Leaf mottling/variegation and shape in the *Ledebouria revoluta* complex – development, stability and putative function.

Elizabeth Mwafongo[1,3], Ane Victoria Vollsnes[2], Charlotte Sletten Bjorå[1], Inger Nordal[2] and Aud Berglen Eriksen[2]

[1]Natural History Museum, P.O. Box 1172 Blindern, NO-0318 Oslo, Norway

[2]Dept. of Biosciences, University of Oslo, P.O. Box 1066 Blindern, NO-0316 Oslo, Norway

[3]Present address: The National Herbarium & Botanic Gardens Headquarters, P.O. Box 528, Zomba, Malawi

Corresponding author: A. B. Eriksen, a.e.b.eriksen@ibv.uio.no

Abstract

The aims of this paper are three-fold: 1. To analyse the development and stability in vegetative traits such as leaf shape, growth and pigmentation patterns in three different morphotypes (defined by leaf shape and pigmentation) of the *Ledebouria revoluta* complex. 2. To discuss the putative function of leaf mottling/variegation. 3. To discuss plasticity in these traits in relation to taxonomy and species delimitation within the complex. Clones were analysed in a cultivation experiment with two nitrogen levels (N1 and N2), three morphotypes (A, B & C) and two light levels (L1 and L2). Anthocyanins were found in hypodermal cells, particularly in the proximal (i.e. young) parts of the leaf. The red pigmentation faded out in distal (i.e. more mature) parts of the leaf. Furthermore, older, outer leaves had more pigmentation than younger, inner leaves. Increased nitrogen availability had no effect on leaf pigmentation. Some plants developed significantly more red pigmentation adaxially at high light intensities, whereas abaxial pigmentation was unaffected by light intensity. The pigmentation of the two sides of a leaf therefore seems to be regulated independently and may accordingly serve different functions for the plants. Adaxial pigmentation (mainly in the form of mottling, pigmentation mainly in spots) may serve as photoprotection, whereas abaxial pigmentation (mainly in the form of variegation, pigmentation mainly in bands) may possibly be aposematic. In the field a high degree of intrapopulational variation in pigmentation patterns was observed. This might be due to local habitat heterogeneity and gene flow or frequency dependent selection. Characters relevant to taxonomy (leaf shape, pigmentation pattern) only changed to a limited extent, suggesting that the vegetative traits are genetically based. The three different morphotypes were easily recognized unrelated to the different treatments. Whether a formal taxonomic status is justified for the different morphotype will need more plant material and genetic data and cannot be decided based on this study.

Keywords:

Anthocyanins; Aposematism; Herbivory; Leaf pigments; Nitrogen; Photoprotection; Plant morphotypes

Highlights

- Pigment development was independent of nitrogen, but depended on light intensity
- Adaxial pigmentation increased with increased light intensity, abaxial was unaffected
- Adaxial pigmentation might act as a sunscreen against high light intensities
- Abaxial pigmentation might protect against herbivory
- Taxonomically important vegetative traits as leaf shape and pigmentation pattern were fairly stable across treatments

1. Introduction

1.1 The genus Ledebouria

The genus *Ledebouria* used to be referred to the family Hyacinthaceae (e.g. Dahlgren et al., 1985; and recently finished or ongoing African Flora projects, such as Flora of Tropical East Africa (Stedje, 1996), Flora of Ethiopia (Stedje, 1997), and Flora Zambesiaca (Stedje and Kativu, in press)). The Angiosperm Phylogeny group (APG IV 2016) sunk this family into Asparagaceae, as subfamily Scilloideae. This subfamily includes bulbous plants with leaves in a basal rosette and a leafless scape. The generic delimitation within the subfamily has undergone dramatic changes through the last decades (Manning et al., 2004, 2009; Pfosser and Speta, 1999; Speta, 1998; Stedje and Kativu, in press), and the final taxonomic classification is not settled. The genus *Ledebouria*, originally described from India, appears, however, to have found its delimitation by consensus, since Jessop (1970) undertook a taxonomic revision, delimitating *Ledebouria* versus *Scilla*.

Within the subfamily Scilloideae, the genus *Ledebouria* is characterized by its inflorescence being a simple raceme, tepals free and reflexed, stamens and style purplish-violet, and seeds subglobose (not flat). The leaves are mottled or variegated with reddish pigmentation as often as not.

Species delimitation in the genus is notoriously difficult, and species numbers from 20 (Stedje, 1996, 1997) to 50 (Speta, 1998) have been proposed. Species of the genus *Ledebouria* inhabit a wide range of habitats from grasslands, rocky outcrops, bushland and woodland. Here they grow in medium and fine sandy soil to clayish soil from sea level up to 2800 m (Stedje, 1996, 1997; Venter, 2008; Stedje and Kativu, in press). Most *Ledebouria* species are adapted to seasonal climates with fairly long dry periods. This appears to be particularly useful in grasslands and fynbos, which are adapted to regular burning in the dry season (Manning et al., 2004). *Ledebouria* species are among the first to sprout immediately when the first rains start and are thus exposed to heavy grazing by both vertebrates and invertebrates.

The *Ledebouria revoluta* complex is a very variable group of plants which may include more than one species, but where further species delimitation is not yet agreed upon (cf. Stedje and Kativu, in press). The species complex has a wide distribution in tropical and southern Africa (own observations; Stedje, 1996, 1997; Venter, 2008; Stedje and Kativu, in press). Plants are used as ornamentals in home gardens or as potted plants, mainly due to the decorative effect of the variegated leaves (Mwafongo, 2009).

1.2 Leaf pigmentation and the particular role of anthocyanins

Leaf pigmentation in different patterns is known in several angiosperm genera, not least within lilies in the wide sense. The terminology when it comes to describe the patterns is confusing. Terms like *blotching*, *flecking*, *maculation*, *mottling*, *spotting*, and *variegation* are found in relevant literature.

Apparently, mottling and variegation are the more general terms. Here we have used the term "mottling" when the pattern mainly consists of more or less irregular spots, and variegation when the pattern displays presence of differently coloured zones (stripes) in the leaves. There are, however, transitional forms, not at least in *Ledebouria*, where often a combination of spots and bands are found (Fig. 1).

>>insert Fig. 1 here

Within the monocots reddish mottling/variegation is known in several Asparagalean families, such as Asparagaceae (*Chlorophytum*, *Drimia*, *Lachenalia* and *Ledebouria*),

Amaryllidaceae (*Haemanthus*, *Scadoxus*) and quite a number of species in Orchidaceae. The phenomenon is also widely spread in Araceae (Arales).

The leaf pigmentation consists of the water-soluble pigment group anthocyanins, which is ranging in colour from red, purple to bluish. The colour is influenced by the chemical structure of the pigment and pH of the cell vacuoles, the main storage compartment of anthocyanins.

The functional role of the pigmentation in reproductive structures is obvious, giving visual guide to pollinators and dispersers (Grotewold, 2006). The physiological roles of anthocyanins in vegetative tissues have, on the other hand, been disputed for more than a century. In leaves, they are often found in tissues close to the upper epidermis (Lee and Collins, 2001; Hormaetxe et al., 2004; Velissarios-Phaedon and Manetas, 2006; Hughes and Smith, 2007; Hughes and Lev-Yadun, 2015). Numerous functions of the foliar anthocyanins have been proposed and may be classified as: (1) protection against abiotic stresses (Chalker-Scott, 1999, Steyn et al., 2002), (2) part of the defence system against herbivory (Manetas, 2006; Hughes and Smith, 2007; Hughes and Lev-Yadun, 2015), or against fungal attacks (Coley and Aide, 1989).

Anthocyanin production may increase in plants subjected to abiotic stresses such as nutrient deficiency, soil salinity, drought, excess temperatures and radiation (UV and visible light), and reactive oxygen species (ROS) (Chalker-Scott, 1999; Steyn et al., 2002; Erilmaz, 2006; Lillo et al., 2008; Basu et al., 2010). Nitrogen availability may have an impact on the anthocyanin content. Hilbert et al. (2003) found that too high soil nitrogen levels caused reduction in the grape anthocyanin content. Further, Peng et al. (2008) showed that anthocyanin production was part of the adaptation to nitrogen limitation in *Arabidopsis*. The protective effect may depend on which specific anthocyanins are produced (Kayanja et al., 2014; Landi et al., 2015) and where they are stored (Kovinich et al., 2015). Anthocyanin content further depends on the factors controlling its degradation (Passeri et al., 2016).

Anthocyanins absorb the highly energetic blue and green light without taking part in photosynthesis, and protect against excess light intensity. Thus, photoinhibition is reduced, but simultaneously, the photosynthetic efficiency is reduced (Hughes et al.,

2014). The chloroplasts in juvenile leaves are not fully developed and are vulnerable to photoinhibition and thus the young leaves may contain anthocyanins more often than mature leaves (Ranjan et al., 2014).

The production of anthocyanins in the leaves correlates with production of other phenolic compounds that act in the chemical defence against herbivores (Lillo et al., 2008; Cooney et al., 2012) as well as against fungi (Coley and Aide, 1989). This correlation between low palatability and anthocyanin colours may impact on herbivore behaviour, reducing feeding on red, purple or bluish plant parts, as suggested by several studies (Maskato et al., 2014; Green et al., 2015). Most insects lack red light photoreceptors (Döring et al., 2009), and for these insects the red colouration of leaves may look unattractive and less bright. Insect herbivores normally camouflaged on plain green leaves, may become more discernible to predators on the red parts of leaves. Experiments with plant phenotypes with red and green leaves showed greater herbivore damage, higher number of leaves attacked and larger area lost due to herbivory on the green phenotype (Karageorgou and Manetas, 2006).

Several hypotheses have been suggested to explain the evolution of intrapopulational polymorphism in leaf mottling/variegation. Smith (1986) provided experimental evidence for the adaptive significance of the phenomenon, and presented several hypotheses to explain such polymorphism. He based his hypothesis on a study of the tropical liana *Blyttneria aculeolata* (Malvaceae), polymorphic in variegation. The variegation was primarily a juvenile character and the variegated forms were more common in open sites. One explanation was that the discoloured parts of the leaf mimicked damage or colonization by herbivores, thus deterring potential new herbivores. Opposite to Smith (1986), Givnish (1990) observed that mottling in temperate herbs was more common in forest habitats than in open sites. He proposed that mottling/variegation serves to camouflage the foliage of certain groups of forest herbs by disrupting their outline as perceived by colour-blind vertebrate herbivores in sun-dappled understories. However, the ecological and thus evolutionary conditions were very different in Smith's tropical flora compared to Givnish's temperate flora.

Allen and Knill (1991), as well as Brown and Lawton (1991), supported the hypothesis of Smith (1986) versus Givnish (1990), that mottling, representing 'pseudodamage' mimicking leaf miners, might be protective against herbivores, being it invertebrates or vertebrates.

Lev-Yadun and Inbar (2002) studied plants where the spots obviously mimicked ants, aphids or caterpillars rather than "pseudo-damage". They suggested that particularly insects would avoid these plants since they were already "infected". When the insect mimicked by the plant is dangerous or aposematic, also larger herbivores might avoid grazing on the plants. Lev-Yadun and Niemelä (2017) elaborated on this tissue and introduce the term "pseudo-variegation" as a plant mimic defense.

Smith (1986) pointed out one weakness in his argumentation, for plain leaves being more attractive than mottled. The herbivores' preference, might relate to the ecological conditions in the habitats of the plain leaf morphs. The herbivores might prefer the shaded areas where the plain leaved forms are overrepresented. Campitelli et al. (2008) designed their sampling to minimize this type of confounding factors, based on studies of a temperate species, *Hydrophyllum variegatum* (Boraginaceae). Their results showed that the non-variegated form displayed nearly twice the amount of damage by herbivory compared to the variegated form. Later, Soltau et al. (2009) tested the defensive potential of leaf variegation by painting artificial variegation on non-variegated leaves of *Caladium steudneriifolium* (Araceae) and found that it indeed reduced herbivore attacks.

Lev-Yadun (2014a) has further discussed the phenomenon of leaf mottling. Two different dazzle effects of "zebralike white leaf variegation" may be involved in defending plants from herbivory, making it hard for herbivores to decide where in a three dimensional space to bite the leaves (large herbivores) or land on them (insects). This matches in a way Givnish's (1990) camouflage hypothesis.

1.3 Aims of the study

From the start the main aim of this study was to analyse the plasticity of characters such as leaf shape and mottling pattern to serve as taxonomic parameters for classification

purposes within the notoriously difficult *Ledebouria revoluta* complex, leading to an enhanced interest in the patterns and processes of leaf pigmentation.

The ecophysiological dynamics of leaf pigments in the complex were thus studied in relation to light, nutrition (nitrogen) and putative functions. The specific aims are accordingly:

1. To analyse the development and stability in vegetative traits such as leaf shape, growth and pigmentation patterns by a cultivation experiment with two light levels, two nitrogen levels and three morphotypes of the *Ledebouria revoluta* complex.

2. To discuss the putative function of leaf mottling/variegation.

3. To discuss plasticity in these traits in relation to taxonomy and species delimitation within the complex.

2. Materials and methods

2.1 Materials

The study was conducted based on the plant material listed in Table 1. All these samples belong in the *Ledebouria revoluta* (L.f.) Jessop complex sensu Stedje and Kativu (in press). Field observations (7 from Zambia and 2 from Tanzania) were documented photographically (Fig. 1). Plants with bulbs, still in the growing phase, were collected in November 2004 in the field of Zambia and Tanzania and stored in paper bags for slow drying. When arrived at the University of Oslo in December 2004, the plants were potted and placed in a greenhouse. The plants were not watered in January and February, to simulate the dry period in the fields of Zambia and Tanzania. From March to December, regular watering of the plants was performed and once a week addition of fertilizer (0.2% Red Superba and 0.5% calcium nitrate (Yara A/S, Norway)). During the winter period, October to March, additional light by high intensity discharge lamps 400 W Powerstar HQI-BT daylight, OSRAM, Germany, 91.3 ± 4.8 (SD) μmol m^{-2} s^{-1} added at plant height) gave a photoperiod of 12 h and the day and night temperatures were kept at about 20 °C and 16 °C, respectively. During summer, the daylight photoperiod is up to 19 h in

Oslo. The plants were given these conditions for three years, and the plants started early to clone by splitting off lateral bulbs. The plants were allocated to three groups according to leaf morphology and mottling pattern to allow statistical analyses; morphotypes A, B and C. A typical representative of morphotype A (Fig. 2a) is characterized by erect linear leaves up to 1 cm wide, with "tiger-like" variegation on the abaxial surface near the leaf base. Morphotype B (Fig. 2b) is characterised by having more or less erect, lanceolate leaves, with abaxial variegation and variable mottling on the adaxial surface. Morphotype C (Fig. 2c) is characterised by semi-erect broadly lanceolate leaves with no or slight variegation stripes abaxially at the leaf base only.

>>insert Table 1 here

>>insert Fig. 2 here

2.2 Growth experiment

The dormant dry bulbs were planted (1 bulb/pot) in 0.75 L plastic pots (12C - 4 3/4", OS Plastic A/S, Denmark) with a 10:2:1 (v/v/v) mixture of sandy peat soil (Herbia P-soil, Nordic Garden A/S, Norway), sand and perlite (Plant perlite, L.O.G, Norway). The growth experiment was conducted in two environmentally controlled growth rooms, each 10 m^2, differing only in light intensity, in the Phytotron at the University of Oslo. The relative humidity was kept above 60% and day and night temperatures were 26 ± 1 °C and 18 ± 1 °C, respectively. The photoperiod was 12 hours and the light source was high intensity discharge lamps (400 W Powerstar HQI-BT daylight, OSRAM, Germany) which gave a photosynthetic photon flux density of 229 ± 10 μmol m^{-2} s^{-1} and 85 ±5 μmol m^{-2} s^{-1} at plant height in the growth rooms with high or low light, respectively. Two days after potting half of the pots were fertilized once with ammonium nitrate, 37.5 mg N/pot, which is equivalent to 100 kg N ha^{-1}. In the other half no extra N was added. The pots were watered daily as required.

The growth experiment had a split plot design, with two light levels (L1 and L2), two nitrogen levels (N1 and N2) nested in light treatment and three morphotypes (A, B & C) with four cloned bulbs of the 9 accessions (Tab. 1).

2.3 Assessment of growth effects and development of leaf mottling

To follow the development of mottling and the growth rate, one of the outer leaves was selected, and the corners of a 1 cm^2 square were marked with Indian ink at the leaf base. The growth of the leaf cells within the square was studied by measuring the size of the marked square every week. Changes in the squares' position relative to the leaf base were used to calculate relative growth rate (RGR). The distance (D) from the base to the lower corners of the square marked on the leaf was measured once a week for six weeks. The relative growth rate between two points in time (t_1 and t_2) was calculated as:

$$RGR = \frac{\log(D_2) - \log(D_1)}{t_2 - t_1}$$

This resulted in five RGR values per leaf, each representing the RGR of one week. For analyses of effects of nitrogen and light on growth rates, the maximum RGR observed per leaf during this period was chosen.

The pattern and the colour of the mottling inside and outside the square was evaluated and registered by photos every week. After seven weeks of growth, the lamina length and lamina width of the oldest leaf was measured. The lamina length from the base to the tip of the leaf and the maximum leaf width were measured. The leaves with lamina length ≥ 1 cm were counted on each bulb. One mature leaf from each plant was harvested at the end of the experiment and both the abaxial and adaxial surfaces were photographed for the study of the mottling/variegation pattern. The images (2560 pixels × 1920 pixels, jpg format) of individual leaves were captured against a black background. To maximise the image resolution, the distance between the camera and the leaf varied according to the length of the leaf, such that the leaf length filled the image length.

Image analysis was performed in ImageJ (http://rsbweb.nih.gov/ij/). The leaf was separated from the background by drawing a segmented line (with the polygon tool) along the rim of the leaf at high magnification. The image was saved as a tiff file and then split into the three channels; red, green and blue. The red mottling/variegation was easily seen as dark areas on the green channel image. The adaxial surfaces were somewhat shiny and not entirely flat, resulting in uneven reflection across the leaf. The blue image was subtracted from the green image, to eliminate the effect of this unevenness. The

resulting image was thresholded to identify the pigmented parts of the adaxial leaf surface. Since the abaxial surfaces were not as shiny, the pigmentation was best identified by thresholding the result of the green channel image subtracted from the red channel image. The resulting number of pixels covering the red pigmentation and the entire leaf were then used to calculate the percentage of the leaf surface covered by red mottling/variegation, hereafter called the degree of pigmentation.

In order to determine the location of the pigmentation in the red parts of the leaves, thin transverse hand-cut sections of fresh leaves were made using a surgical blade, placed on a microscope slide and photographed.

2.4 Statistical analysis

SPSS (Statistical Package for the Social Sciences, version 24) was used for all statistical calculations. Evaluation of assumptions of normality and homogeneity of variance were done before a nested Analysis of Variance (ANOVA) tests were performed. The data about degree of leaf pigmentation were transformed before analysis (ln(x+1)). The general linear model (GLM) command with a nested design was used ($p < 0.05$ for significant results). The general model was

$$Y_{ijk} = \mu + \alpha_i + \beta_{ij} + \varepsilon_{ijk}$$

where Y is the dependent variable, μ is the overall mean, α_i is the effect of the i-th level of light (low or high light), β_{ij} is the effect of the j-th level of nitrogen (low or high nitrogen addition) at the i-th level of light, and ε_{ijk} is the random error.

For the degree of pigmentation, the datasets included only the genotypes that were successfully represented in all four treatment groups (L2N2, L2N1, L1N2, and L1N1). For analyses of the abaxial surfaces, four genotypes from morphotype B and one from each of morphotypes A and C were represented, whereas for the analyses of adaxial surfaces only four genotypes from morphotype B were included in the dataset, as adaxial pigmentation was lacking in the A and C morphotypes. To study the degree of variation of leaf shape and size characters within each group the quantitative data were plotted as box plots, with the median value inside the box and the borders at the 25^{th} and 75^{th} percentiles. Whiskers extend to the minimum and maximum values. Significant

differences ($p < 0.05$) between morphotypes found in Post Hoc Tukey HSD tests are marked with different letters in the boxplots.

3. Results

3.1 Field observations

The observed/collected plants belonging to the *Ledebouria revoluta* complex grow within the category of tropical "wooded grassland", an open park landscape with 10–40% tree cover, or "grassland" with a continuous grass cover and up to 10% tree cover. In both cases the landscape is heterogeneous combining a mosaic of shaded and open patches (Huntley and Walker, 1982; White, 1983).

Plants belonging in the *Ledebouria revoluta* complex observed in the field in Eastern and Central Zambia during field work in November 2004 (Bjorå & Nordal, Table 1), exhibited striking intrapopulational leaf polymorphism, here documented photographically (Fig. 1). Within very short distances, plants (even overlapping) with distinctly different colouration patterns were recorded. An example is shown in Fig. 1a: adaxial patterns of dense maculation (upper left specimen) and dense striation (upper middle specimen), both classified as morphotype B. In the same population (defined as an area of approximately 50 m × 50 m) leaf morphs were collected and documented in Fig. 1b: leaves in the upper row represent the adaxial pattern, leaves from the lower row the abaxial of the same individuals. Out of 9 specimens no one displayed a pattern that might be classified as "similar". Some populations even contained mixed "morphotypes" (as in Fig. 1c: specimens belonging somewhere between morphotype A and B and a specimen which, with our definition, would be classified as morphotype C). Also morphotype A displayed intrapopulational variation with very different intensities of reddish maculation (Fig. 1d).

In a particular population (Hoell & Nordal 43, Table 1), the abaxial surfaces displayed a shape, position and a pattern of mottling and variegation that mimicked a snake with a

raised head. This characteristic appearance was kept when the plants were cultivated in the environmentally controlled growth rooms in the Phytotron (Fig 3).

>>insert Fig. 3 here

No systematic measurement on habitat qualities with reference to light or nutrition was undertaken. Generally, however, the plants with less pigmentation, mainly belonging in morphotype C, were observed growing under trees, rather than in the open sun exposed habitats of morphotype A and B.

3.2 Phytotron experiment: Leaf pigmentation

Quantitative changes in the pigmentation inside and outside the marked squares were observed during a period of six weeks. In morphotype A and B the red pigmentation gradually faded as the leaf grew from the base, and the anthocyanin containing cells moved upwards with a colour change from deep red to pale red and then to green (Fig. 4a,b). In morphotype C (Fig. 4c) there was no colour change in the squares as the leaf elongation had ended at the start of the experiment. Leaf pigmentation occurred more often on the abaxial side of the leaves (33 out of 39 leaves) than on the adaxial sides (17 out of 39). When occurring, it was also more pronounced on the abaxial side, covering 1–28% of the surface, but only 1–6% of the surface on the adaxial side (Figs. 5, 6, 7). The leaves were further divided in three sections, the proximal, the middle and the distal part along the length of the leaf, and the degrees of pigmentation were compared. Pigmentation was by far more prominent in the proximal i.e. youngest part of the leaves (abaxial side: 86 ± 6 % of pigmented area, adaxial side: 77 ± 8 %, mean ± SE), gradually disappearing in the middle and distal parts.

>>insert Fig. 4 here

>>insert Fig. 5 here

>>insert Fig. 6 here

>>insert Fig. 7 here

Leaf mottling of the adaxial leaf surfaces of morphotype B increased significantly, more than three-fold, following exposure to increased light level (p = 0.03, Fig. 8) but was

unaffected by nitrogen. On the other hand, in morphotype B leaf mottling/variegation on the abaxial surfaces was unaffected by both light (13.5 ± 5.7 % and 14.4 ± 3.1 %, mean ± SE for low and high light, respectively) and nitrogen levels (13.8 ± 4.4 % and 14.1 ± 4.7 % mean ± SE) for low and high N, respectively). This indicates that mottling/variegation on the two leaf surfaces were regulated independently. When all clones (A, B, C) were included, there were also high values of abaxial leaf variegation but no effect of light or nitrogen levels (Fig 8). The degree of mottling on abaxial side was, though, significantly lower in morphotype C than in morphotype A and B in the Post Hoc test ($p = 0.024$ and 0.001, respectively).

>>insert Fig. 8 here

The location of the anthocyanins in the leaves, both adaxially and abaxially, was in the hypodermis which is the cell layer immediately below the epidermis. Leaves with larger areas of tinged surfaces had continuous layers of purple hypodermal cells whereas those bearing stripes and/or spots had discontinuous layers (Fig. 9 a, b).

>>insert Fig. 9 here

3.3 Effects of nitrogen and light on leaf size and number

Lamina lengths of the three morphotypes A, B and C overlapped slightly (Fig. 10a). The distribution of lamina lengths in morphotype B was wide and skewed, illustrating that the leaves were of variable length, but that the shorter leaves were most common. The morphotypes differed significantly in lamina length in the ANOVA test. The post-hoc test showed that lamina length in morphotypes A and C did not differ significantly, but that the leaves of morphotype B were significantly longer ($p = 0.002$ for morphotype A and $p < 0.001$ for morphotype C). The effects of light and nitrogen levels on lamina lengths are illustrated in Fig. 10b. None of these factors had a significant effect on lamina length.

>>insert Fig. 10 here

The box plot based on leaf width for all the three morphotypes (Fig. 11a) showed discontinuity between morphotype A and B. It further showed an overlap in the distribution of leaf width between morphotype B and C. Samples belonging to

morphotype A had significantly narrower leaves (≤ 1 cm) than leaves of morphotype B and C in the Post Hoc test ($p < 0.001$ for both comparisons). Neither light nor nitrogen had any significant effects on the width of the leaves (Fig. 11b).

>>insert Fig.11 here

The distribution of the number of leaves produced within each morphotype during the experimental period overlapped, particularly in morphotype A and B (Fig. 12a). Morphotype C clones typically produced three leaves, whereas morphotype A and B produced on average 6 or 4.5 leaves per plant, respectively. The number of leaves produced in morphotype C was significantly lower than in the two other morphotypes ($p = 0.003$ for morphotype A and $p = 0.01$ for morphotype B) Light, had a significantly positive effect on leaf production ($p < 0.05$), whereas nitrogen did not (Fig. 12b).

>>insert Fig. 12 here

3.4 Maximum growth rate of leaves

The maximum relative growth rates (RGR_{max}) of the three morphotypes overlapped (Fig. 13a). However, morphotype C clones had significantly smaller RGR_{max} than morphotype B ($p = 0.02$), possibly because the leaf growth of morphotype C was more or less completed before the measurements were undertaken. Neither nitrogen nor light had significant effects on the relative growth rate ($p > 0.05$, Fig. 13b).

>>insert Fig. 13 here

4. Discussion

4.1 Dynamics of the leaf pigmentation

The present study has demonstrated a dynamic leaf pigmentation system in the *Ledebouria revoluta* complex. The leaves of the plants grow from the base and the

continuous production of new tissues moves the older leaf parts upwards. The red pigmentation of the leaves is primarily expressed in the young tissues near the base. This finding is in accordance with Smith's (1986) and Manetas' (2006) observations that leaf mottling/variegation most often occurs in young leaves.

Cell division/elongation occurs only near the leaf base of the plants, demonstrated by the fact that the size and shape of the marked squares did not change as they moved upwards from the base and the tissue matured. Thus, the loss of anthocyanins within the squares in morphotypes A and B, as the leaf elongated, does not seem to be due to a dilution effect caused by cell expansion (Mwafongo, 2009). The chloroplasts in mature tissues may not need or need less protection against high light and thus the plants may have degraded the anthocyanins, as previously shown in *Jatropha curcas* (Euphorbiaceae) by Ranjan et al. (2014).

The leaf cross sections revealed that in *Ledebouria* anthocyanins are located in the hypodermis. This is consistent with findings from several other plant species (e.g. Velissarios-Phaedon and Manetas, 2006; Hormaetxe et al., 2004; Hughes and Lev-Yadun, 2015), although other species may primarily produce anthocyanins in epidermis cells, spongy mesophyll cells or palisade cells (Lee and Collins, 2001; Hughes and Smith, 2007; Merzylak et al., 2008; Ranjan et al., 2014).

4.2 Putative function of leaf pigmentation

Despite the wide distribution of mottled/variegated leaves in Angiosperms, there is still no consensus on the function of this characteristic. As indicated in the Introduction, explanatory arguments fall into three categories; one related to physiological factors, such as nitrogen and light in the present study, the other two to ecological factors as herbivory and fungal attack. Of the two physiological factors studied, nitrogen and light, light plays the more important role.

Hormaetxe et al. (2004) and Hughes and Smith (2007) indicated that the pigments may play a protective role by decreasing light interception in chloroplasts which are situated below the upper epidermis. In the present study of the *Ledebouria revoluta* complex, and particularly in the morphotype B, increased light intensity caused increased pigmentation

on the adaxial leaf surface, as would be expected if the function of the pigments was photoprotection. It is possible that the regulation of anthocyanin production and metabolism on the adaxial side of the leaf was influenced by light by the same mechanisms as in leaves using anthocyanins for photoprotection. However, if photoprotection was the only, or major, function, anthocyanin expression is expected to occur over continuous leaf areas rather than in patterns of maculation/variegation.

In our study, pigmentation is generally more pronounced abaxially than adaxially in the leaves, a phenomenon not fully in accordance with the photoprotection hypothesis. Further, the abaxial variegation was mainly found in the lower part of the leaves that will receive the least amount of light, again contrary to a putative dominant photoprotective function. Hughes and Smith (2007) demonstrated, however, that abaxial anthocyanin might also play a role in reducing photoinhibition, particularly when the abaxial surface intercepts sunlight due to leaf orientation.

Alternatively, the presence of mottling/variegation has been linked to camouflage especially for those species growing in sun-dappled understoreys, and the hypothesis predicts that mottling should be more common in forest herbs than in plants growing in sun exposed habitats (Givnish, 1990; Lev-Yadun, 2014a). Since most members of the genus *Ledebouria* are found in open and fairly exposed places, this kind of variegation based camouflage probably does not play an important role here.

Ledebouria revoluta plants are among the very first to sprout with the first rains, often after a harsh dry season. Their appearance before the surrounding vegetation, dominated by grasses, makes them prone to herbivory. Studies have further shown that the extent of seasonality in insect numbers reflects the seasonality of rainfall in different areas in that insect populations are depressed during the dry season, with a marked rebound at the beginning of the wet season (reviewed in Coley and Barone, 1996). Herbivory rates are known to mirror this pattern by being lowest in the dry season and highest in the beginning of the rainy season. Variegation/mottling may mimic already attacked plants (e.g. Lev-Yadun and Niemelä, 2017), or signal to herbivores that the plant has a high content of poisonous compounds, like phenolics (Karageorgou and Manetas, 2006), i.e.

being visually aposematic (Lev-Yadun, 2009). Thus, *L. revoluta* may benefit from leaf mottling/variegation in this first part of the rainy season.

The first leaves appearing from the bulbs of *Ledebouria* become the outer leaves as new leaves are produced from centrally placed meristems. These first leaves are the most exposed to possible herbivores and in our experiments, they also had a higher degree of mottling compared to the less exposed inner leaves. Thus, the colouration may represent signals to herbivores, possibly invertebrates or small mammals (Mwafongo, 2009). Several studies have revealed that in natural populations, mottled/variegated leaves receive less herbivore damage compared to plain leaves (e.g. Smith, 1986; Campitelli et al., 2008; Soltau et al., 2009; Green et al., 2015). The mimicry hypothesis of Smith (1986) may be relevant here: If the coloured spots/stripes appear as leaf damage in the eyes of a colour-blind herbivore, the leaf might be avoided, both due to lower resource quantity and possibly higher induced anti-herbivory toxin level.

Some *Ledebouria* species have been recorded to contain bitter and poisonous substances and leaf mottling/variegating might signal this to putative herbivores (Pohl et al., 2000), a typical case of aposematism sensu Lev-Yadun (2009). If Batesian mimicry were involved, less toxic specimens might imitate the more toxic ones for increased protection (Wiens, 1978). Another form of putative Batesian mimicry has been observed. An aposematic snake-like appearance was striking in some of the plants in the present study (Fig. 3). The shape of the leaf and the mottling/variegation obviously mimic spitting cobras (genus *Naja*), of which there are 7 species in tropical and subtropical Africa, all highly poisonous. Visual snake (viper) mimicry by anthocyanin pigmentation as defence has been proposed to exist in certain pods of wild peas (*Pisum*, Fabaceae) growing in the Middle East (Aviezer and Lev-Yadun, 2015; Lev-Yadun 2017). This putative type of defensive animal mimicry is a new observation for plant leaves.

The hypothesis of Lev-Yadun et al. (2004) and Hughes and Lev-Yadun (2015) that leaf colouration might undermine the camouflage of herbivorous invertebrates in relation to predating birds cannot be excluded. In this case, it would probably be more efficient with larger uniformly coloured “killing zones”, rather than mottles and stripes.

The observed intrapopulational polymorphism in colour patterns (cf. Fig. 1) is still not understood. The phenomenon is well known in e.g. cheating orchids, as a way of confusing pollinators. Since they do not produce nectar, it will take longer time for insects to develop avoiding behaviour. The polymorphy in vegetative traits might be due to habitat heterogeneity. The typical habitat for the *Ledebouria* plants is a mosaic, mainly open and dominated by grass, but with scattered clusters of shrubs and trees. This might create selective regimes, favouring mottling in the most sun exposed patches, and opposite under more shaded patches. Gen flow between the subpopulations might then result in polymorphism as e.g. demonstrated in Fig. 1. The advantage of colour polymorphism in a setting with herbivory is not obvious. However, some studies have indicated that herbivores prefer the most common colour morph in different species. Smith (1986) observed that within a given habitat, the rate of herbivory of leaf miners on a given morph increases with increasing relative frequency of that morph. Allen and Knill (1991) supported this view and stated that leaf miner attacks were frequency dependent. As an atypical morph thus increases in abundance, it will be more vulnerable. It pays to be rare. Herbivore pressure would thus tend to prevent exclusion of one morph by the other, and polymorphism is maintained.

4.3 Plasticity of traits

Leaf mottling and leaf shape have played a role when it comes to species delimitation in *Ledebouria* (Venter, 1993, 2008). The three morphotypes A, B, and C, certainly showed differences regarding leaf shape and pigmentation in their response to light and nitrogen. But they kept their main characteristics throughout the experiment. So in a taxonomic context both the leaf shape and the mottling/variegation pattern (although not density of the pigmentation) are fairly stable, indicating different genotypes rather than phenotypes. Morphotype C responded to nitrogen addition by having longer leaves, whereas morphotype A and B did not. None of the morphotypes responded with significant change in leaf width during the different treatments, meaning that leaf width is potentially a more reliable taxonomic character than leaf length.

Light seemed to have a profound quantitative effect on the mottling of clones belonging to morphotype B, as they produced more spots on the adaxial surfaces when exposed to

high light intensity, while there was no effect of light on the abaxial variegation. Further, light had a significant effect on the number of leaves produced in morphotypes A and C as leaf production increased with increased light intensity.

Nitrogen addition did not influence the traits studied. If the high level of nitrogen had led to a great increase in growth, other mineral nutrients, as e.g. phosphorus, might have become limiting to an extent that increased anthocyanin production (Chalker-Scott, 1999), but this did not occur in the current experiment. Further, the low nitrogen level was sufficiently high to avoid nitrogen limitation and subsequent production of anthocyanins (Peng et al., 2008).

4.4 Conclusion

In conclusion, our results reported for the *Ledebouria revoluta* complex showed that (1) the anthocyanin patterns are dynamic, mottling/variegation is pronounced in younger parts of the leaves and fading in older tissues, and that (2) mottling/variegation may partly have a photoprotective effect, but also probably more importantly, an anti-herbivory effect. Adaxial mottling might serve as photoprotection, whereas abaxial variegation may be aposematic. Further, we found that (3) characters relevant to taxonomy, such as leaf shape and pigmentation pattern, only changed to a limited extent in response to light and nitrogen, suggesting that the vegetative traits are genetically based. The three morphotypes were easily recognized, independent of the different treatments. Whether a formal taxonomic recognition is justified, will need much more material and cannot be decided based on this study.

5. Acknowledgements

The research was undertaken with support from the Norwegian Council for Higher Education Programme for Development, Research & Education, NUFU Grants (39/2002: Biodiversity of Southern Africa and 53/2003: Monocots of Eastern Africa). The authors gratefully acknowledge the assistance of Ingrid Johansen and Marit Langrekken from the

Phytotron staff at the University of Oslo. We are further grateful to two anonymous reviewers who have contributed considerably to the improvement of the manuscript.

6. References

Allen, J.A., Knill, R., 1991. Do grazers leave mottled leaves in the shade? Trends Ecol. Evol. 6, 109-110.

APG IV, 2016. http://www.mobot.org/MOBOT/research/APweb.

Aviezer, I., Lev-Yadun, S., 2015. Pod and seed defensive coloration (camouflage and mimicry) in the genus *Pisum*. Isr. J. Plant Sci. 62, 39-51.

Basu, S., Roychoudhury, A., Saha, P,P., Sengupta, D.N., 2010. Differential antioxidative responses of indica rice cultivars to drought stress. Plant Growth Reg. 60, 51–59.

Brown, V.K., Lawton, J.H., 1991. Herbivory and the evolution of leaf size and shape. Phil. Trans. Biol. Sci. 333, 265-272.

Campitelli, B.E., Stehlik, I., Stinchcombe, J. R., 2008. Leaf variegation is associated with reduced herbivore damage in *Hydrophyllum virginianum*. Botany 86, 306-313.

Chalker-Scott, L., 1999. Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. 70, 1-9.

Coley, P.D., Aide, T.M., 1989. Red coloration of tropical young leaves: a possible antifungal defence? J. Trop. Ecol. 5, 293-300.

Coley, P.D., Barone, J. A., 1996. Herbivory and plant defences in tropical forests. Annu. Rev. Ecol. Syst. 27, 305-35.

Cooney, L.J., van Klink, J.W., Hughes, N.M., Perry, N.B., Schaefer, H.M., Menzies, I.J., Gould, K.S., 2012. Red leaf margins indicate increased polygodial content and function as visual signals to reduce herbivory in *Pseudowintera colorata.* New Phytol. 192, 488-497.

Dahlgren, R.M.T., Clifford, H.T., Yeo, P.F., 1985. The families of the Monocotyledons. Springer-Verlag, Berlin.

584 Döring, T.F., Archetti, M., Hardie, J., 2009. Autumn leaves seen through herbivore eyes.
585 Proc. R. Soc. B. 276, 121-127.

586 Eryilmaz, F., 2006. The relationships between salt stress and anthocyanin content in
587 higher plants. Biotechnol. Biotechnol. Equip. 20:1, 47-52.

588 Givnish, T.J., 1990. Leaf mottling: relation to growth form and leaf phenology and
589 possible role as camouflage. Funct. Ecol. 4, 463-474.

590 Green, J.P., Foster, R., Wilkins, L., Osorio, D., Hartley, S. E., 2015. Leaf colour as a
591 signal of chemical defence to insect herbivores in wild cabbage (*Brassica oleracea).* PloS
592 One 10(9), e0136884. Doi:10. 1371/journal.pone.0136884.

593 Grotewold, E., 2006. The genetics and biochemistry of floral pigments. Ann. Rev. Plant
594 Biol. 57, 761-780.

595 Hilbert,G., Soyer,J.P., Molot, C., Giraudon, S., Milin., S., Gaudillere, J.P., 2003. Effects
596 of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot.
597 Vitis. 42, 69-76.

598 Hormaetxe, K., Becerril, J.M., Fleck, I., Pintó, M., J. I., García-Plazaola, J. I., 2004.
599 Functional role of red (retro)-carotenoids as passive light filters in the leaves of *Buxus*
600 *sempervirens* L.: increased protection of photosynthetic tissues? J. Exp. Bot. 56, 2629-
601 2636.

602 Hughes, N.M., Lev-Yadun, S., 2015. Red/purple leaf margin coloration: Potential
603 ecological and physiological function. Env. Exp. Bot. 119, 27-39.

604 Hughes, N.M., Smith, K.W., 2007. Attenuation of incident light in *Galax urceolata*
605 (Diapensiaceae): concerted influence of adaxial and abaxial anthocyanic layers on
606 photoprotection. Am. J. Bot. 94, 784-790.

607 Hughes, N.M., Carpenter, K.L., Keidel, T.S., Miller, C.N., Waters, M.N., Smith, W.K.,
608 2014. Photosynthetic costs and benefits of abaxial versus adaxial anthocyanins in
609 *Colocasia esculenta* "Mojito". Planta 240, 971-981.

610 Huntley, B. J. & Walker, B. H. 1982. Ecology of tropical savannas. Ecological Studies
611 42. ISBN 978-3-642-68786-0

ImageJ. http://rsbweb.nih.gov/ij/

Jessop, J. P., 1970. Studies in the bulbous Liliaceae: *Scilla*, *Schizocarpus*, and *Ledebouria*. J. South Afr. Bot. 36, 233-266.

Karageorgou, P., Manetas, Y., 2006. The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. Tree Physiol. 26, 613-621.

Kayanja, G., Chanoca, A., Riedl, K., Otegui, M.S., Grotewold, E., 2014. Not all anthocyanins are born equal: distinct patterns induced by stress in Arabidopsis. Planta. 240, 931-940.

Kovinich, N., Kayanja, G., Chanoca, A., Otegui, M.S., Grotewold, E., 2015. Abiotic stresses induce different localizations of anthocyanins in Arabidopsis. Plant Signaling Behav., 10:7, e1027850, DOI: 10.1080/15592324.2015.1027850

Landi, M., Tattini, M., Gould, K.S., 2015. Multiple functional roles of anthocyanins in plant-environment interactions. J. Exp. Bot. 119, 4-17.

Lee, D.W., Collins, T.M., 2001. Phylogenetic and ontogenetic influences on the distribution of anthocyanins and betacyanins in leaves of tropical plants. Int. J. Plant Sci. 162, 1141-1153.

Lev-Yadun, S., 2009. Aposematic (warning) coloration in plants. In: Plant-environment interactions. From sensory plant biology to active plant behavior. Baluska F. (ed). Springer-Verlag, Berlin, pp. 167-202.

Lev-Yadun, S., 2014a. Potential defence from herbivory by ‘dazzle effects’ and ‘trickery coloration’ in leaf variegation. Biol. J. Linn. Soc. 111, 692-697.

Lev-Yadun, S., 2014b. Defensive masquerade by plants. Biol. J. Linn. Soc. 113, 1162-1166.

Lev-Yadun, S., 2017. Defensive animal and animal action mimicry by plants. Isr. J. Plant Sci.64,179-209.

Lev-Yadun, S., Inbar, M., 2002. Defensive ant, aphid and caterpillar mimicry in plants? Biol. J. Linn. Soc. 77, 393-398.

Lev-Yadun, S., Niemelä, P., 2017. Leaf pseudo-variegation: definition, common types, and probably the defended models for real defensive leaf variegation mimicking them? Flora 226, 82-88.

Lev-Yadun, S., Dafni, A., Flaishman, M.A., Inbar, M., Izhaki, I., Katzir, G., Ne`eman, G., 2004. Plant coloration undermines herbivorous insect camouflage. BioEssays 26, 1126-1130.

Lillo, C., Lea, U.S., Ruoff, P., 2008. Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway. Plant Cell Env. 31, 587-601.

Manetas Y., 2006. Why some leaves are anthocyanic and why most anthocyanic leaves are red? Flora 201, 163-177.

Manning, C. J., Forest, F., Devey, D.S. Fay, M. F., Goldblatt, P., 2009. A molecular phylogeny and a revised classification of Ornithogaloideae (Hyacinthaceae) based on an analysis of four plastid DNA regions. Taxon 58, 77-107.

Manning, C. J., Goldblatt, P., Fay, M.F., 2004. A revised generic synopsis of Hyacinthaceae in sub-Saharan Africa, new combinations and the new tribe *Pseudoprospereae*. Edinb. J. Bot. 6, 533-568.

Maskato, Y., Talal, S., Keasar, T., Gefen, E., 2014. Red foliage color reliably indicates low host quality and increased metabolic load for development of an herbivorous insect. Arthropod-Plant Interact. 8, 285-292.

Merzylak, M.N., Melø, T.B., Naqvi, K.R., 2008. Effect of anthocyanins, carotenoids, and flavonols on chlorophyll fluorescence excitation spectra in apple fruit: signature analysis, assessment, modelling, and relevance to photoprotection. J. Exp. Bot. 59, 349-359.

Mwafongo, E., 2009. Studies of *Albuca* and *Ledebouria* (Hyacinthaceae) in the Flora Zambesiaca area: aspects of systematics, ecophysiology and ethnobotany. Ph. D. thesis, University of Oslo. ISSN 1501-7710.

Passeri, V., Koes, R., Quattrocchio, F.M., 2016. New challenges for the design of high value plant products: Stabilization of anthocyanins in plant vacuoles. Front. Plant Sci. 7, 153. doi: 10.3389/fpls.2016.00153.

669 Peng, M., Hudson, D., Schofield, A., Tsao, R., Yang, R., Gu, H., Bi,Y-M., Rothstein, S.J.
670 2008. Adaptation of *Arabidopsis* to nitrogen limitation involves induction of anthocyanin
671 synthesis which is controlled by the NLA gene. J. Exp. Bot. 59, 2933-2944.

672 Pfosser, M., Speta, F., 1999. Phylogenetics of Hyacinthaceae based on plastid DNA
673 sequences. Ann. Missouri Bot. Gard. 86, 852-875.

674 Pohl, T.S., Crouch, N.R., Mulholland, D.A., 2000. Southern African Hyacinthaceae:
675 Chemistry, bioactivity and ethnobotany. Curr. Org. Chem. 4, 1287-1324.

676 Ranjan, S., Singh, R., Singh, M., Pathre, U.V., Shirke, P.A., 2014. Characterizing
677 photoinhibition and photosynthesis in juvenile-red versus mature-green leaves of
678 *Jatropha curcas* L. Plant Physiol. Biochem. 79, 48-59.

679 Smith, A.P., 1986. Ecology of a leaf color polymorphism is a tropical forest species –
680 Habitat segregation and herbivory. Oecologia 131, 283-287.

681 Soltau, U., Dötterl, S., Liede-Schumann, S. 2009. Leaf variegation in *Caladium*
682 *steudneriifolium* (Araceae): a case of mimicry? Evol. Ecol. 23, 503-512.

683 Speta, F., 1998. Hyacinthaceae. In: Kubitzki, K. (Ed.), The Families and Genera of
684 Vascular Plants. Springer, Berlin, pp. 261-285.

685 Stedje, B., 1996. Hyacinthaceae. In: Polhill, R.M. (Ed.), Flora of Tropical East Africa.
686 A.A. Balkema, Rotterdam.

687 Stedje, B., 1997. Hyacinthaceae. In: Edwards, S., Sebsebe, D., Hedberg, I. (Eds.), Flora
688 of Ethiopia and Eritrea, Vol. 7, National Herbarium, Addis Ababa University, Addis
689 Ababa, pp. 138-147.

690 Stedje, B., Kativu, S., (in press). Hyacinthaceae. In: Timberlake, J. (Ed.), Hyacinthaceae.
691 Flora Zambesiaca. Royal Botanical Gardens, Kew.

692 Steyn, W.J., Wand, S.J.E., Holcroft, D.M., Jacobs, G., 2002. Anthocyanins in vegetative
693 tissues: a proposed unified function in photoprotection. New Phytologist. 155, 349-361.

694 Velissarios-Phaedon, K., Manetas, Y., 2006. Mesophyll versus epidermal anthocyanins as
695 potential in vivo antioxidants: evidence linking the putative antioxidant role to the
696 proximity of oxy-radical source. J. Exp. Bot. 57, 2203-2210.

697 Venter, S., 1993. A revision of the genus *Ledebouria* Roth (Hyacinthaceae) in South
698 Africa. M. Sc. Thesis, University of Natal, Pietermaritzburg.

699 Venter, S., 2008. Synopsis of the genus *Ledebouria* Roth (Hyacinthaceae) in South
700 Africa. Herbertia 62, 85-155.

701 White, F. 1983. The vegetation of Africa. A descriptive memoir to accompany the
702 UNESCO/AETFAT/UNSO vegetation map of Africa. Chapman & Hall, London

703 Wiens, D., 1978. Mimicry in plants. Evol. Biol. 11, 365-403.

704

705

706 **Table 1**. List of accessions of specimen in the *Ledebouria revoluta* complex used in the
707 Phytotron experiment. Voucher specimens deposited in Herb. O. (Natural History
708 Museum of University of Oslo). Accessions included in the cultivation experiment
709 marked with an asterisk. The notations i and ii refer to different genotypes within the
710 same population.

Morphotype	**Collector**	**Origin, year**
A	Bjorå & Nordal 576i*	Zambia E, 2004
A	Bjorå & Nordal 576ii*	Zambia E, 2004
A	Bjorå & Nordal 664	Zambia N, 2004
A	Nordal 2013	Zimbabwe S, 1988
B	Bjorå & Nordal 532*	Tanzania T2, 2004
B	Bjorå & Nordal 575*	Zambia E, 2004
B	Bjorå & Nordal 600	Zambia E, 2004
B	Bjorå & Nordal 656i*	Zambia E, 2004
B	Bjorå & Nordal 656ii*	Zambia E, 2004
B	Bjorå & Nordal 669	Zambia C, 2004
B	Hoell & Nordal 43	Zambia N, 2005
B	Stedje 341	Zambia N, 2002
C	Bjorå & Nordal 586*	Zambia E, 2004
C	Bjorå & Nordal 624A*	Zambia E, 2004
C	Bjorå & Nordal 683*	Tanzania T6, 2004

Legends and figures

Figure 1. Intrapopulational leaf polymorphism in plants belonging in the *Ledebouria revoluta* complex from Zambia. a & b) Bjorå & Nordal 669, c) Bjorå & Nordal 600, d) Bjorå & Nordal 664. Voucher information in Table 1.

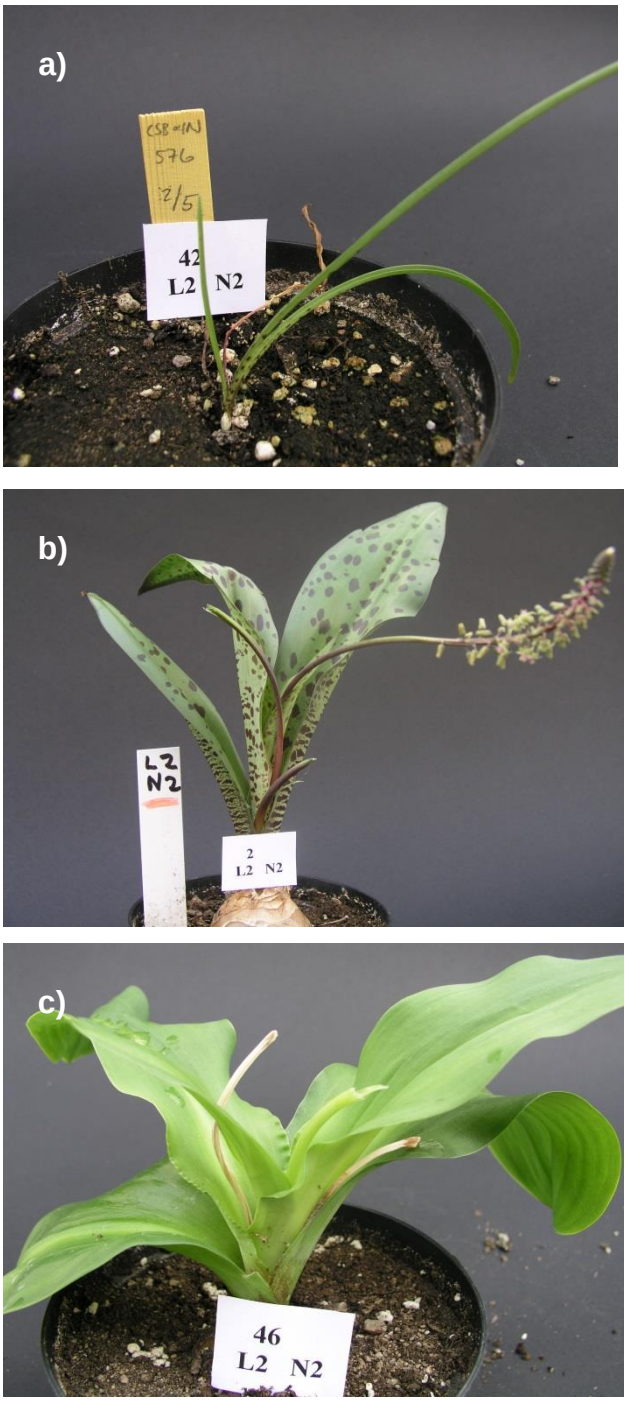


Figure 2. Representatives of the three morphological forms in the *Ledebouria revoluta* complex a) morphotype A, characterised by erect and narrow leaves with randomly distributed variegation bands on the abaxial surface (Bjorå & Nordal 576i), b) morphotype B characterised by having erect leaves broader than 2 cm with abaxial tiger

stripes (Bjorå & Nordal 532), c) morphotype C characterised by semi-erect broad leaves with/without variegation abaxially at the leaf base only (Bjorå & Nordal 586).

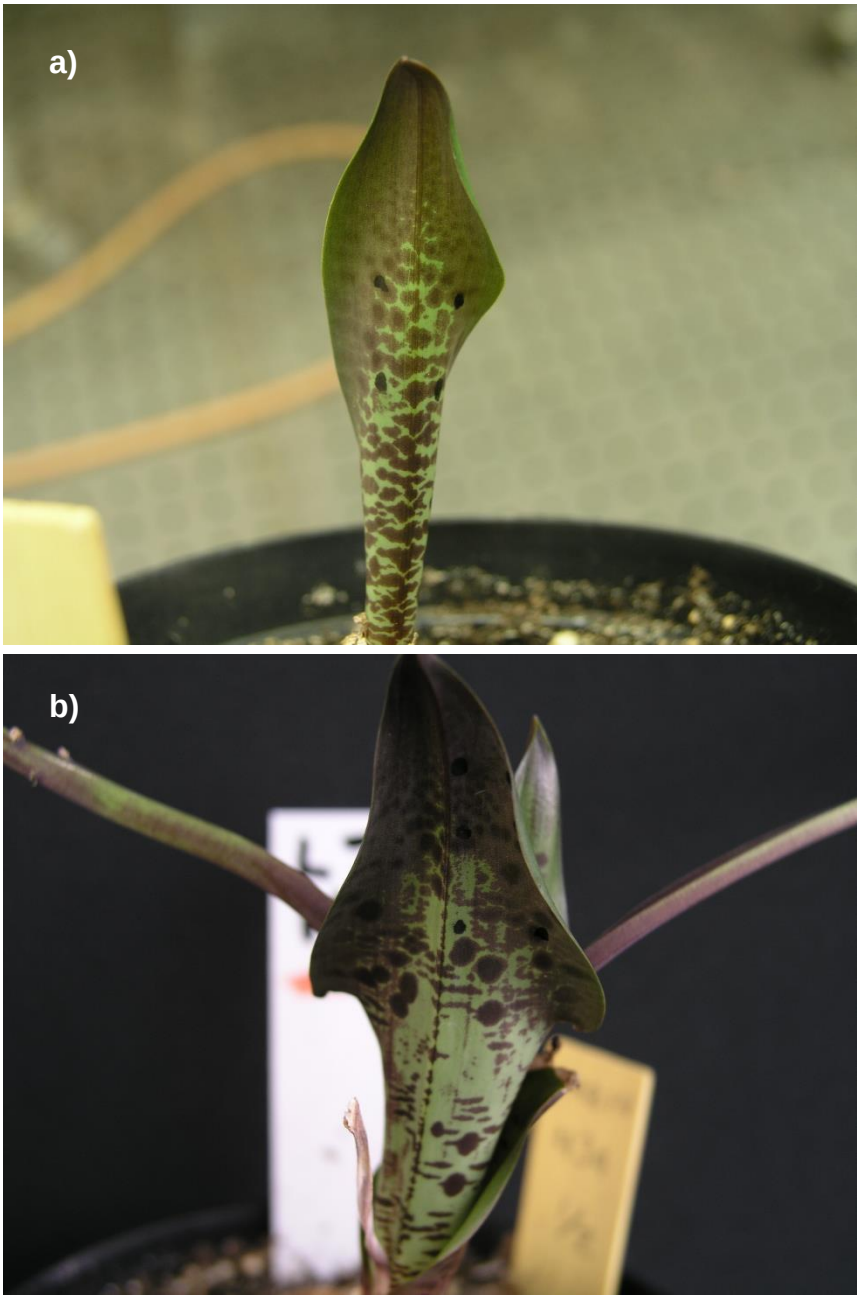


Figure 3. Leaves of Ledebouria mimicking Cobra snakes. The plants were cultivated in the Phytotron, University of Oslo. (Hoell & Nordal 43, see Table 1).

728

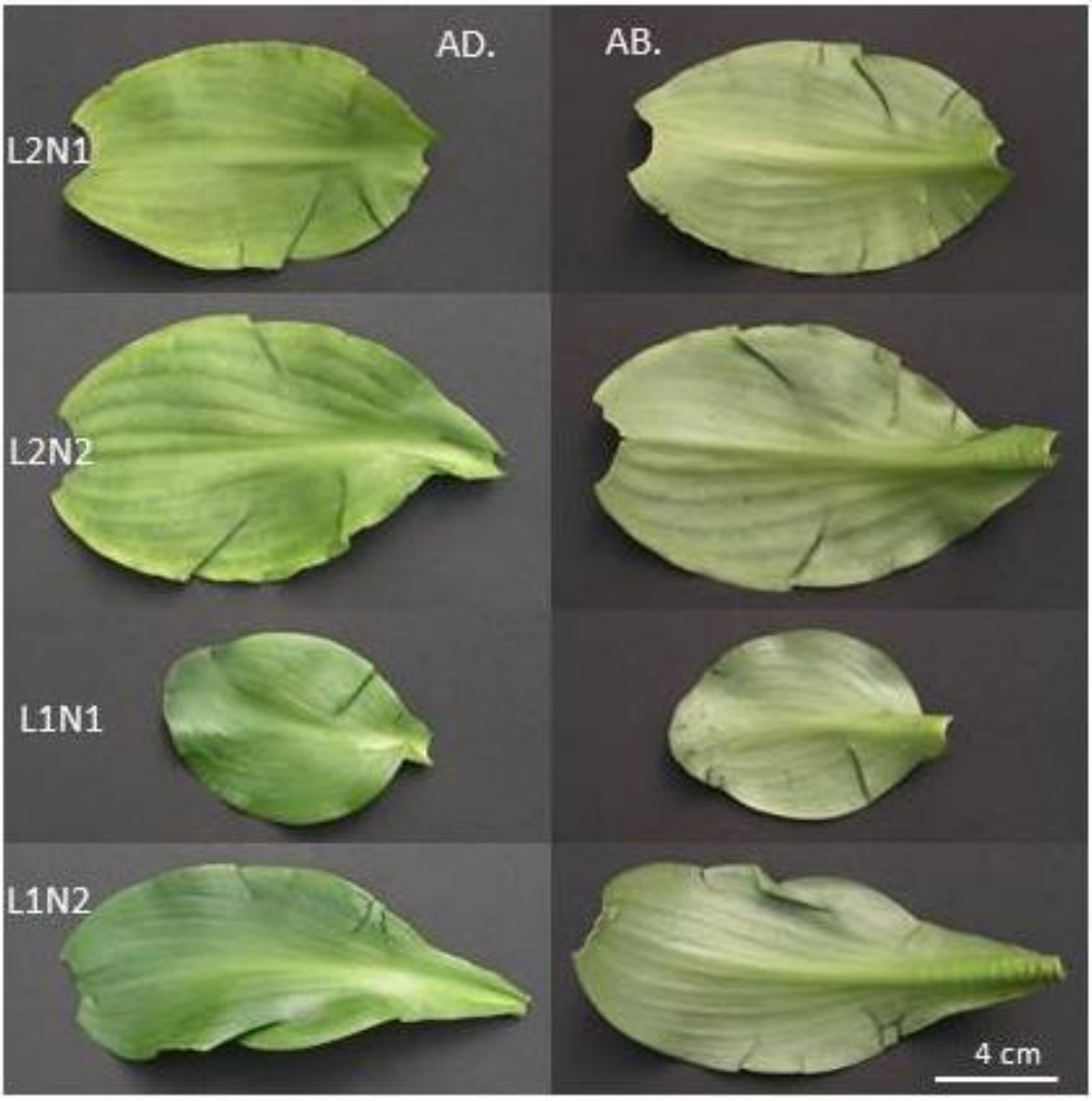
729 **Figure 4.** Development of leaf mottling/variegation inside the 1 cm ^{2} square during a
730 period of six weeks (explanation in text). A) Morphotype A, Bjorå & Nordal 576i. B)
731 Morphotype B, Bjorå & Nordal 656i. C) Morphotype C, Bjorå & Nordal 586. Voucher
732 information in Table 1

733

734 **Figure 5.** A representative of morphotype A (Bjorå & Nordal 576i, Table 1) of
735 *Ledebouria revoluta* showing responses of 4 individual clones to different light and
736 nitrogen treatments in adaxial (AD.) and abaxial (AB.) leaf surfaces of the same leaf.
737 Treatments with high or low light (L2 and L1 respectively) and high or low nitrogen (N2
738 and N1, respectively) are indicated next to the image pairs.

739

740 **Figure 6.** A representative of morphotype B (Bjorå & Nordal 656i, Table 1) of
741 *Ledebouria revoluta* showing responses of 4 individual clones to different light and
742 nitrogen treatments in adaxial (AD.) and abaxial (AB.) leaf surfaces of the same leaf.
743 Treatments with high or low light (L2 and L1 respectively) and high or low nitrogen (N2
744 and N1, respectively) are indicated next to the image pairs.

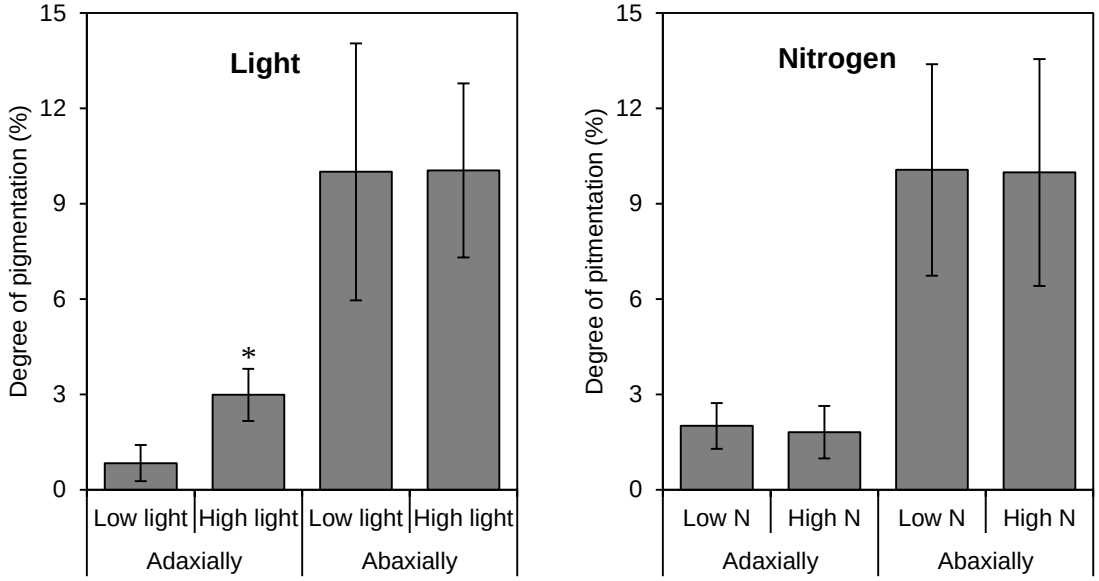


745

746 **Figure 7.** A representative of morphotype C (Bjorå & Nordal 683, Table 1) of
747 *Ledebouria revoluta* showing responses of 4 individual clones to different light and
748 nitrogen treatments in adaxial (AD.) and abaxial (AB.) leaf surfaces of the same leaf.
749 Treatments with high or low light (L2 and L1 respectively) and high or low nitrogen (N2
750 and N1, respectively) are indicated next to the image pairs.

751

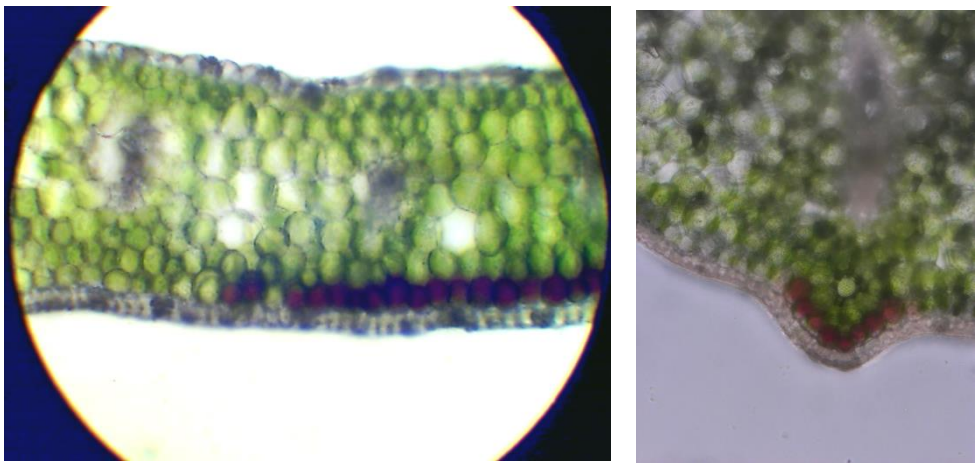
752 a) b)



753

754 **Figure 8.** The degree of pigmentation (%) of leaf surface on adaxial and abaxial sides of
755 mottled/variegated *Ledebouria revoluta* leaves as affected by (a) light and (b) nitrogen
756 (N) addition (mean ± SE, n = 8 (adaxially, morphotype B clones 532, 575, 656i, 656ii) or
757 12 (abaxially, morphotype A clones 576i / 576ii, morphotype B clones 532, 575, 656i,
758 656ii, and morphotype C clone 683). *: significant difference ($p < 0.05$) between low and
759 high level of the treatment, from ANOVA.

760 a) b)



761

762 **Figure 9.** Transverse sections of fresh *Ledebouria revoluta* leaves examined under bright
763 field showing the position of the anthocyanin cells in the hypodermis. (a) Nordal 2013

764 from Zimbabwe, (b) Stedje 341 from Zambia. Scale bar in (a) also holds for the image in
765 (b).

766

767

768 **a)** **b)**

769

770

771

772

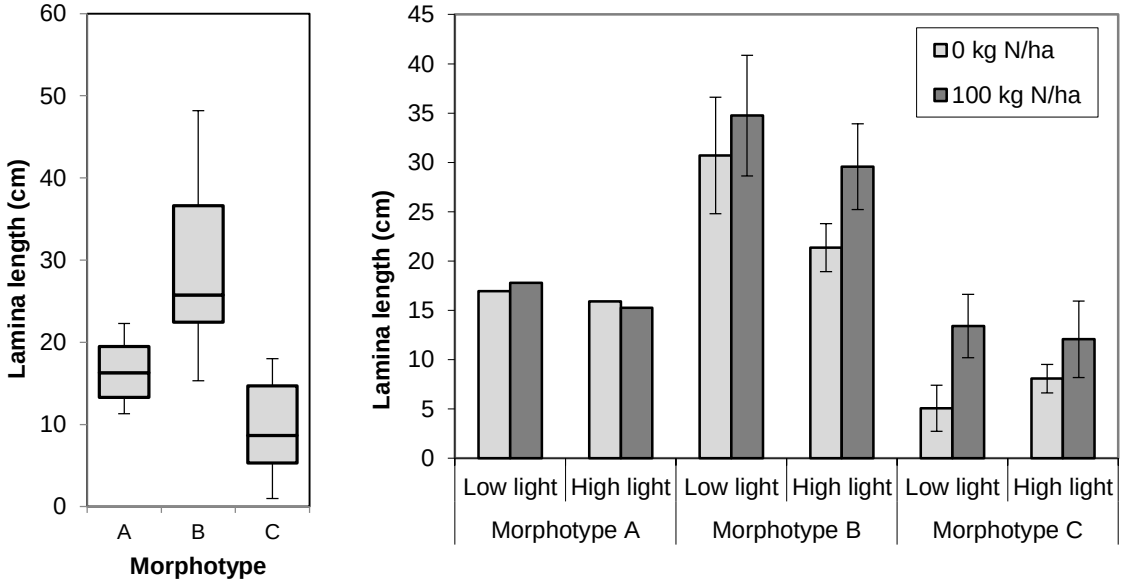
773

774

775

776

777



778

779 **Figure 10** (a) Variations in the lamina length for the three morphotypes A, B and C of
780 *Ledebouria revoluta*. Significantly different values are marked with different letters. (b)
781 Effects of light and nitrogen addition on lamina length of the three morphotypes (Mean ±
782 SE, n = 3–4 leaves in morphotype C and B, and mean of n = 2 leaves in morphotype A).

783

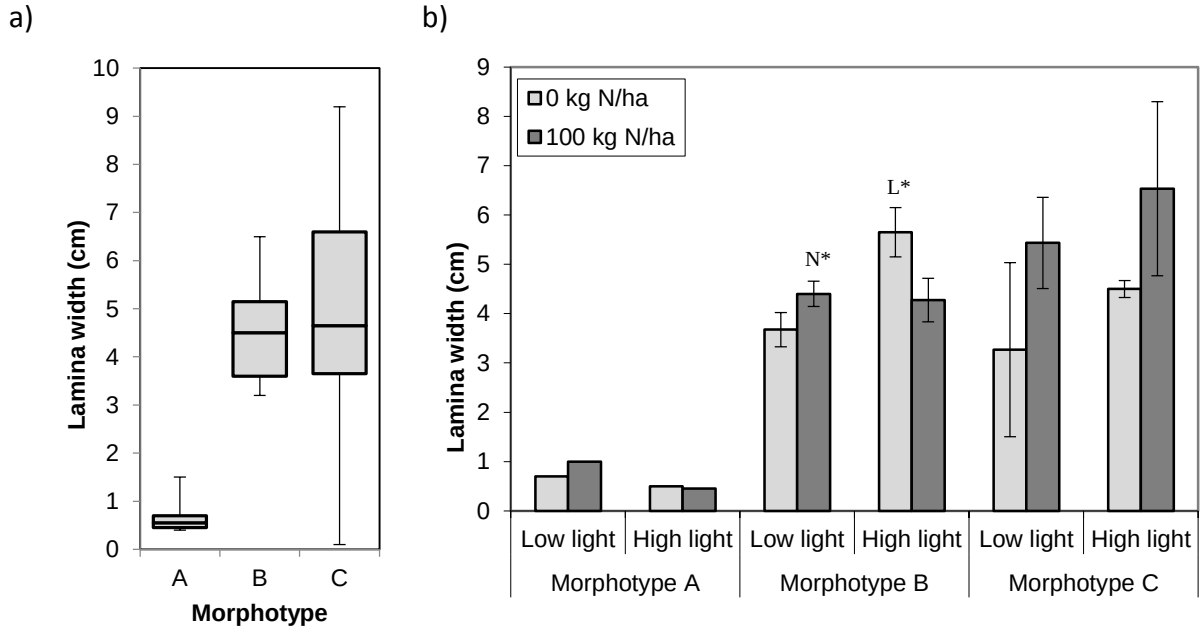


Figure 11. (a) Variations in the lamina width for the three morphotypes A, B and C of *Ledebouria revoluta*. Significantly different values are marked with different letters. (b) Effects of light and nitrogen addition on lamina width of the three morphotypes (Mean ± SE, n = 3–4 leaves in morphotype C and B, and mean of n = 2 leaves in morphotype A).

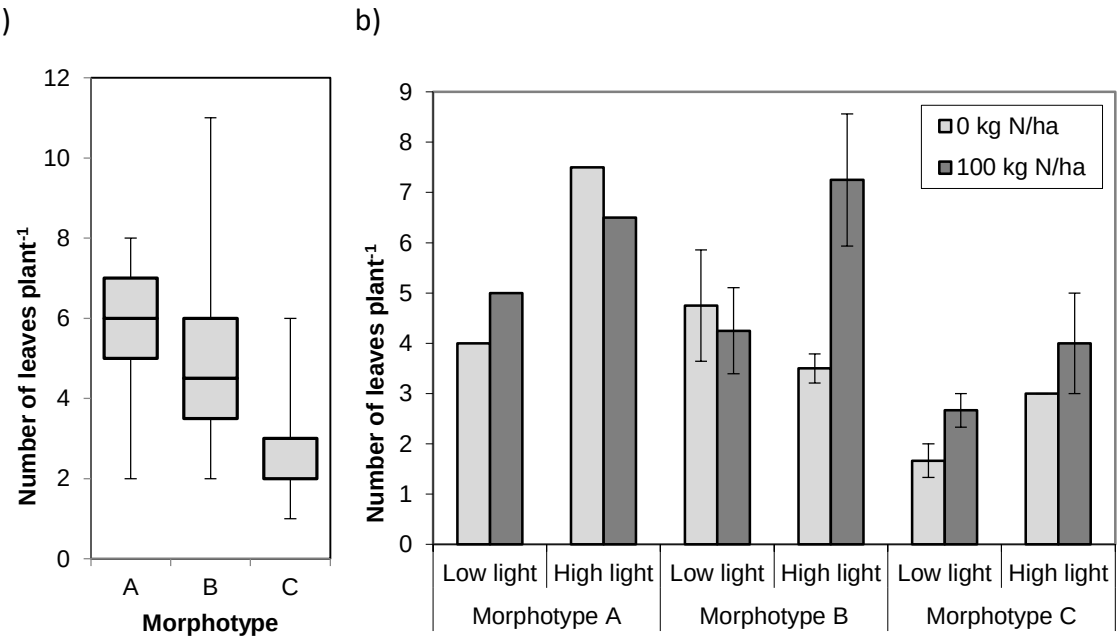


Figure 12. (a) Variation in the number of leaves for the three morphotypes A, B and C. Significantly different values are marked with different letters. (b) Effects of light and nitrogen addition on number of leaves on the three morphotypes. (Mean ± SE, n = 3–4 leaves in morphotype C and B, and mean of n = 2 leaves in morphotype A).

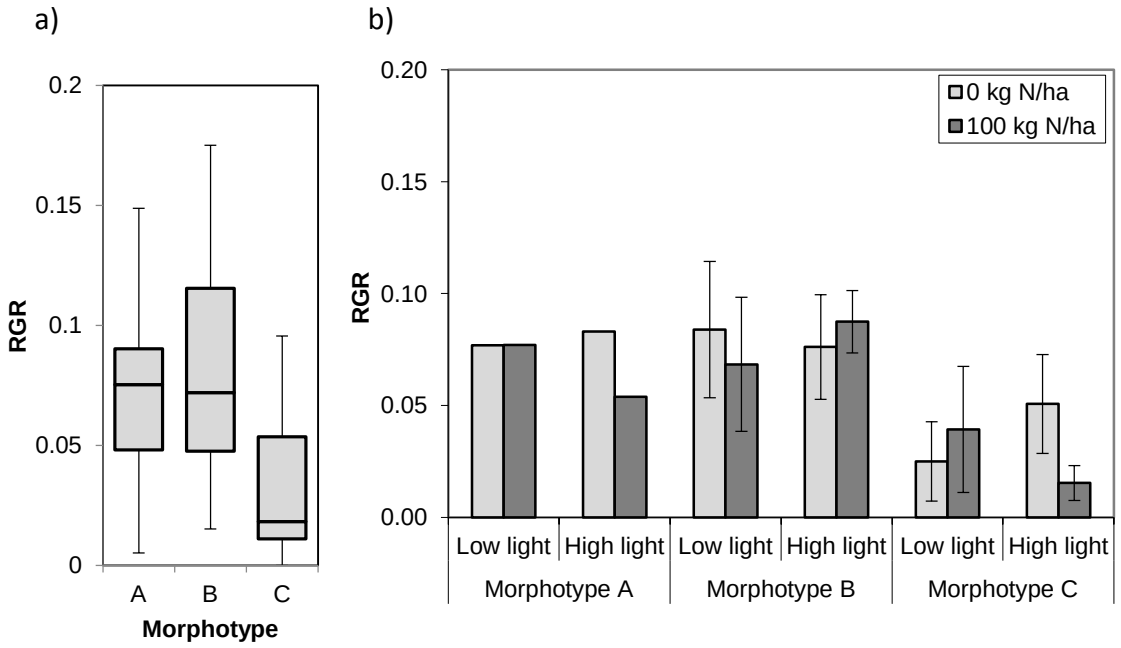


Figure 13. (a) Variation in the relative growth rate for the three morphotypes A, B and C of *Ledebouria revoluta* . Significantly different values are marked with different letters. (b) Effects of light and nitrogen addition on relative growth rate of the three morphotypes (Mean ± SE, n = 3–4 leaves in morphotype C and B, and mean of n = 2 leaves in morphotype A).