

Taxonomic significance of the abaxial lemma surface in southern African members of *Helictotrichon* (Poaceae)

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Background: *Helictotrichon* (excluding *Amphibromus*, *Avenula*, *Helictochloa* and *Tricholemma*), a genus of temperate C₃ grasses, is represented by 14 species in southern Africa. Members of the genus are difficult to identify at species level on the basis of macromorphology alone.

Objectives: The primary objective of this study was to investigate the usefulness of the micromorphology of the lemma surface for differentiating amongst the southern African members of *Helictotrichon*.

Method: Lemma surfaces were studied with scanning electron microscopy (SEM). Lemmas were obtained from herbarium specimens housed in the National Herbarium, Pretoria (PRE). These were mounted on aluminium stubs using double-sided adhesive tape, sputter-coated with gold, and photographs were taken using a J840 scanning electron microscope.

Results: Based on whether the lemma surface is smooth, papillate, scaberulous, scabrid or a combination of these, five groups of species are distinguished. All species, except *Helictotrichon barbatum* which has a smooth lemma surface, have small prickles (scaberulous). *Helictotrichon leoninum*, *Helictotrichon quinquesetum* and *Helictotrichon rogerellisii* have only small prickles, whilst *Helictotrichon dodii*, *Helictotrichon hirtulum*, *Helictotrichon namaquense* and *Helictotrichon roggeveldense* have, in addition, medium to large prickles (scabrid). *Helictotrichon capense*, *Helictotrichon longifolium*, *Helictotrichon longum* and *Helictotrichon turgidulum* have a combination of small prickles and papillae whilst *Helictotrichon galpinii* and *Helictotrichon natalense* have a combination of all three. A key to the groups and photos of the different types of surfaces are provided.

Conclusion: The micromorphology of the lemma surface was shown to be of considerable taxonomic significance and to be extremely useful for differentiating amongst species.

Introduction

Helictotrichon Besser ex Schult. & Schult.f. (Mashau, Fish & Van Wyk 2010b) [excluding *Amphibromus* Nees, *Avenula* (Dumort.) Dumort., *Helictochloa* Romero Zarco and *Tricholemma* (Röser) Röser] (Romero-Zarco 2011; Röser *et al.* 2009) is a genus of C₃ grasses belonging to the subfamily Pooideae and tribe Poeae (Soreng, Davis & Voionmaa 2007) in the Poaceae. The genus, with about 40 species worldwide (Gibbs Russell *et al.* 1990; Mabberley 2008), is most diverse in the temperate regions of the northern hemisphere, especially Europe, from where it extends southwards through the East African mountains (with about five species) to southern Africa, with 14 species in the Flora of southern Africa (FSA) region and one species in Madagascar. Biogeographically the link between the African and Mediterranean members of the group is via Frank White's Afromontane Archipelago-like Regional Centre of Endemism (Van Wyk & Smith 2001).

Schweickerdt (1937) revised *Helictotrichon* in South Africa and recognised 12 species, one of which was only known from the type collection, and a number of other species only from a very few herbarium specimens. The present availability of more herbarium collections has necessitated a revision of the group for the FSA region, this having already resulted in the description of two new species (Mashau, Fish & Van Wyk 2010a).

Southern African members of *Helictotrichon* have an inflorescence which is a narrow, open, contracted to spike-like panicle with solitary and pedicelled spikelets. Spikelets are 7 mm – 30 mm long (excluding awns), laterally compressed and disarticulate above the glumes and between the 2–6 florets. Glumes are usually shorter than the spikelet, equal or unequal,

hyaline or subhyaline, and the apex is acute, acuminate or shortly awned. Lemmas are firmer than the glumes, being firmly membranous to leathery, and they have scarious or hyaline apices. The lemma is rounded dorsally, 5–11-nerved, the apex acute or acuminate and 2-lobed (rarely 4-lobed). The awn, which is longer than the body of the lemma, is geniculate and twisted, or only slightly so, and the callus is hairy. The palea is shorter than the lemma, 2-keeled and ciliate.

As many species of *Helictotrichon* are difficult to identify using only macromorphology, the primary objective of this study was to investigate the usefulness of the micromorphology of the lemma surface for differentiating amongst the southern African members of *Helictotrichon*. The elucidation of new features to facilitate identification will contribute towards knowledge of the southern African flora in general and infrageneric diversity of *Helictotrichon* in particular. Moreover, most species of *Helictotrichon* are relatively valuable grazing grasses as they remain green until late in winter. Reliable species identification will therefore benefit many end users of plant names, including ecologists, pasture scientists and farmers.

Research methods

Materials

The abaxial lemma surface features were studied in 14 species of *Helictotrichon* (Table 1) from the FSA region. Lemmas were dissected from herbarium specimens housed in the National Herbarium (PRE), Pretoria (Table 1).

Procedures

Dry lemmas (24 samples) were mounted on aluminium stubs using double-sided adhesive tape, sputter-coated with gold and viewed under a Jeol J840 scanning electron microscope (SEM). The entire lemma, as well as enlargements of the middle-dorsal and distal (below the awn insertion) areas were photographed and compared. Terminology used to describe the surface features follows Ellis (1979).

Ethical considerations

The study did not involve the collection of any fresh samples as only previously collected dried herbarium material was used.

Results

The lemma provides some of the most useful diagnostic features to differentiate amongst the species of *Helictotrichon* in the FSA region. In some species the micromorphology of the abaxial lemma surface is variable from the base to the point of awn insertion, but the particular pattern of variability appears to be constant for a species. Taxonomically useful lemma characters as seen with the SEM are compared in Table 2. The main character states are whether the lemma surface is smooth, papillate, scaberulous (prickles small) or scabrid (prickles medium or large) (Figures 1a–h and 2a–f). Prickles are described as small if they are shorter than 42 µm, and as medium or large if they are longer than 56 µm. Based on lemma surface micromorphology the species can be classified into five groups. These are keyed out in Box 1.

Group 1: Lemma surface smooth

Helictotrichon barbatum. A smooth lemma is the most obvious character distinguishing *H. barbatum* from all other southern African members of *Helictotrichon* (Figure 1a).

Group 2: Lemma surface papillate and scaberulous

Helictotrichon capense, *Helictotrichon longifolium*, *Helictotrichon longum* and *Helictotrichon turgidulum* (Figure 1b, g & h; 2f).

Group 3: Lemma surface papillate, scaberulous and scabrid

Helictotrichon galpinii and *Helictotrichon natalense* are the only two species which have a combination of all the features that may be present on the lemma surface, namely papillae, small prickles and medium or large prickles (Figure 1d; 2b).

TABLE 1: List of species and specimens (all in the National Herbarium, Pretoria) examined using scanning electron microscopy.

Species	Specimens
<i>H. barbatum</i> (Nees) Schweick.	Acocks 18632
<i>H. capense</i> Schweick.	Story 3803 & 4509
<i>H. dodii</i> (Stapf) Schweick.	Adamson 1230 & 1367
<i>H. galpinii</i> Schweick.	Ellis 1389 & 1401
<i>H. hirtulum</i> (Steud.) Schweick.	Smook 5859; Ellis 589
<i>H. leoninum</i> (Steud.) Schweick.	Spies 3164; Ellis 5124
<i>H. longifolium</i> (Nees) Schweick.	Scheepers 1820; Smook 1184
<i>H. longum</i> (Stapf) Schweick.	Acocks 19730; Van Breda 4168
<i>H. namaquense</i> Schweick.	Ellis 5997
<i>H. natalense</i> (Stapf) Schweick.	Rennie 1534; Huntley 415
<i>H. quinquesetum</i> (Steud.) Schweick.	Ecklon 929
<i>H. rogerellisii</i> Mashau, L.Fish & A.E.van Wyk	Ellis 4663
<i>H. roggeveldense</i> Mashau, L.Fish & A.E.van Wyk	Acocks 17178; Ellis 5117
<i>H. turgidulum</i> (Stapf) Schweick.	Burrows 2196; Moss 1224
<i>H.</i> , <i>Helictotrichon</i> .	

TABLE 2: Comparison of the abaxial lemma surface micromorphology in southern Africa species of *Helictotrichon*.

Species	Lemma surface topography			
	Smooth	Papillate	Scaberulous (prickles small, shorter than 42 µm)	Scabrid (prickles medium to large, longer than 56 µm)
<i>H. barbatum</i>	†	-	-	-
<i>H. capense</i>	-	†	†	-
<i>H. longifolium</i>	-	†	†	-
<i>H. longum</i>	-	†	†	-
<i>H. turgidulum</i>	-	†	†	-
<i>H. galpinii</i>	-	†	†	†
<i>H. natalense</i>	-	†	†	†
<i>H. dodii</i>	-	-	†	†
<i>H. hirtulum</i>	-	-	†	†
<i>H. namaquense</i>	-	-	†	†
<i>H. roggeveldense</i>	-	-	†	†
<i>H. leoninum</i>	-	-	†	-
<i>H. quinquesetum</i>	-	-	†	-
<i>H. rogerellisii</i>	-	-	†	-

H., *Helictotrichon*.

†, indicates presence of state.

Group 4: Lemma surface scaberulous and scabrid

Helictotrichon dodii, *Helictotrichon hirtulum*, *Helictotrichon namaquense* and *Helictotrichon roggeveldense* (Figure 1c & e; 2a & e).

Group 5: Lemma surface scaberulous

Helictotrichon leoninum, *Helictotrichon quinquesetum* and *Helictotrichon rogerellisii* (Figure 1f; 2c & d).

Discussion

Although Schweickerdt (1937) included the lemma surface characters for each species description in his key, he only used it to separate some species that are closely related and then usually only as one of the many distinguishing characters used. In southern Africa, Chippindall (1955) closely followed Schweickerdt's key, whilst Stapf (1899) mentioned the lemma surface in the species descriptions but did not use it at all in the key. Descriptive terms such as granulate, finely or coarsely granular, scaberulous and scabrid are used by the various authors, but there is no doubt that they all refer to the prickles as seen under the SEM and referred to as such in the present contribution.

Helictotrichon dodii, with its very dense contracted panicle and long lemma lobes (6 mm – 8 mm, including awn), is a fairly well defined species. However, it does resemble *H. turgidulum*, which has shorter lemma lobes (3 mm – 5 mm, including awn); it also resembles *H. natalense*, which has a shorter spikelet (7 mm – 9 mm), and *H. longifolium*, which has setaceous leaves.

In addition to all these characters, the SEM shows that the lemma surface of *H. dodii* has small (scaberulous) as well as medium to large prickles (scabrid). This makes it easily distinguishable from *H. turgidulum*, *H. natalense* and *H. longifolium*, which have lemma surfaces with small prickles (scaberulous) mixed with papillae. *Helictotrichon*

capense and *H. longum*, both winter rainfall species, fall in the same group as *H. longifolium*, a summer rainfall species from the mountains of Lesotho, KwaZulu-Natal and Eastern Cape, and *H. turgidulum*, the most widespread but predominately summer rainfall species.

Helictotrichon roggeveldense resembles *H. namaquense* in having a scabrid lemma and conspicuously hairy keels of the palea, but the two species differ in a number of other characters easily seen under the light microscope, such as inflorescence pulvinii and anther length (Mashau *et al.* 2010a). *Helictotrichon roggeveldense* occurs in the Sutherland District (Roggeveld Subcentre, Hantam-Roggeveld Centre of Endemism) (Van Wyk & Smith 2001) whilst *H. namaquense* is found mainly in the Kamiesberg, Namaqualand (Kamiesberg Centre of Endemism), with an outlier on the Hantamsberg, Calvinia. The SEM shows that the lemma surface of these two species has a combination of small (scaberulous) and medium to large prickles (scabrid).

Helictotrichon galpinii is easily distinguished by the broad glumes that are as long as the spikelet and the apparently scabrid lemma surface which is easily seen under a light microscope. However, the SEM images show the lemma surface of *H. galpinii* matching that of *H. natalense* as both have a combination of all three lemma surface types, namely papillate, small and medium to large prickles, present. *Helictotrichon natalense* occurs at lower altitudes (1900 m) than *H. galpinii* and both are summer rainfall species.

Helictotrichon rogerellisii is similar to *H. longifolium* in that both species have setaceous leaves and loosely flowered spikelets. However, they differ in their distribution (geographical range) as *H. rogerellisii* has only been found in the Bredasdorp District, Western Cape (Agulhas Plain Subcentre, Cape Floristic Region), in fynbos, mainly in shallow, humic soils between limestone outcrops on coastal plain. *Helictotrichon longifolium* is found along the

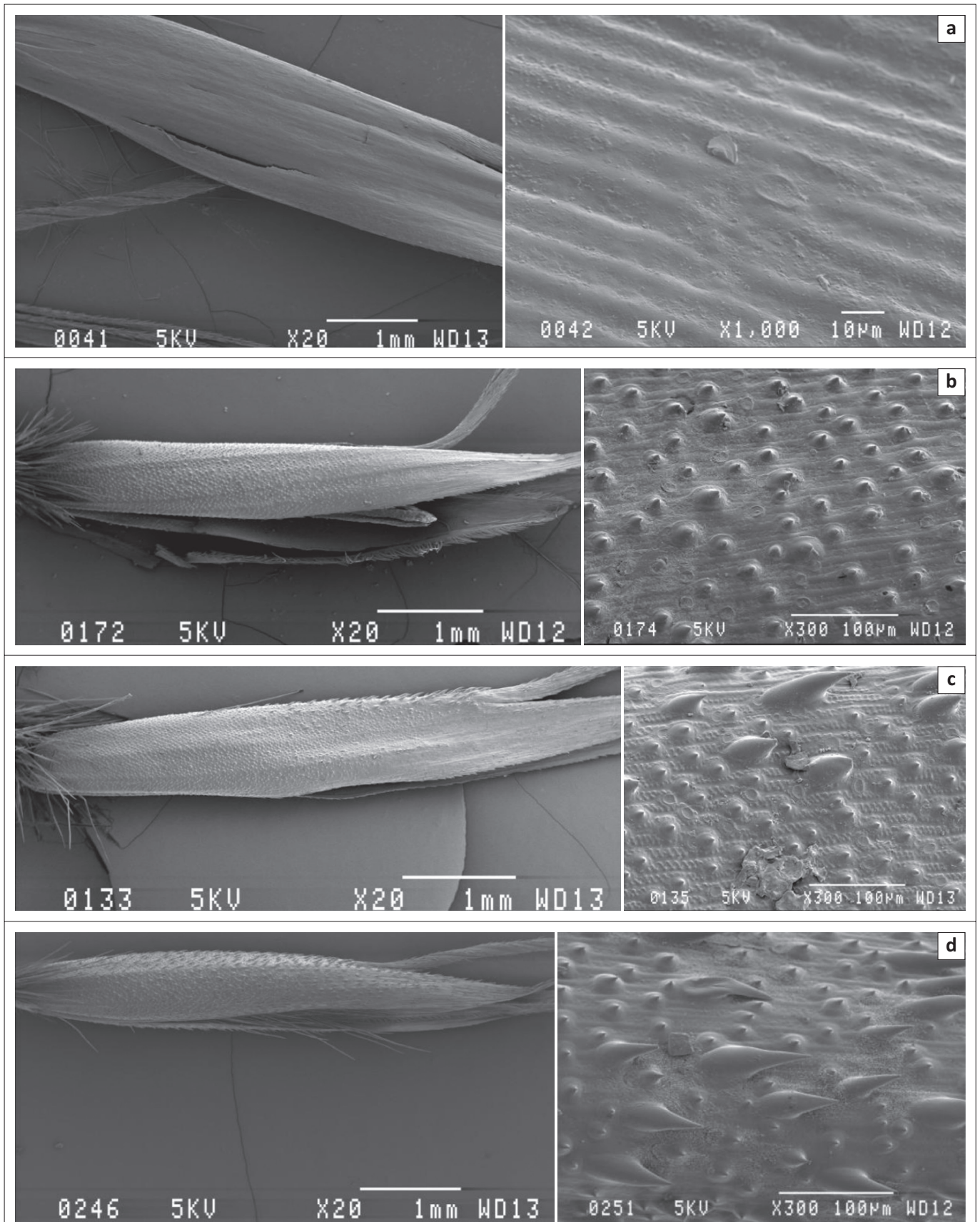


FIGURE 1: Scanning electron microscopy micrographs of the abaxial lemma surface in species of *Helictotrichon*: (a) *Helictotrichon barbatum*, lemma smooth, (b) *Helictotrichon capense*, lemma papillate and scaberulous, (c) *Helictotrichon dodii*, lemma scaberulous and scabrid, (d) *Helictotrichon galpinii*, lemma ± papillate, scabrid and scaberulous, (e) *Helictotrichon hirtulum*, lemma scaberulous and scabrid, (f) *Helictotrichon leoninum*, lemma scaberulous, (g) *Helictotrichon longifolium*, lemma ± papillate and scaberulous and (h) *Helictotrichon longum* lemma papillate and scaberulous.

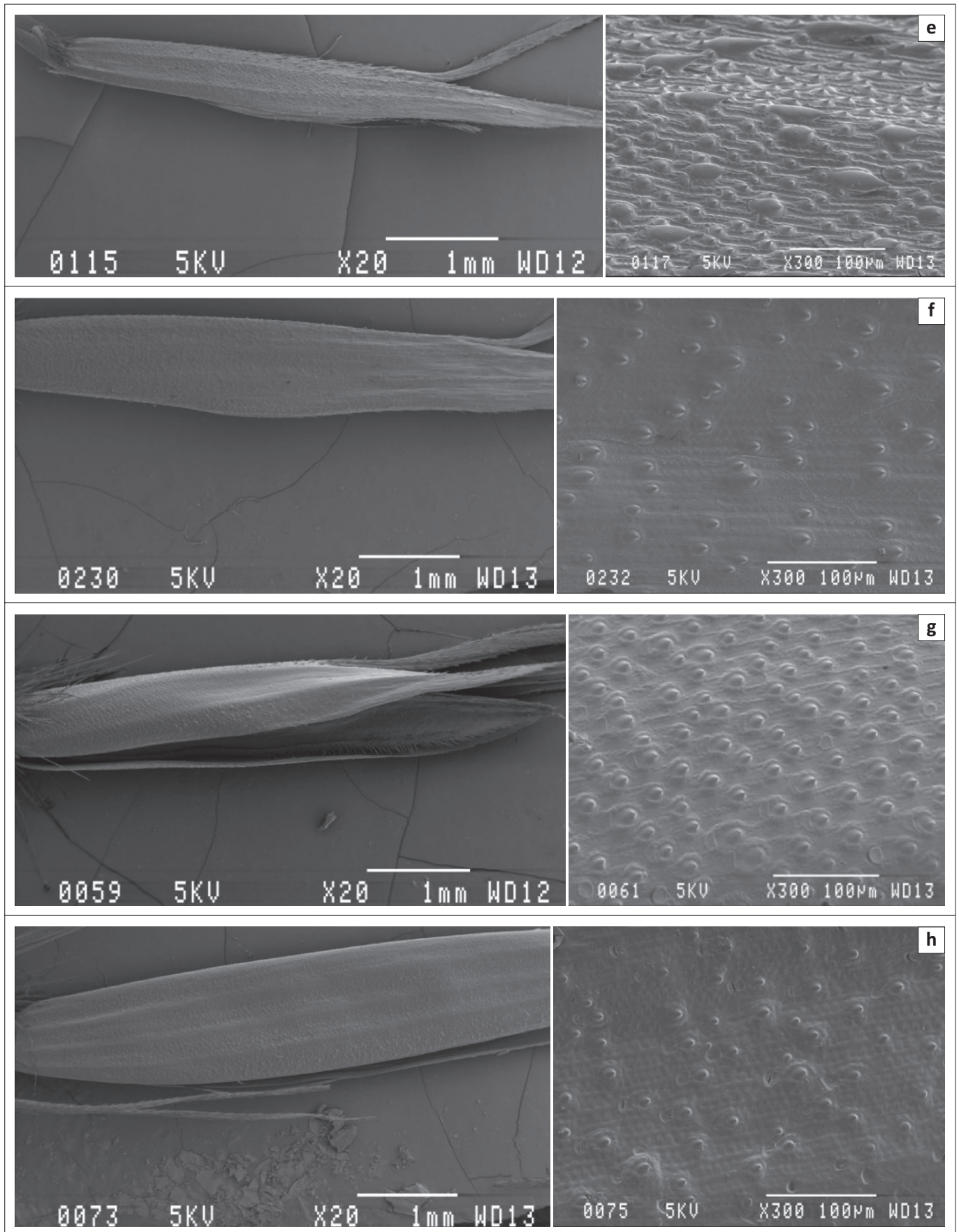


FIGURE 1 (Continues...): Scanning electron microscopy micrographs of the abaxial lemma surface in species of *Helictotrichon*: (a) *Helictotrichon barbatum*, lemma smooth, (b) *Helictotrichon capense*, lemma papillate and scaberulous, (c) *Helictotrichon dodii*, lemma scaberulous and scabrid, (d) *Helictotrichon galpinii*, lemma \pm papillate, scabrid and scaberulous, (e) *Helictotrichon hirtulum*, lemma scaberulous and scabrid, (f) *Helictotrichon leoninum*, lemma scaberulous, (g) *Helictotrichon longifolium*, lemma \pm papillate and scaberulous and (h) *Helictotrichon longum* lemma papillate and scaberulous.

Drakensberg Range, centred in Lesotho (Drakensberg Alpine Centre), especially in grasslands, and mainly on moist and rocky mountain slopes. These two species also differ in a number of other characters (Mashau *et al.* 2010a) and under the SEM the lemma surface of *H. longifolium* has papillae and small prickles (scaberulous) (Group 2) whereas *H. rogerellisii* has small prickles only (Group 5).

Using macromorphological characters, *H. leoninum* is the only southern African species with the rachilla internodes glabrous – this character state makes the species distinctly

different from all others. The lemma surface of *H. leoninum*, *H. quinquesetum* and *H. rogerellisii* are covered only with small prickles (scaberulous; Group 5). All three species occur in the winter rainfall area in and around Cape Town, to as far east as Bredasdorp.

Although the lemma surface in the FSA species of *Helictotrichon* is not sufficiently variable to distinguish between all species, it is still a very useful character for identification. It also allows for species that are possibly closely related to be grouped together. The use of the SEM

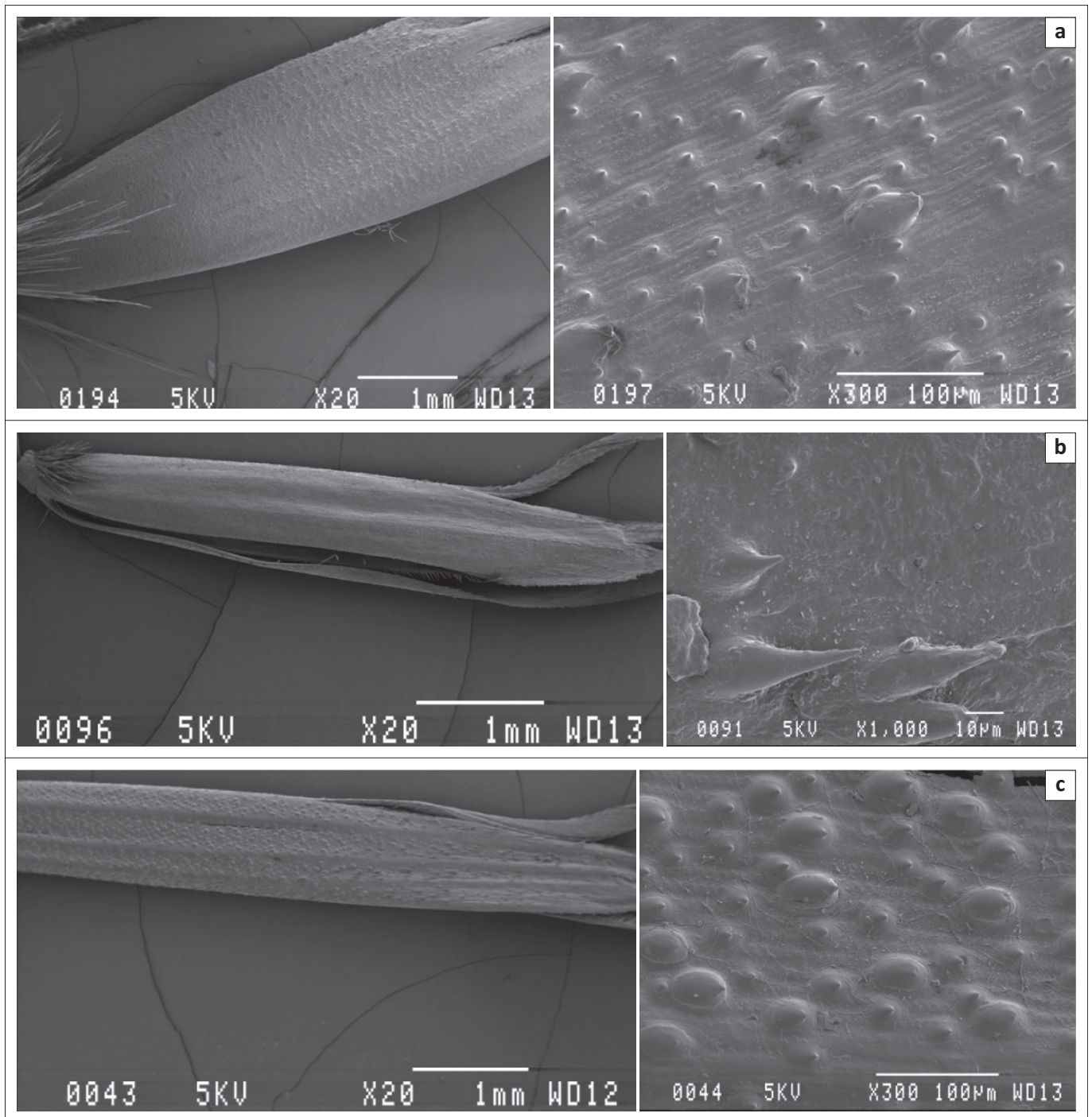


FIGURE 2: Scanning electron microscopy micrographs of the abaxial lemma surface in species of *Helictotrichon*: (a) *Helictotrichon namaquense*, lemma scaberulous and scabrid, (b) *Helictotrichon natalense*, lemma ± papillate, scabrid and scaberulous, (c) *Helictotrichon quinquesetum*, lemma scaberulous, (d) *Helictotrichon rogerellisii*, lemma scaberulous, (e) *Helictotrichon roggeveldense*, lemma scaberulous and scabrid and (f) *Helictotrichon turgidulum*, lemma papillate and scaberulous.

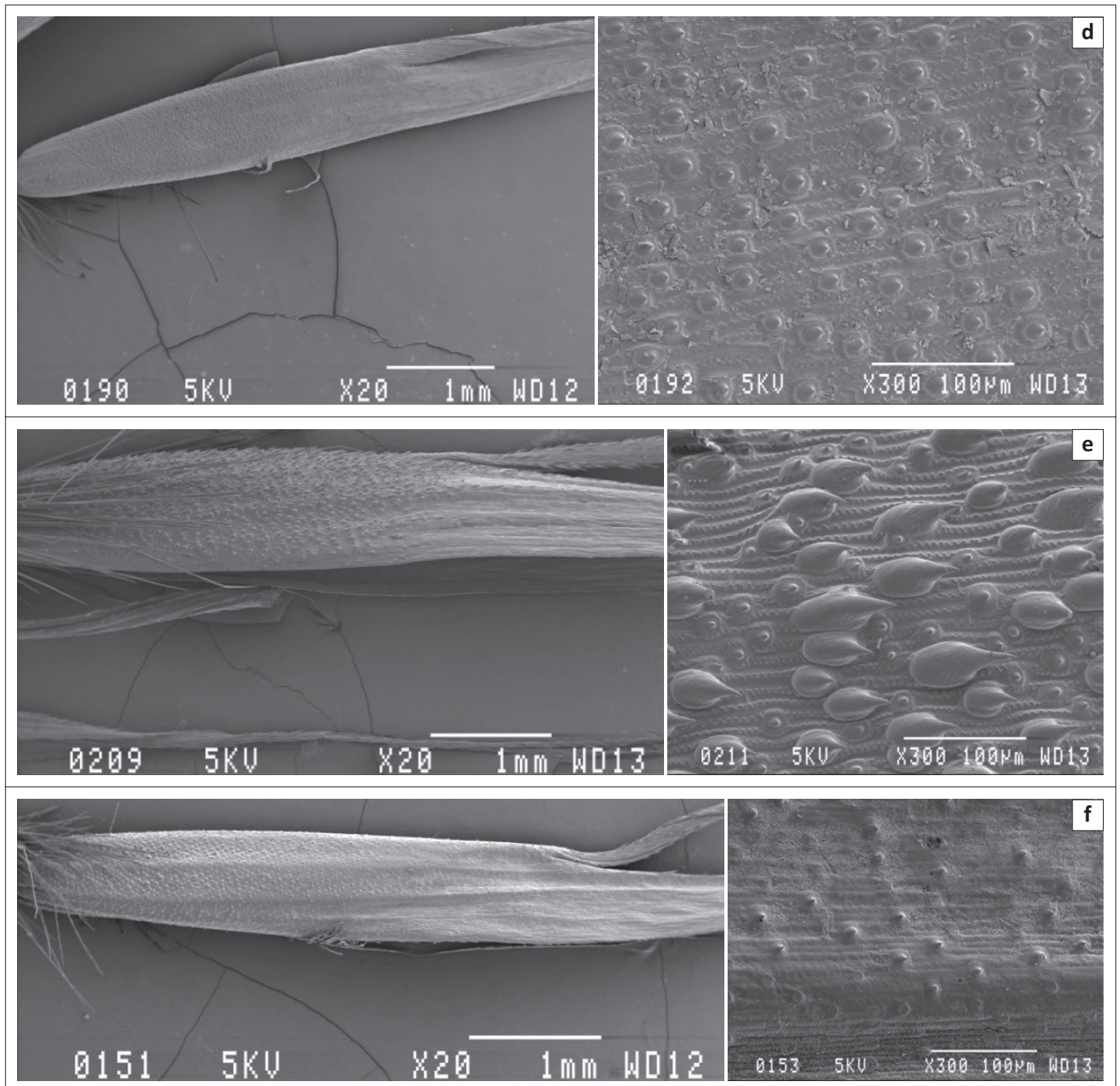


FIGURE 2 (Continues...): Scanning electron microscopy micrographs of the abaxial lemma surface in species of *Helictotrichon*: (a) *Helictotrichon namaquense*, lemma scaberulous and scabrid, (b) *Helictotrichon natalense*, lemma \pm papillate, scabrid and scaberulous, (c) *Helictotrichon quinquesetum*, lemma scaberulous, (d) *Helictotrichon rogerellisii*, lemma scaberulous, (e) *Helictotrichon roggeveldense*, lemma scaberulous and scabrid and (f) *Helictotrichon turgidulum*, lemma papillate and scaberulous.

BOX 1: Key to the species groups of *Helictotrichon* based on the micromorphology of the abaxial lemma surface.

- | | |
|--|---------|
| 1A. Lemma surface smooth | Group 1 |
| 1B. Lemma surface papillate, scaberulous or scabrid: | |
| 2A. Lemma surface papillate and scaberulous or scabrid: | |
| 3A. Lemma surface papillate and scaberulous..... | Group 2 |
| 3B. Lemma surface papillate, scaberulous and scabrid..... | Group 3 |
| 2B. Lemma surface not papillate, but scaberulous or scabrid: | |
| 4A. Lemma surface scaberulous and scabrid..... | Group 4 |
| 4B. Lemma surface scaberulous..... | Group 5 |

enhances this character especially to distinguish whether the lemma surface is smooth, papillate or has small prickles mixed with larger prickles – details not seen or not easily identified under the light microscope.

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Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors' contributions

A.C.M. (South African National Biodiversity Institute) prepared the samples and analysed the data, A.E.v.W. (University of Pretoria) was project leader and supervisor, and L.F. (South African National Biodiversity Institute) acted as mentor and made conceptual contributions.

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