The Species Delimitation of *Lachenalia unifolia* and *L. hirta*

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**Abstract**

The genus *Lachenalia* consists of bulbous geophytes growing mainly in the Western Cape. This genus produces beautiful pot plants and is, therefore, very valuable as an export product to countries like the Netherlands. *Lachenalia unifolia* and *L. hirta* differ mainly in regard to their hairiness, whereas many other morphological characters correspond. Several cytogenetic studies indicated that *L. unifolia* has somatic chromosome numbers of \(2n = 16, 21, 22, 24\) and \(26\), whereas *L. hirta* has \(2n = 18, 22\) and \(24\). This study indicated that the majority of specimens from both species are diploids, with \(2n = 2x = 22\). DNA amplification fingerprinting indicated that the genetic variation between the two species is only marginally higher than the variation within any of these species. Consequently, this study suggests that these species probably represent two subspecies of the same species, rather than two separate species.

**INTRODUCTION**

The genus *Lachenalia* Jacq. f. ex Murray, or the Cape cowslip, contains species of considerable character and beauty. The genus is endemic to southern Africa. The colourful flowers are ideal for pot plants. New hybrids are being developed and exported to the Netherlands. This genus is economically important (Niederwieser et al. 1998).

*Lachenalia hirta* (Thunb.) Thunb. is closely related to *L. unifolia* Jacq. The flowers are similar in shape and colour. The leaves of *L. hirta* are hairy, compared to the smooth leaves of *L. unifolia*. Both species have either no spots on the leaves, or maroon spots or stripes. The environment, i.e. shade or sun, influences this feature. It is, therefore, problematic to use prominent features such as these for classification and identification. *Lachenalia hirta* is closely related to *L. unifolia* and it is uncertain whether these two species are one variable species or two separate species (Duncan 1988).

The morphological similarities between these species are emphasized by their taxonomical classification, where two classification systems included them in the same taxonomic group. Three different subgeneric classification systems have been described for *Lachenalia*, i.e. the systems of Baker (1897), Crosby (1986) and Duncan (1988). Baker (1897) included both *L. unifolia* and *L. hirta* in the subgenus Chloriza. Crosby (1986) used *L. unifolia* as the typical species of his *L. unifolia* group in his subgeneric classification of the genus, whereas *L. hirta* remained unclassified in his treatment. Duncan (1988) classified both *L. unifolia* and *L. hirta* into subgroup 1e. The subgeneric classification system of Crosby (1986) was used during this study.

Various taxonomic aids can be used to determine species boundaries. In this study cytogenetics, electron microscopic studies (seeds and leaves) and DNA amplification fingerprinting (DAFs) are used for the delimitation purposes. Previous cytogenetic studies indicated somatic chromosome numbers of 16, 21, 22, 24 and 26 for *L. unifolia* (Moffett 1936; De Wet 1957; Ornduff and Watters 1978; Crosby 1986; Hancke 1991; Johnson and Brandham 1996) and \(2n = 18, 22\) and \(24\) for *L. hirta* (De Wet 1957; Ornduff and Watters...
The aim of the study is to use cytogenetics, scanning electron microscopy and DAFs, to determine whether the delimitation of *L. hirta* and *L. unifolia* is correct.

**MATERIALS AND METHODS**

Two specimens of *L. hirta* and 18 of *L. unifolia* were used. Bulbs were obtained from the Roodeplaat Agricultural Research Institute and planted in the nursery at the University of the Orange Free State. These plants were used to collect the necessary cytogenetic and molecular material.

Root tips were collected, pretreated, fixed (Spies et al. 2000) and squashed in 2% aceto-orcein (Darlington and LaCour 1976) and permanently mounted in Euparal.

Leaf material were fixed in 3% glutaraldehyde, dehydrated and critical-point dried before the surfaces were examined under a Jeol 6400 WINSEM scanning electron microscope. Leaves and seeds were coated with gold before being studied.

Leaf material was stored in a saturated Sodium Chloride-CTAB solution and DNA was extracted (Rogstad 1992). PCR reactions were optimized according to the Taguchi method (Cobb and Clarkson 1994), using a Perkin Elmer thermocycler. Eight DAF primers {DAF1 - 5'-AACGGGTG-3'; DAF2 -5'-GTAACGCC-3'; DAF3 - 5'-GAGGGTG-3'; DAF5 -5'-GGAACGCC-3'; DAF6 - 5'-GTTACGCC-3'; DAF7-5'-CTGGACTA-3'; DAF8 - 5'-GTAACGCC-3'; DAF9 - 5'-GTACTGCC-3'} were used individually and in four different multiplex combinations {DAF1 and DAF2; DAF3 and DAF5; DAF6 and DAF7; DAF8 and DAF9}. The amplification products were separated on a 5% poly-acrylamide gel (Caetano-Anollés et al. 1993). The DNA amplification products were visualized using a silver staining method (Bassam et al. 1991).

Pair-wise genetic distances were calculated with the formula

\[ D = -\ln(F), \]

where \( F = 2X_{1,2}/(X_1 + X_2) \) and \( X_{1,2} \) represents the number of corresponding fragments present in both specimens, \( X_1 \) the number of fragments in the first specimen and \( X_2 \) the number of fragments in the second specimen (Nei 1987).

**RESULTS AND DISCUSSION**

**Cytogenetics**

A somatic chromosome number of \( 2n = 22 \) (Table 1) was observed for both *L. unifolia* (Figure 1A-C) and *L. hirta* (Figure 1D) and confirm the chromosome numbers of previous studies (Moffett 1936; De Wet 1957; Ornduff and Watters 1978; Crosby 1986; Hancke 1991; Johnson and Brandham 1996) for both species. Various other chromosome numbers were also described in the literature, i.e. \( 2n = 16, 18, 21, 24, 26 \) and 44 (Moffett 1936; De Wet 1957; Ornduff and Watters 1978; Crosby 1986; Hancke 1991; Johnson and Brandham 1996). The deviating chromosome numbers may be attributed to misidentified specimens (especially specimens with possibly \( 2n = 2x = 16, 2n = 2x = 18, 2n = 3x = 21, 2n = 4x = 24 \) and \( 2n = 2x = 26 \)), the presence of putative B-chromosomes without recognizing them as such (perhaps \( 2n = 2x = 22+2B \)) (Hancke and Liebenberg 1990; Johnson and Brandham 1996) or simply to erroneous chromosome counts due to the very small chromosomes present in *Lachenalia*. The overwhelming number of reports suggesting multiples of 11 (Moffett 1936; De Wet 1957; Ornduff and Watters 1978; Crosby 1986; Hancke 1991; Johnson and Brandham 1996; this study), clearly indicate that both species have a basic chromosome number of 11.

**Seed Morphology**

Seed morphology (i.e. the seed patterns and -textures of the testa as seen under the scanning electron microscope) is often used in this genus as a taxonomical aid (Duncan 1989; Barker 1989; Dold and Phillipson 1998). The seed of *L. unifolia*, and all of its varieties, have a terminal, ridged arillode (Barker 1989). New species have already been assigned to different subgeneric groups based on seed characters. No study has
unfortunately been done on the whole genus (Crosby 1986). Scanning electron microscope work has only been done on some Western (Barker 1989) and Eastern Cape species (Dold and Phillipson 1998).

This study concentrated on three species in the *L. unifolia* group (Crosby 1986): i.e. *L. juncifolia*, *L. unifolia* and *L. mediana*, as well as the unclassified *L. hirta*. The seeds of *L. hirta* are ± 1 mm long, with minor irregular ridges over the surface (Figure 2). It has a short arillode. The seeds of *L. hirta*, *L. juncifolia* and *L. unifolia* are similar in size (± 1 mm) and shape (Figure 2). The arillode is minute in these species with a base adjacent to the arillode (Barker 1989). The minor ridge connecting the arillode with the base is similar in these species. It is possible to classify *L. hirta* into the *L. unifolia* group according to the seed characters, but other criteria are needed to support this theory.

However, although *L. mediana* belongs to the *L. unifolia* group, its seed differs from the other two species in this group (*L. unifolia* and *L. juncifolia*). The body of the seed is the same size of that of the other species (1 mm), but is globose with a long, terminal inflated arillode (Barker 1989). This species is either miss-classified, or the use of seed characters as diagnostic criteria must be reviewed, or the classification system of Crosby (1986) is not a natural system.

The seeds of *L. unifolia* have smooth surfaces (Figure 2A) compared to the rough surfaces of *L. hirta* (Figure 2B). The shape is very similar. *Lachenalia juncifolia*, *L. unifolia* and *L. hirta* bare remarkable similarities with one another, whereas the seed of *L. mediana* shows no resemblance with them. This suggests that *L. hirta* belongs to the *L. unifolia* group and *L. mediana* probably to another group, but further morphological and molecular studies need to be done to determine the real classification. Further studies on the leaf surfaces will determine its contribution to classification.

### Leaf Surfaces

Trichomes are important for the comparative systematic investigations of angiosperms. The length, size and density of the trichomes vary in response to varied environmental conditions. Delimitation of species, genera or families can be done on the basis of the type of trichomes. Expected but absent trichomes in a species, may be because of an inherited trait that was not inherited because of a mutation or genetic change (Metcalfe and Chalk 1979). Trichomes were observed in only one accession of *L. hirta* and *L. unifolia*. The trichomes of *L. hirta* are divided into the long multicellular category and are thickened (Figure 3). The trichomes of *L. unifolia* are small and unicellular and occur on the abaxial side of the leaf. Specimens of both species from different localities differ, i.e. some specimens have trichomes whereas others don’t (Figure 3).

### Genetic Distances

Small genetic distances were obtained for *L. unifolia* specimens within a locality. This indicates little variation and is confirmed by the fact that this genus propagates mostly through bulbs. The genetic distances between *L. unifolia* specimens from different localities were, although small, greater than within a specific locality. This suggests a rapid evolution of this genus between different geographical areas.

*Lachenalia hirta* (D = 0.53) shows more variation within the species than *L. unifolia* (D = 0.33). The genetic distance between the two species is higher than within the *L. unifolia* specimens from different localities (Figure 4). The average genetic distance between *L. hirta* and *L. unifolia* is 0.46. This indicates that the two species are closely related and, although these two species differ slightly, they may form the extreme groups within a single species. Sequence data and crosses between these species should contribute to the species delimitation in this case.

### CONCLUSIONS

Both *L. hirta* and *L. unifolia* are diploids with 2n = 2x = 22, indicating that these species correspond at a cytogenetic level.

The seed morphology of these two species is very similar in shape and size. This,
together with the variation and consequent overlapping of leaf surface morphology within the species, indicate that these species are closely related. The plant morphology and the shape and size of the \textit{L. hirta} seeds, indicate that \textit{L. hirta} should at least be classified in the same subgeneric group.

The molecular studies indicate that the genetic distance between \textit{L. hirta} and \textit{L. unifolia} is greater than within \textit{L. unifolia}. The distance between the two species is small and they are closely related.

**ACKNOWLEDGMENTS**

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**Literature Cited**


Table 1. List of specimens studied, their voucher numbers, somatic chromosome numbers and localities

<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher</th>
<th>2n</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. hirta</em></td>
<td>Spies 6858</td>
<td>22</td>
<td>WESTERN CAPE.–3218 (Clanwilliam): 3 km from Elandsbaai (-AD).</td>
</tr>
<tr>
<td></td>
<td>Spies 6859</td>
<td>22</td>
<td>WESTERN CAPE.–3219 (Wuppertal): between Pakhuis Pass and Biedouvallei, Clanwilliam district (-AA).</td>
</tr>
<tr>
<td><em>L. unifolia</em></td>
<td>Spies 6865</td>
<td>22</td>
<td>NORTHERN CAPE.–2917 (Springbok): Wildepaardehoek (-DC).</td>
</tr>
<tr>
<td></td>
<td>Spies 6866</td>
<td>22</td>
<td>NORTHERN CAPE.–3017 (Hondeklipbaai): along the way to Soebatsfontein in the Kamieskroon district (-BA).</td>
</tr>
<tr>
<td></td>
<td>Spies 6878, 6879</td>
<td>22</td>
<td>NORTHERN CAPE.–3017 (Hondeklipbaai): Soebatsfontein road, 5 km from Kamieskroon (-BB).</td>
</tr>
<tr>
<td></td>
<td>Spies 6861</td>
<td>22</td>
<td>WESTERN CAPE.–3218 (Clanwilliam): Paleisheuwel (-BC).</td>
</tr>
<tr>
<td></td>
<td>Spies 6872</td>
<td>22</td>
<td>WESTERN CAPE.–3318 (Cape Town): Darling (-AD).</td>
</tr>
<tr>
<td></td>
<td>Spies 6874</td>
<td>22</td>
<td>WESTERN CAPE.–3318 (Cape Town): Jonkershoek forestry station (-DD).</td>
</tr>
<tr>
<td></td>
<td>Spies 6873, 6881</td>
<td>22</td>
<td>Unknown – grown from seeds</td>
</tr>
<tr>
<td><em>L. unifolia</em> var. unifolia</td>
<td>Spies 6898</td>
<td>22</td>
<td>WESTERN CAPE.–3218 (Clanwilliam): Along road between Klawer and Clanwilliam (-BB).</td>
</tr>
<tr>
<td></td>
<td>Spies 6889</td>
<td>22</td>
<td>WESTERN CAPE.–3219 (Wuppertal): At the farm of Strauss, 6 km from the Wuppertal, Biedouvallei crossing (-AC).</td>
</tr>
<tr>
<td></td>
<td>Spies 6891</td>
<td>22</td>
<td>Unknown – grown from seeds</td>
</tr>
<tr>
<td><em>L. aff. unifolia</em></td>
<td>Spies 6899</td>
<td>22</td>
<td>WESTERN CAPE.–3318 (Cape Town): Tienie Versveldt Nature Reserve, Darling (-AD).</td>
</tr>
</tbody>
</table>
Figures


Fig. 2. Seed morphology of *L. unifolia* (left) and *L. hirta* (right).

Fig. 3. Leaf surfaces of *L. unifolia* (top) and *L. hirta* (below).
Fig. 4. Map indicating the positions and genetic distances among specimens from the same locality (written in the circle) and between different localities (written along the line). *Lachenalia unifolia* localities are indicated by a U and *L. hirta* specimens by an H in the circles.