Character Variation and a Cladistic Analysis of the Genus *Lachenalia* Jacq. f. ex Murray (Hyacinthaceae)

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Abstract
    The horticulturally important and botanically diverse genus *Lachenalia* Jacq. f. ex Murray is endemic to southern Africa, and is the largest member (115 species) of the Hyacinthaceae. The genus is morphologically and cytologically variable. Species delimitation is usually unambiguous but in some instances there is gradation between species. Other species display extensive variation which has led to considerable taxonomic confusion. The close taxonomic relationship with the small endemic, sympatric genus *Polyxena* Kunth is discussed, and its inclusion within *Lachenalia* is supported. The aim of the study was to examine the potential of morphological characters in producing a natural classification of *Lachenalia*. A morphological cladistic analysis of *Lachenalia* has not previously been attempted. A wide range of characters was analysed, including macromorphology, micromorphology, anatomy and palynology. Two parsimony analyses were performed using the endemic southern African genus *Massonia* Thunb. ex Houtt as the ancestral outgroup; an initial analysis in which the characters were unweighted, and a weighted analysis using successive approximations character weighting. The high level of homoplasy evident in both analyses indicates that morphological characters on their own are insufficient to trace the evolutionary history of *Lachenalia*, and other approaches such as molecular and cytological methods will need to be used in conjunction with morphology in order to generate a robustly supported, phylogeny of the genus. The study was based on species in their natural habitats as well as under cultivation, and from representative herbarium specimens.

INTRODUCTION

Lachenalia is endemic to southern Africa, and is the largest member (115 species including 134 taxa) of the family Hyacinthaceae (Duncan, 1996, 1998). The genus was last revised by Baker (1897), in which 42 species were recognised. *Polyxena* is a paraphyletic genus (Pfosser and Speta, 1999; Pfosser et al., 2003; van der Merwe, 2002).

The distribution of *Lachenalia* extends from the western and southern parts of Namibia southwards into the northwestern, western, southwestern, southern, southeastern and central parts of South Africa (Duncan, 1988). The genus is geophytic, deciduous, and is usually winter-growing. It occurs in a wide range of habitats and its centre of diversity is in the Succulent Karoo, in the mountains and valleys of the winter rainfall region of the southwestern Cape (Duncan, 2004).

The unusually high level of morphological variation in *Lachenalia* has led to considerable taxonomic confusion, with overemphasis of minor morphological differences (Duncan, 1992). Variation within a species occurs in several macro-morphological characters. Variable species often display population stability in features such as bulb and flower shape, and seed morphology. However, species such as *L. bulbifera*, *L. contaminata*, *L. elegans*, *L. mutabilis*, *L. orchioides*, *L. pustulata* and *L. violacea* are exceedingly variable.

The karyology of *Lachenalia* is unusually variable, including different basic

chromosome numbers, different ploidy levels and B-chromosomes within some species. The basic chromosome numbers x = 5, 6, 7, 8, 9, 10, 11, 13 and 15 have been recorded, with x = 7 and x = 8 being the most frequent (Johnson and Brandham, 1997). Ploidy levels are predominantly diploid, but tetraploid, hexaploid and octoploid counts have been made. Morphological variation within *Lachenalia* appears to be better correlated to geographical distribution than with ploidy level, as species with similar ploidy levels but with different geographical distribution, differ morphologically (Kleynhans and Spies, 1999).

The aim of this study was to examine the potential of morphological characters in producing a natural classification of *Lachenalia*.

MATERIALS AND METHODS

The study was based on wild collections and the extensive, cultivated collection at Kirstenbosch National Botanical Garden in Cape Town, as well as the comprehensive collection of herbarium specimens housed in the Compton Herbarium (NBG).

Characters

A morphological cladistic analysis of *Lachenalia* and *Polyxena* has not previously been attempted. Seventy-three characters were defined for use in the cladistic analysis, and comprised fifty-seven qualitative and sixteen quantitative characters. Qualitative characters such as bulb and seed shape were used where these formed clear evolutionary states. Quantitative characters such as tepal and filament length were used only where dimensional data formed clear, discontinuous units. The characters comprised thirty-eight binary and thirty-five multistate character states. They were coded to form a data matrix, and polarised using the outgroup comparison method. The endemic southern African genus *Massonia* was chosen as the outgroup. It forms part of the tribe Massonieae and shares the most recent common generic ancestor with *Lachenalia* and *Polyxena*, according to the molecular phylogenies of Wetschnig et al. (2002).

Cladogram Construction

The computer program PAUP (Version 4.0b10) phylogenetic analysis using parsimony was used for the cladistic analyses (Swofford, 1999). Due to the large data set (139 taxa comprising the ingroup, 1 taxon comprising the outgroup) and the relatively low number of 73 available morphological characters, the heuristic method was used to search for the most parsimonious trees. Two parsimony analyses were performed, an initial analysis in which characters were unweighted (analysis 1), and a weighted analysis (analysis 2) using the successive approximations character weighting scheme (SACW).

RESULTS AND DISCUSSION

Analysis 1

Using the data matrix for all *Lachenalia* and *Polyxena* taxa, an initial parsimony analysis was performed in which all 73 characters were unordered, unweighted and parsimony-informative, with the trees rooted using the outgroup comparison method. The analysis generated a strict consensus tree of 71153 equally parsimonious trees (Fig. 1), each with a shortest possible length of 1054 steps.

The results of the unweighted analysis suggested that *Polyxena* is paraphyletic with *Lachenalia*, with *P. longituba*, *P. ensifolia* and *P. maughanii* forming the most basal clade, and *P. paucifolia* and *P. corymbosa* placed immediately above, forming the second and third most basal clades. The uppermost major clade containing most *Lachenalia* species was a polytomy, and included four synapomorphies: a terminal clade in which the sister species *L. buchubergensis* and *L. nordenstamii* were supported by seeds with long chalazal collars; a terminal clade in which the sister species *L. barkeriana* and *L. pusilla* were supported by concrescent leaf bases; all the *L. aloides* taxa, *L. bulbifera* and *L. rubida* were supported by medial inner filament insertion; and five *L. aloides* taxa, *L. bulbifera* and *L. rubida* were supported by long filaments.

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The strict consensus tree showed a low level of resolution and indicated a high level of homoplasy across the tree, evident in numerous parallelisms and reversals. Major nodes were unsupported, with only a low level of support for nodes closer to the taxa. As a measure of confidence to test the stability of resolved nodes within the unweighted tree, a Bremer support analysis (also known as 'decay index') (Bremer, 1988, 1994) was performed, for which the 'subparsimonious search procedure' was employed. Under this method, support for each clade resolved by the data set is quantified as the difference in length between the most parsimonious trees and the shortest tree in which the clade is not resolved. Higher scores imply more support, and the analysis indicated a general trend of low support for most major nodes and highest support for nodes closer to the taxa (Fig. 1).

Analysis 2

Due to the high level of homoplasy present in the unweighted trees in Analysis 1, a weighted analysis using the successive approximations character weighting scheme (SACW) was performed, in an attempt to resolve some of the homoplasy.

As in analysis 1, all 73 characters were unordered and parsimony informative, with the trees rooted using the outgroup comparison method. The analysis resulted in 73 equally parsimonious trees, and a strict consensus tree was generated (Fig. 2), each tree having a shortest possible length of 972 steps.

The strict consensus tree showed a greatly improved level of resolution. The major polytomy in the unweighted analysis was resolved in the weighted analysis, but homoplasy remained at a high level. *Polyxena* remained embedded within *Lachenalia*. The reduction in value placed on highly homoplasious characters had the effect of repositioning the clades, resulting in a significantly altered tree topology. However, if the outgroup is disregarded, and the ingroup is treated as unrooted in both analyses, a comparison of the relationships within the ingroup in both analyses revealed that most taxa remained placed adjacent to one another, indicating no major conflict in the topologies of the two trees. The weighted tree comprised six synapomorphies. In addition to the four synapomorphies obtained in the unweighted tree, fifty-two taxa in the uppermost part of the tree were supported by seeds with inflated strophioles, and a clade near the base of the tree comprising twenty taxa was supported by cup-shaped lower bracts.

For the most part, the consensus trees corroborate conventional wisdom in terms of the relationships between species, and almost all infraspecific taxa were grouped adjacent to one another. With regard to vegetative characters, a clear pattern that emerged in both the unweighted and weighted consensus trees was the convergent evolution of character states in response to conditions of aridity. With regard to reproductive characters, a clear pattern that emerged in both consensus trees was the convergent evolution of character states in response to sunbird pollination for all the species known to be pollinated by sunbirds (*L. aloides*, *L. bulbifera*, *L. reflexa*, *L. rubida* and *L. viridiflora*) as well as the putatively sunbird-pollinated *L. algoensis*.

CONCLUSION

A number of evolutionary pressures have driven divergence of vegetative and reproductive characters in *Lachenalia*. The convergent adaptation to conditions of aridity appears to be the main reason for homoplasy in whole sets of vegetative characters. Similarly, the convergent modification of flowers to similar pollinators is probably the main reason for homoplasy in whole sets of reproductive characters.
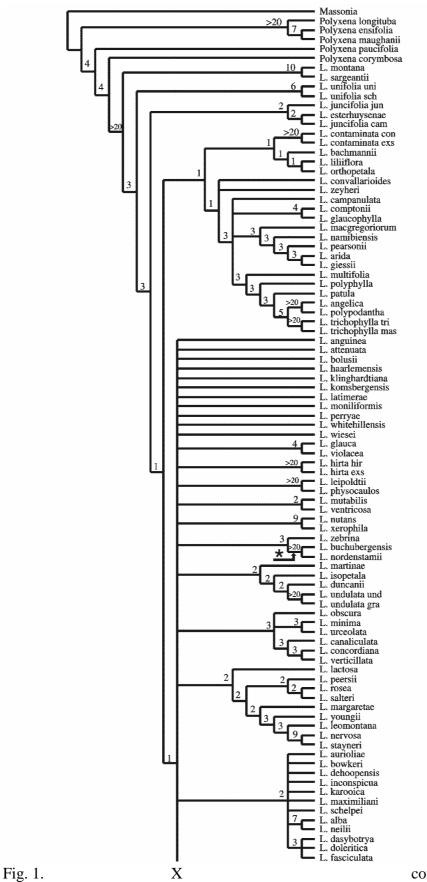
The high degree of variation in morphological characters in *Lachenalia* was reflected in the overwhelmingly homoplasious results obtained in the cladistic analyses. Most morphological characters were ambiguous as they have evolved repeatedly across evolutionary time.
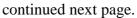
In relation to the large number of taxa analysed, the number of available morphological characters suitable for cladistic analysis is insufficient. With the high level of homoplasy contained in the consensus trees it is not possible to confidently develop

hypotheses on the evolution of most of the characters. However, it is clear that the paraphyletic evolutionary development of *Polyxena* indicates its correct placement within *Lachenalia*.

Pfosser and Speta (1999) have shown that it is difficult to find morphological characters yielding synapomorphies for groups of taxa within the Hyacinthaceae. In *Lachenalia*, morphological characters alone are insufficient to trace the evolutionary history of the genus, and other approaches such as molecular and cytological methods will be needed in conjunction with morphology in order to generate a robust phylogenetic tree of the genus.

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Fig. 1.   X

Fig. 1. Strict consensus of 71153 equally parsimonious trees generated by cladistic analysis of morphological matrix for *Lachenalia* and *Polyxena*, using *Massonia* as outgroup (Duncan, 2004). Numbers above branches are Bremer support (decay) indices. Asterisks indicate synapomorphies: * = seeds with long chalazal collars (character 73, state 1), ** = concrescent leaf bases (character 10, state 1), *** = medial inner filament insertion (character 55, state 1), **** = long filaments (character 52, state 1).

Massonia
Polyxena longituba
Polyxena ensifolia
Polyxena maughanii
Polyxena paucifolia
Polyxena corymbosa
L. sargeantii
L. montana
L. esterhuysenae
L. juncifolia cam
L. juncifolia jun
L. polyphylla
L. comptonii
L. contaminata con
L. contaminata exs
L. patula
L. bachmannii
L. liliiflora
L. orthopetala
L. zeyheri
L. convallarioides
L. campanulata
L. macgregoriorum
L. glaucophylla
L. multifolia
L. angelica
L. polypodantha
L. arida
L. giessii
L. namibiensis
L. pearsonii
L. moniliformis
L. hirta hir
L. hirta exs
L. anguinea
L. nutans
L. xerophila
L. duncanii
L. whitehillensis
L. attenuata
L. haarlemensis
L. komsbergensis
L. latimerae
L. perryae
L. wiesei
L. zebrina
L. buchubergensis
L. nordenstamii
L. maximiliani
L. mathewsii
L. isopetala
L. trichophylla tri
L. trichophylla mas
L. unifolia uni
L. unifolia sch
L. bowkeri
L. dehoopensis
L. inconspicua
L. karooica
L. aurioliae
L. doleritica
L. schelpei
L. undulata und
L. undulata gra
L. minima
L. urceolata
L. obscura
L. canaliculata
L. fasciculata
L. concordiana
L. verticillata
L. ameliae
L. namaquensis
L. framesii
L. valeriae
L. splendida
L. carnosa
L. congesta
L. kliprandensis
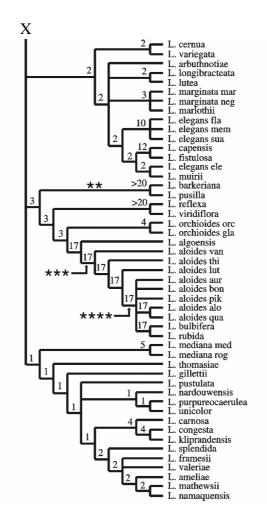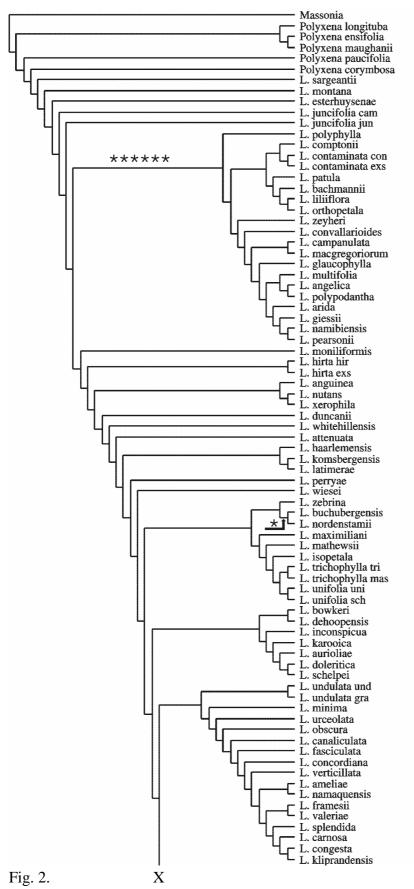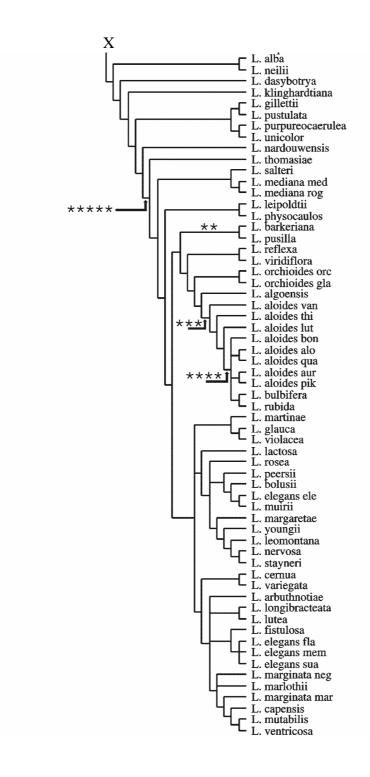
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Fig. 2. X

Fig. 2. Strict consensus of 73 equally parsimonious trees generated by cladistic analysis of successively weighted morphological matrix for *Lachenalia* and *Polyxena*, using *Massonia* as outgroup. Asterisks indicate synapomorphies: * = seeds with long chalazal collars (character 73, state 1), ** = concrescent leaf bases (character 10, state 1), *** = medial inner filament insertion (character 55, state 1), **** = long filaments (character 52, state 1), ***** = seeds with inflated strophioles (character 71, state 1), ****** = cup-shaped lower bracts (character 34, state 1).

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