L. pusilla* ($x = 7$), which was basal to this group. All species having a basic chromosome number of $x = 7$, were distributed in different sister subclades, of which the two largest $x = 7$ subclades includes 25 and 10 taxa respectively. The second largest group in the cladogram (the *Lachenalia* 2 group), consisted of 48 poorly resolved taxa having chromosome numbers of $x = 6$, 7, 8, 9, 10 and 13. This group has no consistent pattern regarding chromosome numbers. These results led the author to conclude that hybridization might have played a role in speciation and that the genus might represent a hybrid swarm.

In the cladogram based on *ITS1-2* sequencing data (Hamatani *et al.* 2008), a monophyletic group for $x = 8$ (supported with a bootstrap value of 83.3) as well as for $x = 7$ forming a polytomy was obtained. Two species, *L. muirii* and *L. pusilla* both with a basic number of 7, grouped with the $x = 8$ clade, but formed the base for the rest of the $x = 8$ species. The *ITS1-2* region seemed to have more variation in the $x = 8$ taxa than in the $x = 7$ taxa, since the clade for $x = 8$ was better resolved. A similar observation was made by Spies (2004) with the *trnL-F* sequences.

The good correlation between basic chromosome numbers and phylogenetic groupings could in the future be used to confirm basic numbers for species. A single count of $2n = 32$ was reported for *L. giessii* but based upon a close phylogenetic grouping with $x = 11$ (Spies 2004), it seems that this species could also be regarded as $x = 11$ ($2n = 33$) rather than $x = 8$ ($2n = 32$). In this review it was included as a tetraploid of $x = 8$ for the purpose of calculations, but this species should be investigated further. Similarly *L. capensis* groups with the $x = 7$ group (Spies 2004) thus supporting the chromosome counts of Johnson and Brandham (1997) and Spies *et al.* (2008) and suggesting that *L. capensis* could be a basic $x = 7$ rather than a basic $x = 8$ as reported by Hamatani *et al*. (1998). Further investigations and correct identification of species are, however, essential to solve the inconsistent reports in chromosome numbers in some species.

Basic chromosome number and cross-ability

Kleynhans *et al.* (2009) presented data showing that the success rate of crossing combinations increased when crosses were made between species containing the same basic chromosome number. The information from additional crosses made in the last five years were added to this data and the number of successful crosses between species with the same basic chromosome number was substantially higher than between species from different basic chromosome numbers (**Table 2**). The success rate of crossing combinations dropped to below 20% when species with different basic chromosome numbers were crossed. The only exception to this is the combination of basic $x = 10$ crossed with basic $x = 8$ (**Table 2**). The two successful crosses resulted from a *L. alba* x *L. unicolor* and *L. alba* x *L. pustulata* combination (specific results not shown).

The increased success rate reported between species with the same basic chromosome number were a confirmation of a report by Crosby (1986) who also indicated that species cross more readily within certain basic chromosome number groupings. Based on differences in the cross-ability and morphology the latter author also split the basic $x = 7$ group of species into two different groups. The existence of different groupings within the basic $x = 7$ was confirmed by Spies (2004) as discussed above. Meiotic data presented by Hancke and Liebenberg (1998), as discussed above, also indicated differences between especially the species *L. mutabilis* and *L. aloides* as illustrated by structural chromosome changes. Kleynhans *et al.* (2009) used the three basic clades as well as the phylogenetic groupings within the basic $x = 7$ group as reported by Spies (2004) and presented data that showed improved cross-ability when crosses were made between individual species within the same phylogenetic groupings. The cross-ability was at least 10 to 20% higher when crossing combinations were attempted within the groups, than between groups. The cross-ability data thus supported phylogenetic groupings as identified by Spies (2004).

The close relationship illustrated in the phylogenetic trees, between species with basic $x = 8$ was also confirmed by the cross-ability data with a success rate of 46% (**Table 2**). The only success rate higher than this was that between species with basic $x = 11$. This data, however, only included 3 crossing combinations in comparison to the 157 combinations within the basic $x = 8$ group and would most probably decline with the inclusion of additional crossing combinations. The relationship among species with $x = 8$ was further illustrated by Du Preez *et al.* (2002). In this meiotic study several hybrids between different species with $x = 8$ were investigated and all hybrids produced 8 bivalents. Hybrids resulting from these crosses are also fertile and was successfully utilized in further crossing combinations (results not shown).

Evolution and relatedness of different basic chromosome numbers

The largest number of species in *Lachenalia* are found within the basic $x = 7$ and 8 groups. Molecular data from *ITS1-2* (Hamatani *et al*. 2009) and *trnL-F* (Spies 2004) sequences indicated a strong relationship between these two basic chromosome number groups and that these groups might have evolved from a common ancestor. Cross-ability data confirmed a relationship between these two basic chromosome number groups with higher success rates (18% for $x = 8$ crossed with $x = 7$), than most of the other between group success rates (**Table** 2). The existence of genome affinity indices of 0.9 in three interspecific dibasic hybrids (Hancke *et al.* 2001), as discussed above, also confirmed this relationship.

Karyomorphological data presented by Hamatani *et al*. (2009) using FISH and DAPI staining to determine the chromosomal evolution of the $x = 7$ and $x = 8$ groups confirmed the results found from both the phylogeny and the cross-ability. The results of this study between a group of $x = 7$ (consisting of *L. muirii*, *L. aloides* var. *aloides*, *L. aloides* var. *aurea*, *L. longibracteata*, *L. variegata*, *L. viridiflora*, *L. mutabilis*, *L. rubida*, and *L. pusilla*) and $x = 8$ (consisting of *L. carnosa*, *L. liliflora*, *L. namaquensis*, *L. splendida* and *L. unicolor*) led to the conclusion, that there was little morphological chromosome variation within the $x = 8$ group and that this group was derived from an ancestral species followed by ongoing speciation.

The $x = 7$ group showed much more variation, with four karyotype patterns indicating several morphological alterations of chromosomes within this group. This was in contrast with the *ITS1-2* region data that seemed to have more variation in the $x = 8$ taxa than in the $x = 7$ taxa, since the clade for $x = 8$ was better resolved than the polytomic $x = 7$ clade (Hamatani *et al*. 2009).

Hamatani *et al.* (2008, 2009) suggested several theories for the evolution of the $x = 7$ and 8 groups. Both groups might have evolved from a common ancestor (as indicated in sequencing data) or they could be the product of mutation or putative hybridization between species in the same geographical distribution area. Reduction in chromosome number either by losing a chromosome or by translocation might have contributed to speciation in these two groups. Hancke *et al.* (2001) speculated that $x = 7$ evolved from $x = 8$ through a reduction in chromosome number based on the homoeology between two chromosomes in the $x = 7$ and three chromosomes in the $x = 8$ species studied.

Five of the nine species in the $x = 7$ group (*L. aloides* var. *aloides*, *L. aloides* var. *aurea*, *L. longibracteata*, *L. variegata*, *L. viridiflora*) had very similar chromosome morphology (Hamatani *et al.* 2009) and seemed to be closely related. The close relationship between *L. aloides* and *L. viridiflora* can be confirmed from crossing data with a success rate of between 25 and 100% depending on the reciprocal direction (data not shown) and the production of fertile F_1 hybrids with seven bivalents in meiotic analysis (Hancke and Liebenberg 1998).

According to (Hamatani *et al*. 2009) the chromosome morphology of *L. mutabilis* and *L. rubida* were very similar, but differed from the above group, and the authors concluded that these species probably originated from a single ancestral species. For the purpose of this review a selection of *ITS1-2* sequences representing only those species used in the FISH study (Hamatani *et al*. 2009) were obtained from Genbank and a phylogram was constructed (**Fig. 5**). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The ITS phylogram yielded similar monophyletic groupings than the *ITS1-2* cladogram (Hamatani *et al*. 2009) and included both *L. mutabilis* and *L. rubida* within the $x = 7$ clade. Both these species have a similar branch length that was much longer than the other species in the clade, which supported the similarity in chromosome morphology. This relationship cannot be confirmed from crossing data (success rate of only 10%), neither by the data presented by Spies (2004) or Hamatani *et al.* (2008).

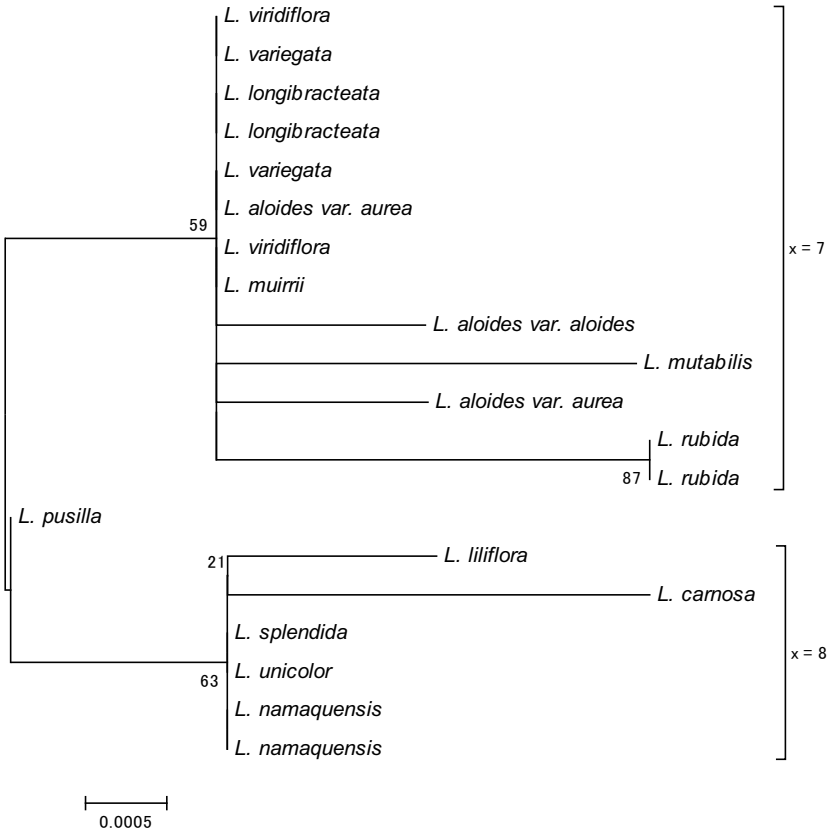

Fig. 5 Evolutionary relationships of 17 taxa based on the *ITS1-2* region. The phylogram was constructed using the Maximum Likelihood option of MEGA 5 (Tamura *et al*. 2011) to compare the evolutionary development of the $x = 7$ and 8 groups.

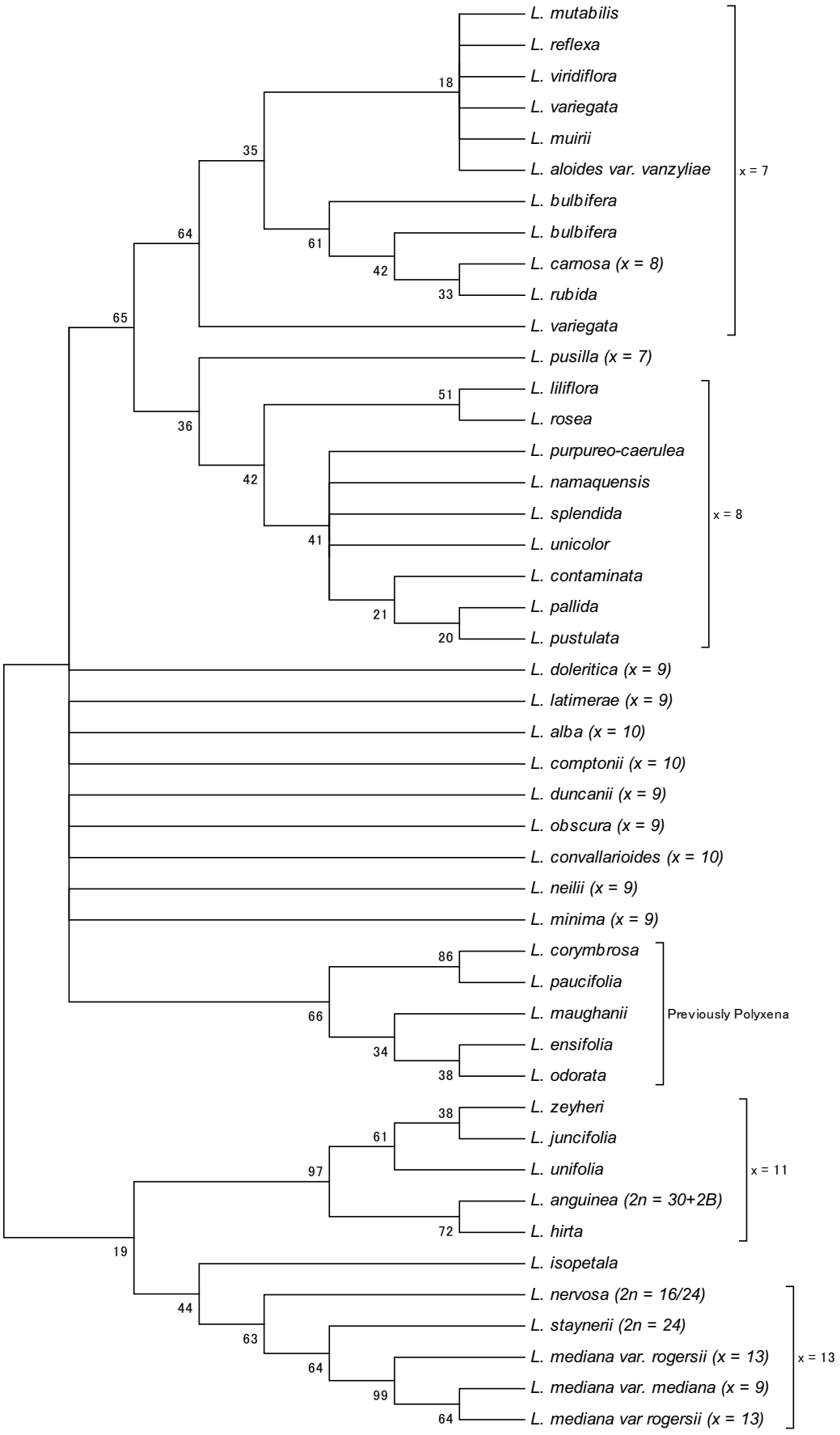

Fig. 6 Evolutionary relationships of 43 taxa based on the *trnL-F* region (Spies 2004), inferred using the Maximum Likelihood option of MEGA 5 (Tamura *et al.* 2011).

The remaining two species in the $x = 7$ group that were investigated (Hamatani *et al*. 2009), *L. muirii* and *L. pusilla*, shared chromosomal characteristics with species in both the $x = 7$ and 8 groups. The relationship to both $x = 7$ and 8 of *L. muirii* and *L. pusilla* was confirmed by Hamatani *et al.* (2008). Hamatani *et* al. (2009) suggested that *L. pusilla* might be intermediate between the $x = 7$ and $x = 8$ group. None of the crosses made with *L. pusilla* as either parent

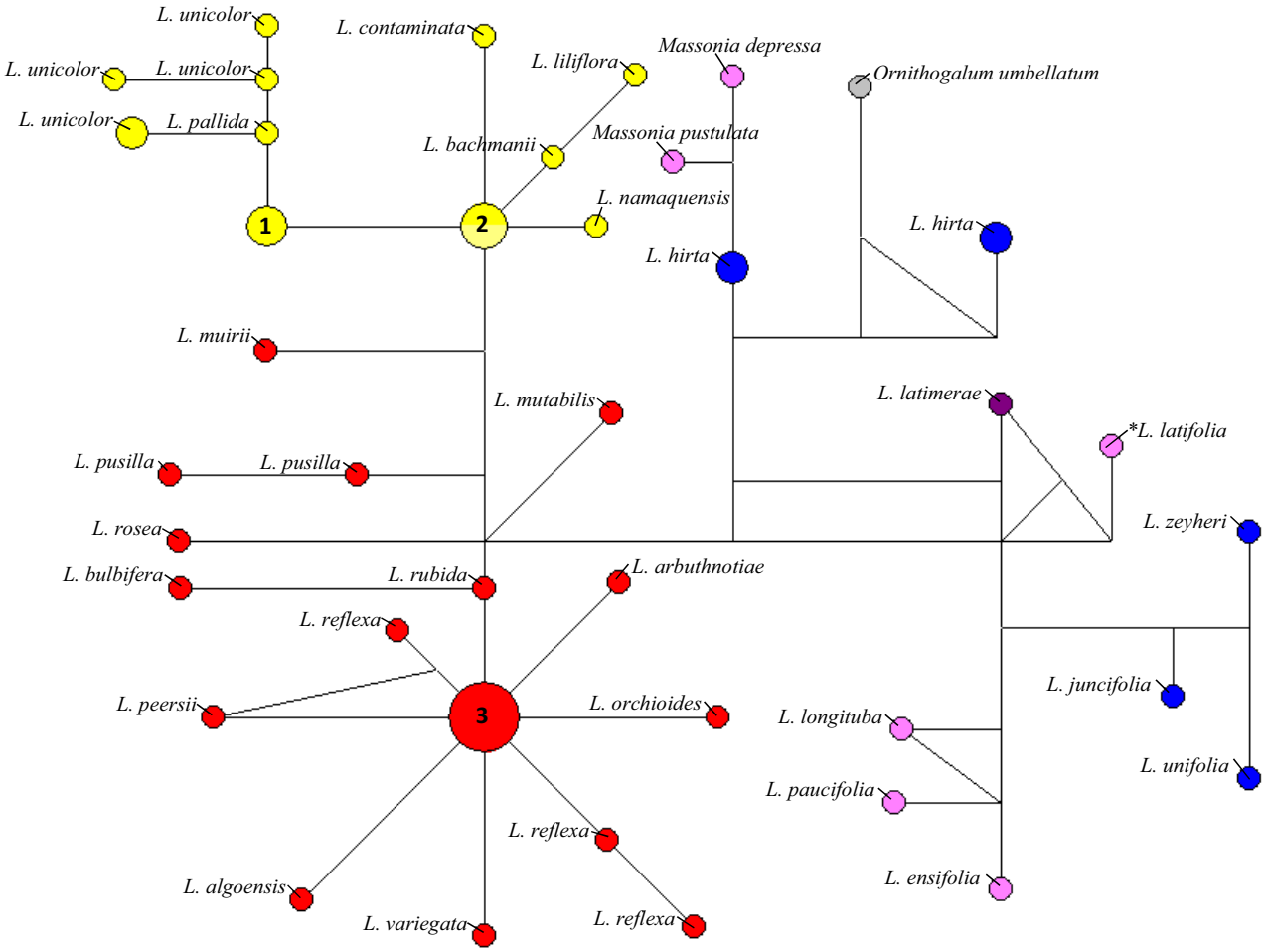

Fig. 7 Network of *Lachenalia* species based on *ITS* data using NETWORK 4.6.1.0 (Fluxus Technology, 2012). The correct current citation of *L. latifolia* (indicated with *) is *L. nervosa*. Colour codes: Red, x = 7; Yellow, x = 8; Blue, x = 11; Purple, 2n = 24/26/28; Grey, x = unknown. Node 1, *L. pustulata* and *L. purpureo-caerulea*; Node 2, *L. carnosa* and *L. splendida*; Node 3, *L. aloides* var. *aloides*, *L. aloides 'Pearsonii'*, *L. aloides* var. *luteola*, *L. aloides* var. *vanzyliae*, *L. aloides* var. *quadricolor*, *L. aloides* var. *aurea*, *L. viridiflora*, *L. orchioides* var. *orchioides* and *L. longibracteata*.

were successful, neither with x = 7 nor with x = 8 species. The cross-ability data available can thus not shed any light on the position of *L. pusilla.*

There seem to be an evolutionary relationship between some of the other basic chromosome number groups and even with other genera. For better insight in the evolution of the rest of the chromosome numbers, sequences from Spies (2004) were selected to represent a broad spectrum of chromosome numbers in the genus. Sequences were selected based on the cladogram produced by Spies (2004), but all sequences forming a polytomy were excluded, and a new cladogram (**Fig. 6**) was constructed.

Although many of the clades are not well supported, the new *trnL-F* cladogram (**Fig. 6**) supports the suggestion that the genus evolved from a common ancestor. The basic numbers x = 7 and 8 evolved from a common predecessor, even though many of the clades are not well supported, thus confirming the data presented above. The higher basic numbers (x = 9, 10, 11 and 13) form a poorly supported monophyletic clade (bootstrap value 57). It seems as if the higher numbers evolved independently from the lower numbers in at least two separate events. The basic numbers x = 9 and 10 forms a polytomy in the higher clade and seems to be the bridge from the lower to the higher numbers or *vice versa* (**Fig. 6**). Because none of the x = 9 or 10 taxa are well resolved, this group might be a recent group. The low level of variation in these two basic numbers indicates that evolution was recent and these numbers have not evolved into two definite clades.

A median-joining network (Bandelt *et al.* 1999) was constructed from the *ITS* data (Hamatani *et al.* 2008) (**Fig. 7**) as well as from 43 *trnL-F* sequences (Spies 2004) (**Fig. 8**). The *trnL-F* network suggests that x = 11 and x = 8 have evolved independently from a common ancestor, and that x = 9 and 10 could have evolved from any one of these two numbers. The ITS network (**Fig. 7**) could not confirm or reject this, due to the lack of x = 10 species and the inclusion of only a single x = 9 species. Both the networks support a close relationship between the x = 7 and 8 groups. The cross-ability success rate of 33% between basic x = 10 and basic x = 8 (**Table** 2) could be a confirmation of the possible bridge between x = 7 and 8 and the higher numbers. The ITS network also supported the relationship between *L. mutabilis* and *L. rubida* (**Fig. 5**) and the *trnL-F* network positioned *L. pusilla* in an ancestral position to x = 7 and 8 thus supporting the molecular cytogenetic data.

Dysploidy (through the fusion of acrocentric chromosomes at the centromere to form larger metacentric to submetacentric chromosomes) has been shown to be important in the chromosomal evolution of other plant families, e.g. the *Commelinaceae* (Jones 1976). If dysploidy is the mode of speciation in *Lachenalia* a study on the chromosome morphology of species with higher basic chromosome numbers compared to lower basic chromosome numbers could assist in confirming the hypotheses. A study of *L. latimerae* (x = 9 according to Hamatani *et al.* 2007) indicated that this species has three large chromosomes, of which two are very similar, with the third one having a satellite (Hamatani *et al.* 2007). The chromosome morphology thus, supports the theory of dysploidy, but it must be further investigated with chromosome banding techniques. A second hypothesis is the possibility that *L. latimerae* could have resulted from a hybridization event (Hamatani *et al.* 2007) between x = 7 and x = 11, resulting in a gametic number of n = 18. If this

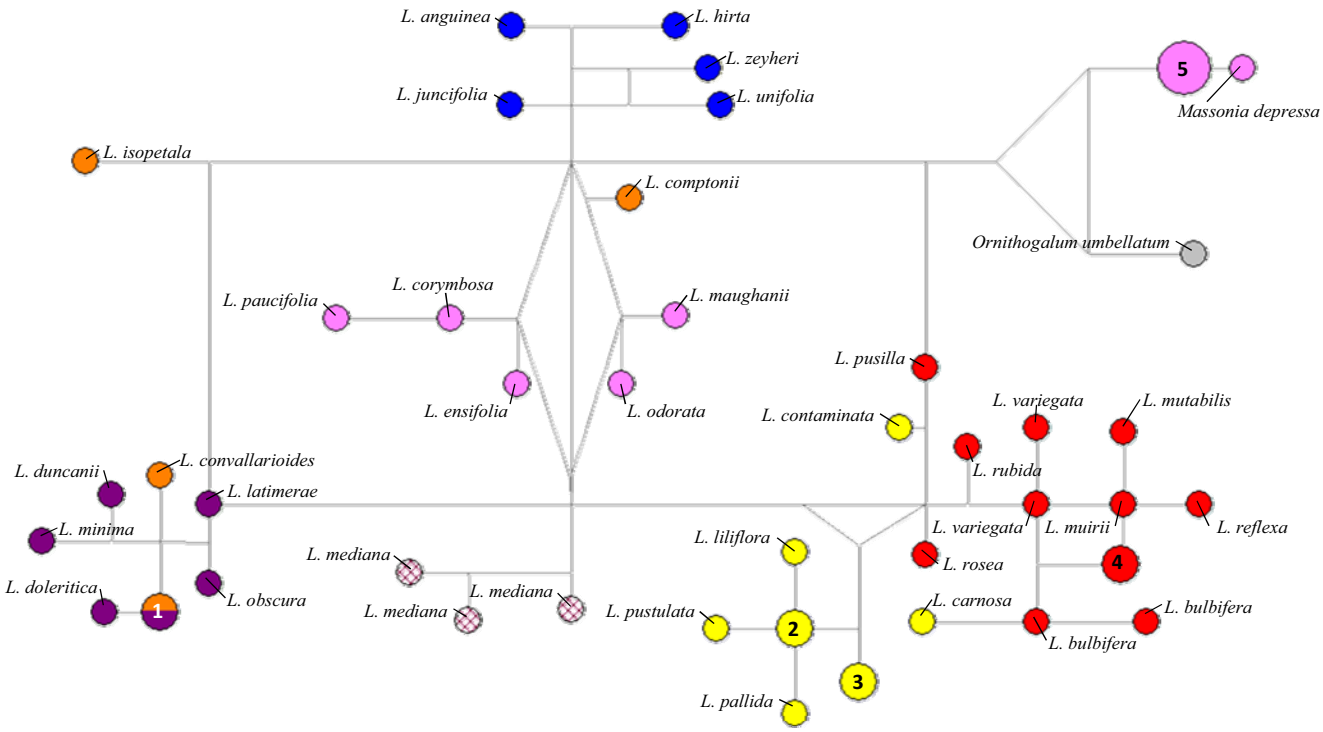

Fig. 8 Network of *Lachenalia* species based on *trnL-F* data using NETWORK 4.6.1.0 (Fluxus Technology, 2012). Colour codes: Red, *x* = 7; Yellow, *x* = 8; Blue, *x* = 11; Light purple, 2n = 24/26/28; Dark purple, x = 9; Orange, x = 10; Diagonal crosses, x = 9 or 13; Grey, *x* = unknown. Node 1, *L. neilii*; *L. alba*; Node 2, *L. purpureo-caerulea*; *L. unicolor*; Node 3, *L. namaquensis*; *L. splendida*; Node 4, *L. viridiflora*; *L. aloides* var. *vanzyliae*; Node 5, *Massonia pustulata*; *M. depressa*; *M. echinata*; *M. jasminiflora.*

theory is correct for other *x* = 9 species, one would expect at least some of the *x* = 9 species to group with either *x* = 7 or *x* = 11 in the chloroplast cladogram. All the *x* = 9 species fall between the *x* = 7/8 groups and the higher numbers, but because the *trnL-F* cladogram (**Fig. 6**) is not supported with high bootstrap values, neither the dysploid theory nor the hybridization theory could be proven. The *trnL-F* median-joining network (**Fig. 8**) is inconclusive in this matter, since the evolutionary direction for *x* = 9 can be from either *x* = 11 or *x* = 7/8 or both (thus hybridization).

The group *x* = 11 is very well supported with a bootstrap value of 94 in the *trnL-F* cladogram (**Fig. 6**), suggesting a strong relationship within this group. The close relationship within this group is also supported by the morphological cladogram constructed by Duncan (2005), even though these species do not form a monophyletic group. The evolution of *x* = 11 is not clear, but from the cladograms obtained in the different studies i.e. morphological (Duncan *et al*. 2005), *ITS* (Hamatani *et al*., 2008) and *trnL-F* (Spies 2004), *x* = 11 (and *x* = 13) is basal to the lower numbers and it seems that species with *x* = 11/13 is the intermediate between the outgroup species (which have higher numbers) and the lower numbers in the genus. The network drawn from the *ITS* sequences provides evidence of the link between the higher basic numbers in *Lachenalia* and outgroup species used in this study. The outgroup for the *ITS* network (**Fig. 7**) is *Massonia* and *Ornithogalum umbellatum*. The latter species has a high degree of cytogenetical variation (Czapik 1968) with numbers of 2n = 18-30 and B-chromosomes reported. Hamatani *et al*. (2008) obtained the *ITS* sequences for *L. hirta* (*x* = 11) by cloning the maternal and paternal genomes. One genome was cloned in some specimens and seem to have evolved from *Massonia*, while the other genome have evolved from *Ornithogalum* this may be the reason why different specimens form two different nodes in the network.

Existence of basic chromosome numbers

The evolution and even existence of certain chromosome numbers (such as *x* = 5, 6, 12, 13 and 15) have not been investigated to the same extend as *x* = 7, 8, 9 and 11. With basic chromosome numbers of 5, 6, 7, 8, 9, 10, 11, 12, 13 and 15 recorded, it is still speculated whether basic numbers of *x* = 5, 6, 10, 12, 13 and 15 exists.

There are very few reports for *n* or *x* = 5 in *Lachenalia*, and usually when *x* = 5 has been reported for a species, it was based only on one accession. Both *L. violacea* and *L. aloides* are *x* = 7 species, with a single 2*n* = 15 reported, indicating possible miss counts in these species. *Lachenalia mutabilis* has chromosome counts of *x* = 5, 6 and 7. This is the only species where numerous counts have been recorded for all three these numbers. This species is morphologically distinct and wrong identification could not attribute to the differences in counts. All reports for *x* = 5 for *L. mutabilis* are from the same geographical distribution area (Clanwilliam in the Western Cape Province), but there are also reports of *x* = 7 from Clanwilliam. Other species from the Clanwilliam district include *x* = 7 (*L. elegans* var. *sauveolens*, *L. thomasiae* and *L. violaceae*); *x* = 8 (*L. unicolor*); *x* = 10 (*L. marginata* and *L. undulata*) and *x* = 11 (*L. hirta* and *L. unifolia*). It was suggested that the three basic numbers for *L. mutabilis* form an aneuploidy series (Spies *et al*. 2000), but there is no proof of what attributed to the chromosome diversity in this species. Based on molecular systematics, *L. mutabilis* specimens always group with other *x* = 7 species, regardless of their chromosome number (Spies 2004; Hamatani *et al*. 2008); are karyotypically similar to *L. rubida* (*x* = 7) and has the highest number of *x* = 7 counts recorded, thus supporting the theory of an aneuploid series in the species.

Johnson and Brandham (1997) studied the karyotypes of *x* = 7-13 and 15, and reported that all the species studied formed structural diploids and thus concluded that 2*n* = 20 rather represents a diploid based on *x* = 10 than a tetraploid based on *x* = 5. They did state that 2*n* = 30 (*x* = 15) could be an allotetraploid derived from taxa with *x* = 7 and 8, following hybridization and doubling of the chromosome number. Considering this theory, it would be expected that *x* = 10 taxa have a phylogenetic grouping either with *x* = 7 or *x* = 8 taxa, but this have not been observed in the *trnL-F* cladogram (Spies 2004). The fact that the cross-ability

between $x = 10$ and 8 is relatively high could be an indication of the validity of this theory. The existence of the basic number $x = 10$, however, seem to be a reality, proven by the fact that some species has chromosome counts of $2n = 20$, 40 (*L. alba*) and $2n = 30$, 40 (*L. isopetala* – not grouped in this study) indicating the existence of polyploids. After all the evidence, it is still not clear whether $x = 5$ exist in any other species than *L. mutabilis*.

Reports for six species with either $x = 6$ or $2n = 24$ were mostly based on only one accession and differed from the majority number of counts for these species. *Lachenalia nervosa* has counts of $n = 8$ and $2n = 24$, indicating that this species has a basic number of $x = 8$ and have a triploid somatic number. *Lachenalia stayneri* is also $2n = 24$, and the lack of meiotic studies in this species may lead to the conclusion that this species represents a tetraploid based on $x = 6$ or also a triploid with $x = 8$. Therefore $x = 6$ should also be considered as a basic number. Based on *trnL-F* sequences, both these species indicate close relations with *L. mediana* ($x = 9$ and 13) and do not group with $x = 8$ (Spies 2004). Therefore, species with $2n = 24$ cannot be considered as "typical" $x = 8$ species, and might even be considered as being miss counts based on $x = 13$. None of the $2n = 24$ species has its own monophyletic grouping and it seems as if $x = 6$ does not exist except maybe in *L. mutabilis*.

Somatic counts of $2n = 28$ and 56 have been reported by several authors (Moffett 1936; de Wet 1957; Crosby 1986; Hancke and Liebenberg 1990; Johnson and Brandham 1997; Hamatani *et al*. 1998; Kleynhans and Spies 1999; Spies *et al*. 2002; Hamatani *et al*. 2007; Spies *et al*. 2008, 2009), but it has not been proven whether the basic chromosome number of $x = 14$ exists. Somatic numbers of $2n = 28$ as sole chromosome number have been reported for *L. cernua* and *L. longituba*. Both these species were included in the basic group $x = 7$ for the purpose of this review, but additional accessions of these species, as well as meiosis and cytomorphological data will have to be studied to determine the actual basic chromosome number.

Existence of hybrid species

The question of natural hybridization in the genus has been raised several times. Both the morphological and *trnL-F* cladograms had monophyletic groups consisting of a mixture of chromosome numbers $x = 6$, 7, 8, 9, 10 and 13 and no consistent patterns regarding similar groupings. Spies (2004) concluded that hybridization might have a role in speciation, but it was not proven.

Some species (*L. pusilla*, *L. rosea* and *L. carnosa*) do not follow the rule of grouping into monophyletic groups with similar chromosome numbers (**Fig. 6**). Considering the positions of these species in the networks drawn (**Figs. 8, 9**) the first two species is intermediate to the $x = 7$ and $x = 8$ groups in both networks. The position of *L. carnosa* ($x = 8$) fluctuate between $x = 7$ (**Fig. 8**) and $x = 8$ (**Fig. 7**). Within the *trnL-F* cladogram, *L. carnosa*, *L. rubida* and *L. bulbifera* is a sister clade with the rest of the $x = 7$ species. *Lachenalia rubida* is intermediate to $x = 7$ and 8 in both networks. To conclude, based on karyotypic and molecular data, some species are intermediate between $x = 7$ and 8, and can either be considered as predecessor species or as hybrid species.

Lachenalia carnosa ($x = 8$) is an example of a possible hybrid species, grouping with either $x = 7$ or 8, depending on the type of sequencing data (nuclear or cytoplasmic). Spies (2004) reported what seemed to be B-chromosomes in the meiotic divisions if *L. carnosa*, which may have been unidentified univalents, also observed in cultivated *Lachenalia* hybrids (Hancke and Liebenberg 1998). Cross-ability data, however, strongly links *L. carnosa* with other members of the $x = 8$ group, successfully crossing with at least five different $x = 8$ species (data not shown), producing regular meiosis with 8 bivalents (Du Preez *et al*. 2002) as well as fertile hybrids. Natural hybridization may be present in the genus *Lachenalia* but this should be investigated further.

CONCLUSION

This review accentuates the complex nature of the genus *Lachenalia*. Besides the extensive morphological variation that complicates the taxonomy of the genus, the genus is also exceptionally diverse in chromosome numbers. *Lachenalia* has different basic chromosome numbers ($x = 5$, 6, 7, 8, 9, 10, 11, 12, 13 and 15 reported in literature), contains polyploidy (ranging from triploids to octoploids), and includes B-chromosomes. Chromosome counts for the 89 species reported in literature varied from $2n = 10$ to 56 and from $n = 5$ to 28. Polyploidy was reported in 19 taxa (23%), and is most common in the $x = 7$ group.

The low cross-ability (only 18% successful inter-species crosses) reiterates this variation and stresses the importance of investigating the variation in order to develop breeding strategies to overcome the existing crossing barriers. Morphological and molecular phylogenetic studies confirm the complexity of the genus, but also assisted in drawing some conclusions on the relationship between species within the genus and the possible evolutionary history of the genus.

Phylogenetic studies has assisted in finding the phylogenetic position of *Lachenalia* in relation to other genera (Pfosser and Speta 1999; Pfosser *et al.* 2003; Manning *et al.* 2004) and placed the genus within the Asparagaceae family (APG III group 2009). Morphological (Duncan *et al.* 2005) and phylogenetic studies within the genus (Spies 2004; Hamatani *et al.* 2008) supported the inclusion of *Polyxena* in *Lachenalia*, and this inclusion increased the number of recognised *Lachenalia* species to 126.

Molecular studies on the *trnL-F* as well as *ITS* regions revealed monophyletic groupings of species containing the same basic chromosome numbers. This indicated a strong correlation between the phylogeny and basic chromosome numbers in the genus, although there were some exceptions in the larger *trnL-F* data set (Spies 2004). The good correlation between basic chromosome numbers and phylogenetic groupings could in the future assist to confirm basic numbers for species. The improved cross-ability when crosses were made between individual species within the same phylogenetic groupings confirms the phylogeny. Phylogenetic groupings, thus has to be taken into account when crossing combinations are planned to achieve better crossing success rates in the breeding programme.

When comparing the different studies, *Lachenalia* might have evolved from a common ancestor and the two largest basic chromosome number groups, $x = 7$ and 8 have evolved from a common predecessor. The studies also indicated a close relationship between these two basic numbers, which is supported by higher success rates in cross-ability between these two groups. It seems as if the higher basic numbers ($x = 9$, 10, 11 and 13) evolved independently from the lower numbers and that basic numbers $x = 9$ and 10 could be the bridge from the lower to the higher numbers or *vice versa* (**Fig. 6**), but evidence of this is not conclusive (**Figs. 7, 8**).

Dysploidy and hybridization might be the modes of speciation in some *Lachenalia* species but this could not be proven with molecular data and further studies are required to draw conclusions. The existence of some of the basic chromosome numbers reported (such as $x = 5$, 6, 10, 12 and 15) can been disputed. Only a few species can be linked to $x = 5$ and 6 and it is possible that these two basic numbers only exist as part of an aneuploid series in the species *L. mutabilis.* Further studies on species from these disputed basic chromosome numbers is needed to resolve the existence of all the reported numbers.

This review indicates that different genetic studies on *Lachenalia* reveal similar results and stresses the importance of assessing the variation within complex genera to aid in decisions around breeding programme strategies. It is clear that inter-species crosses within phylogentic groups in

the genus can improve the success rate of crossing combinations, but there are still many questions that remain unanswered. Further multidisciplinary studies are needed in the genus *Lachenalia* to solve the evolutionary history of this complex genus, to answer questions around species placement and the existence of basic chromosome number groups and to overcome crossing barriers.

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