

# *Triquetrella mxinwana*, a new moss species from South Africa, with a phylogenetic and biogeographic hypothesis for the genus

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## SUMMARY

We describe *Triquetrella mxinwana*, a new species presently known only from the winter rainfall area of South Africa. Within this region it is common in karroo and renosterveld, but it also occurs in fynbos. It differs from other *Triquetrella* species in the several low, bifid, papillae on each laminal cell, but is similar to the Australian *Leptodontium paradoxum*, differing in size and anatomical details. Although sporophytes of *L. paradoxum* are unknown, those of *T. mxinwana* have the peristome of *Triquetrella* rather than *Leptodontium*. Phylogenetic analysis of data from three chloroplast (*rps4*, *trnL-F* and *psbA-trnH*) and one nuclear (ITS1) loci confirms that *T. mxinwana* and *L. paradoxum* are sister taxa and together are sister to the rest of *Triquetrella*. This placement also better reflects gametophyte morphology, which, though intermediate between *Triquetrella* and *Leptodontium*, better fits the former. Divergence levels among species of *Triquetrella* are very low and molecular clock approaches indicate that all divergences are of Pliocene–Pleistocene age. The estimated time of the split between *T. mxinwana* and its nearest relative is concordant with independent paleoclimatic estimates of the time of onset of winter rainfall conditions in the Cape. The disjunctive distribution of the genus in areas of Mediterranean climate is attributed to dispersal rather than ancient vicariance.

**KEYWORDS:** *Triquetrella*, Mediterranean climate disjuncts, Pliocene–Pleistocene dispersal, Cape flora, xerothermic Pangaeian elements, *Leptodontium*, new South African *Triquetrella*.

## Introduction

*Triquetrella* Müll.Hal. is a small genus of morphologically similar species found on soil or rock, and largely restricted to dry, Mediterranean climates in Europe (Spain), South Africa, western North America (California), southern South America, Australia, Tasmania and New Zealand (Stark, 1980; Magill, 1981; Casas, Cros & Muñoz, 1993; Zander, 1993). A distinctive suite of features characterizes the genus, at least as conceived by Zander (1993). The stems are triangular in section, with the central strand lacking (or extremely reduced). Leaves are arranged in three distinct rows that often spiral about the stem and have long, broadly decurrent, margins that remain on the stem when the leaves are stripped off, resulting in distinctive curling fragments attached to stem sections. The costae are percurrent, with two stereid bands (the ventral of few cells or occasionally absent) in section. Laminal cells have a single narrow, spiculose papilla that is simple or branching from the base and the basal cells are little or not differentiated. Perichaetial leaves are enlarged, sheathing

the base of the seta, and have smooth, elongate laminal cells. The peristome is whitish, consisting of 16 short teeth that are split to the base, and smooth or weakly spiral-striate. The laminal KOH colour reaction is orange or yellow-orange.

Of the species listed by Zander (1993), *T. spiculosa* Thér. has been placed in synonymy with *Zygodon pichinchensis* (Churchill, Griffin & Muñoz, 2000). We have also been able to examine Type material (BM, H-Br and G) of the Australian species *T. fragilis* Müll.Hal. and *T. richardsiae* Müll.Hal. as well as of the South American *T. filicaulis* Dusén, and in our opinion these are indistinguishable, respectively, from *T. papillata* (Hook. f. & Wilson) Broth. and *T. patagonica* Müll.Hal. *Triquetrella tasmanica* (Broth.) Granzow, transferred to the genus from *Anomodon*, is, on the basis of our unpublished analyses of *rps4* and *trnL-F* sequences, a member of the ‘ciliate arthrodonts’ (*sensu* Cox & Hedderson, 1999) rather than the Dicranidae, and can therefore also be eliminated from consideration. There thus remain five species in the genus: *Triquetrella arapilensis* Luisier, *T. californica* (Lesq.) Grout, *T. papillata*, *T.*

*patagonica* and *T. tristicha* (Müll.Hal.) Müll.Hal., all of which have a single, usually branching but occasionally simple, papilla per lumen.

*Triquetrella* differs from *Leptodontium* in: (1) the short, triangular cauline leaves form three distinct rows; (2) the occasional presence of a stem central strand (in *T. californica*) as opposed to its consistent absence; and (3) sharp, spiculate upper laminal papillae, as opposed to the usually multiplex or, in sect. *Leptodontium*, blunt and simple to columnar papillae found in *Leptodontium*. Although Eddy (1990) suggested, without further elaboration, that *Triquetrella* might be misplaced in the Pottiaceae, molecular analyses that have included representatives of the genus consistently refute this (Werner *et al.*, 2004; T. A. Hedderson & R. A. Zander, unpublished); in all such analyses, *Triquetrella* is resolved as sister to *Leptodontium*, and this clade is well nested within Pottiaceae.

*Leptodontium paradoxum* I. G. Stone & G.A.M.Scott is an Australian species that is somewhat intermediate between the two genera (Stone & Scott, 1981). Although it has many of the features of *Leptodontium*, including multiple papillae per cell, it shares with *Triquetrella* two traits rare in the former genus: entire leaves and a differentiated dorsal epidermis (Zander, 1993). Sporophytes, which would help determine placement, have not been found for this species.

An apparently undescribed South African species, represented by a considerable number of specimens gathered over the span of many years by numerous collectors, is similar to this Australian species. However, the South African material is uniformly smaller, by about half, than the Australian species in leaf dimensions and in diameter and length of stems, and also differs in its anatomical details. While only perigonia, not perichaetia, have been found in Australia, some of the South African specimens include sporophytes and these demonstrate the peristome of *Triquetrella*, not that of *Leptodontium*.

In this paper we present our arguments for recognizing the new South African species. We use molecular data to

evaluate phylogenetic relationships between *Triquetrella* and *Leptodontium* and, in particular, we determine the positions of *L. paradoxum* and the new species. Since we have complete sampling of the known *Triquetrella* species we also comment on the biogeographical implications of the phylogeny.

## Methods

### *Taxon and locus sampling*

We sampled all five of the species remaining in *Triquetrella*, *L. paradoxum*, the putative new species and two additional species of *Leptodontium*. In the case of *Triquetrella*, five of the species are represented by at least two accessions (Table 1). *Tetrapterum tetragonum* was used to root the tree, on the basis of our unpublished phylogeny of the Pottiaceae. Using other potential Pottiaceae outgroups (e.g. *Didymodon* spp., *Trichostomum* spp., *Tortula* spp.) for which we have *rps4* and/or *trnL-trnF* information did not affect placement of the root.

The four DNA regions sampled include three from the chloroplast (the *psbA-trnH* spacer, the *trnL-trnF* region and the *rps4* gene region) and a single nuclear locus (the first internal transcribed spacer (*ITS1*) of the 18S–26S rRNA cistron). All have been used previously for recovering species and lower-level phylogenies (e.g. Sang, Crawford & Stuessy, 1997; Chandler, Bayer & Crisp, 2001; Cox & Hedderson, 2004; Hedderson & Nowell 2006).

### *Sequence generation and alignment*

DNA extraction, PCR amplification of target regions, and subsequent sequencing of PCR products followed procedures outlined in Hedderson & Nowell (2006). Primers 18KRC (Hedderson & Nowell, 2006) and ITS2 (Baldwin, 1992) were used to amplify the nuclear ribosomal ITS1

**Table 1.** Voucher information and Genbank Accession numbers for specimens sampled. Vouchers deposited at BOL unless otherwise indicated.

Species	Voucher specimen	rps4	TrnL-F	PsbA-trnH	ITS1
<i>Triquetrella arapilensis</i>	Spain, Ciudad Real. <i>Fuertes s.n.</i> MA	AM491747	AM491741	AM497952	AM497938
<i>T. californica</i>	U.S.A., California. <i>Whittemore 6712</i> , MO	AM491748	AM491742	AM497953	AM497939
<i>T. papillata 1</i>	Australia, New South Wales. <i>Coveny 17500</i> , MO	AM491749	AM491743	AM497954	AM497940
<i>T. papillata 2</i>	Australia, W. Australia. <i>Moore s.n.</i> , MO	AM491750	AM491744	AM497955	AM497941
<i>T. patagonica 1</i>	Chile, Ñuble. <i>Ireland 32767</i>	AM491752	AM491745	AM497956	AM497942
<i>T. patagonica 2</i>	Chile, Concepción. <i>Ireland 32281</i>	AM491751	AM491746	AM497957	AM497943
<i>T. sp. nov. 1</i>	South Africa, Western Cape. <i>Hedderson 13514</i>	AM491753	AM497784	AM497958	AM497944
<i>T. sp. nov. 2</i>	South Africa, Western Cape. <i>Hedderson 13539</i>	AM491754	AM497785	AM497959	AM497945
<i>T. sp. nov. 3</i>	South Africa, Western Cape. <i>Hedderson 15208</i>	AM491755	AM497786	AM497960	AM497946
<i>T. tristicha 1</i>	South Africa, Western Cape. <i>Hedderson 13843</i>	AM491756	AM497787	AM497961	AM497947
<i>T. tristicha 2</i>	South Africa, Western Cape. <i>Hedderson 14527</i>	AM491757	AM497788	AM497962	AM497948
<i>Leptodontium paradoxum 1</i>	Australia, New South Wales. <i>Vitt 27377</i> , MO	AM491758	AM497789	AM497963	AM497949
<i>L. paradoxum 2</i>	Australia, Victoria. <i>Stajsic 2559</i> , MEL	AM491759	AM497790	AM497964	AM497950
<i>L. wallisii</i>	Equador, Azuay. <i>Price 27</i> , MO	AM491760	AM497791	AM497965	NA
<i>L. gemmascens</i>	U.K., Somerset. <i>Hedderson 12996</i> , Herb TAH	AM491761	AM497792	AM497966	AM497951
<i>Tetrapterum tetragonum</i>	South Africa, Western Cape. <i>Hedderson 14305</i>	AM491762	AM497793	NA	NA

region. The chloroplast *rps4*, *trnL-F* and *psbA-trnH* regions were amplified using primers given in Nadot *et al.* (1995), Taberlet *et al.* (1991) and Sang *et al.* (1997), respectively. Assembled sequences were aligned manually using MegAlign (Lasergene System Software). In addition to nucleotide polymorphisms, we identified 21 insertion/deletion events that were coded as presence/absence characters. The *psbA-trnH* and *ITS* regions for *Tetrapterum* were highly divergent from, and could not be reliably aligned against, those of the ingroup taxa. These were therefore treated as missing for the outgroup. Low levels of divergence (see below) rendered alignment of ingroup sequences unproblematic.

#### Data analyses

The data were analysed under the parsimony criterion, as well as under a maximum likelihood criterion employing a Bayesian approach to estimate jointly the topology, branch lengths and parameters of the substitution model. Parsimony analyses used the branch and bound algorithm of PAUP 4.0b10 (Swofford, 1998) to recover all optimal trees. Nodal support was evaluated by the parsimony jackknife (Farris *et al.*, 1996) as implemented in PAUP 4.0b10, using 1000 replicates. At each replicate 36.79% of characters were deleted, and the 'emulate Jac' option was enforced.

Bayesian analyses were undertaken using MrBayes 3 (Ronquist & Huelsenbeck, 2003). Prior analyses using Modeltest (Posada & Crandall, 1998) indicated that a general time-reversible model of DNA substitution, incorporating among-site rate heterogeneity as modelled by a gamma distribution plus a proportion of invariant characters (GTR+I+G) provided the best fit to each of the separate DNA regions. Parameters were unlinked across the four data partitions. The analysis used four chains (one cold and three heated), and was run for 2000 000 generations. Model parameters, including trees, were sampled every 10 generations. The number of generations needed to reach stationarity (i.e. the 'burnin') was estimated by visual inspection of the plot of ML scores at each sampling point. The trees of the burnin from each chain were excluded from the tree set. The remaining trees were combined to form the full sample, which we assume to represent the posterior probability distribution of trees.

#### Results

The four regions yielded 119 variable characters, including 21 indels (Table 2). Of these, 41 characters have potential phylogenetic information under the parsimony criterion.

Topologies recovered under parsimony and Bayesian analyses are identical (Fig. 1). The traditionally defined *Triquetrella* species form a strongly supported group to which an equally well-supported clade, comprising *Leptodontium paradoxum* and the new South African species, is placed with strong support as sister.

Accessions of the new South African species and *L. paradoxum* form reciprocally monophyletic groups (in each case with strong support) united, respectively, by five and six unique changes including at least one character from each locus used. One of the unique changes characterizing the *L. paradoxum* accessions is the insertion of a codon (ACA, i.e. threonine) at a point corresponding to amino acid position 37 relative to *Funaria hygrometrica* Hedw. Reciprocal monophyly of the two species, at least as sampled here, is probabilistically assured because the accessions are supported by two internodes with Bayesian posteriors of 0.98 and 1, the joint probability of which supports recognition of the two lineages as a set at the 95% level.

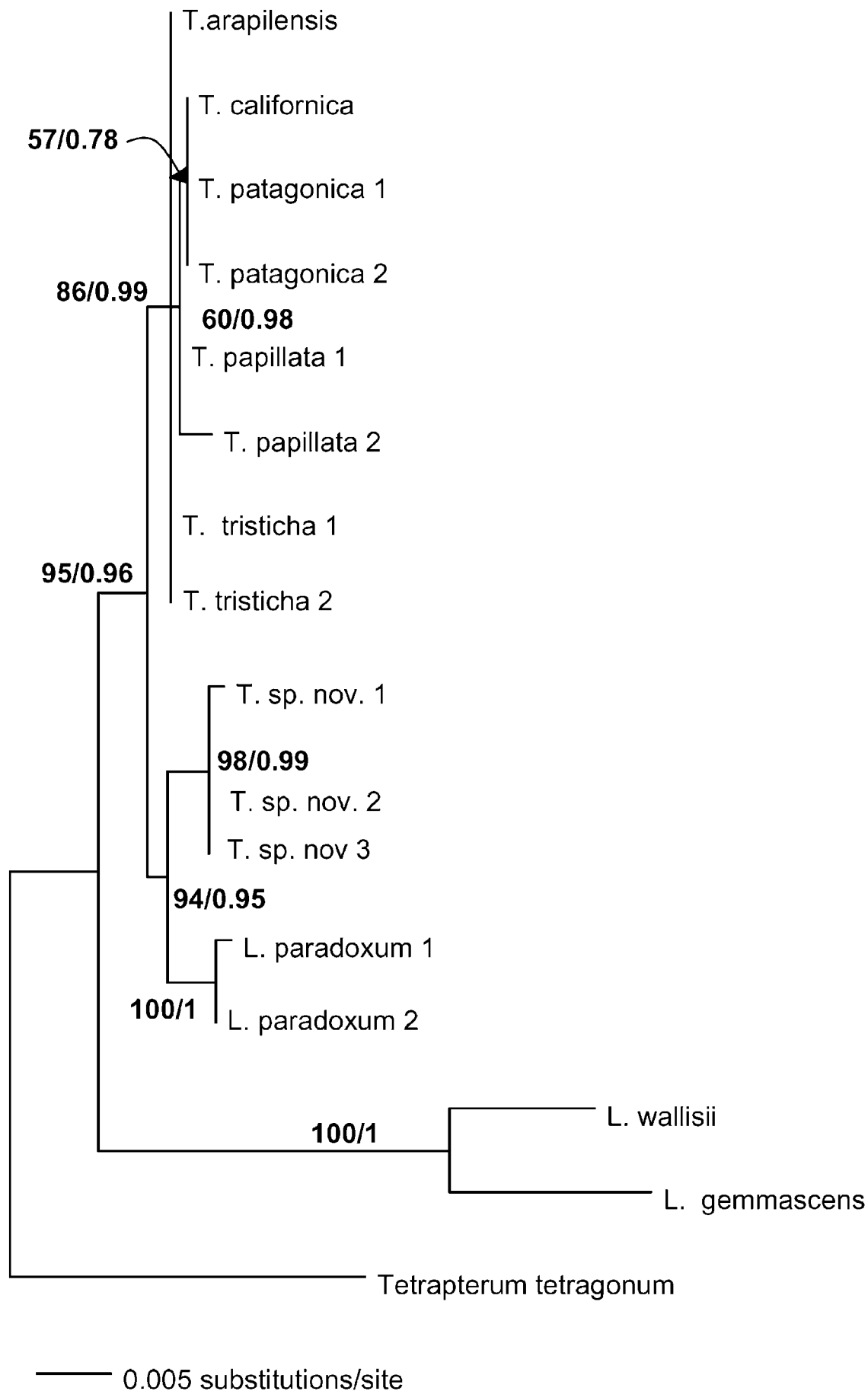
The remaining *Triquetrella* species show few differences across the four regions analysed here. *Triquetrella arapilensis* and the two *T. tristicha* specimens are identical. *Triquetrella californica* and the two *T. patagonica* accessions are identical to each other, and are united relative to the rest of the species by a single, homoplasious, indel in the ITS region. These two are further united with the *T. papillata* accessions by a single, albeit unique, third-base transition in the *rps4* region.

#### Discussion

The data from the four DNA loci examined here provide strong support for the placement of *L. paradoxum* and the multi-papillose South African specimens in the relationship of *Triquetrella* rather than *Leptodontium*. On the basis of the phylogeny presented above, morphological synapomorphies for this expanded concept of *Triquetrella* would include triangular (to rarely five-sided) stems usually lacking a central strand, ovate-triangular leaves with distinctively decurrent margins, and the form of the

**Table 2.** Summary of phylogenetic characteristics for each of the four DNA regions utilized in this study. The values given for the ITS region exclude the 18S portions of the amplicon.

DNA region	Alignment length	Indels	Variable/%	Phylogenetically informative/%	CI
<i>rps4</i>	613	1	39/6.4	21/3.4	0.90
<i>trnL-F</i>	437	9	39/8.9	14/3.2	0.79
<i>psbA-trnH</i>	172	4	11/6.4	08/4.6	1.00
<i>ITS1</i>	292	7	29/9.9	09/3.1	0.90
Total	1867	21	118/6.3	52/2.8	0.87



**Figure 1.** Phylogenetic relationships of *Triquetrella* recovered under Bayesian analysis of sequence variation at *ITS1 rps4*, *trnL-F* and *psbA-trnH* loci. Topologies recovered under the parsimony criterion were identical. Numbers given at nodes are jackknife proportions/Bayesian posterior probabilities.

perichaetial leaves and peristome. The remaining features listed in the introduction as characteristic of *Triquetrella* are potential synapomorphies (at least at this level of universality) for the traditionally defined genus, hereafter referred to as the 'core' *Triquetrella* clade. Thus the character of multiple papillae per cell is to be seen as a symplesiomorphy shared by *T. paradoxa* (formally transferred below) and the multi-papillose Cape material, whilst spiculose papillae are a synapomorphy for the core clade. Given its position in the phylogeny, the occasional presence of a central strand in *T. californica* must be interpreted as a reversal and raises some interesting questions about the genetic control of morpho-anatomical characters in Pottiaceae, suggesting that the loss of this trait may be due to functional changes in a single gene (cf. Zander, 2006).

Given the distinction of the molecular markers, the consistent trait of relatively small size of mature plants in all the many South African collections compared with *T. paradoxa* (leaves about one-third the length of those of the latter), and the distinctive anatomical details listed below, we here describe the species as *T. mxinwana*. The Australian populations differ in the gain of a codon in the *rps4* coding region relative to all the other accessions. Insertion-deletion events are rare in this region, and in broad samplings across all the mosses (Goffinett, Shaw & Hedderson, 2001; Hedderson *et al.*, 2004) only two have been identified previously - one characterizing the Funariaceae, and the other *Octoblepharum*. We take this as further support for recognition of two separate multipapillose species.

Strong similarities among the species of *Triquetrella* have been noted previously (Casas *et al.*, 1993; Zander, 1993) and, except for the *T. mxinwana* and *T. paradoxa* pair, the species are much alike. For the core species, somewhat subtle differences, as summarized by Casas *et al.* (1993), exist in length of the theca, leaf shape, acumination of the leaf apex, length of the decurrencies, and in presence or absence of the stem central strand. It is not clear, on the other hand, how reliably one could differentiate among these without information on provenance.

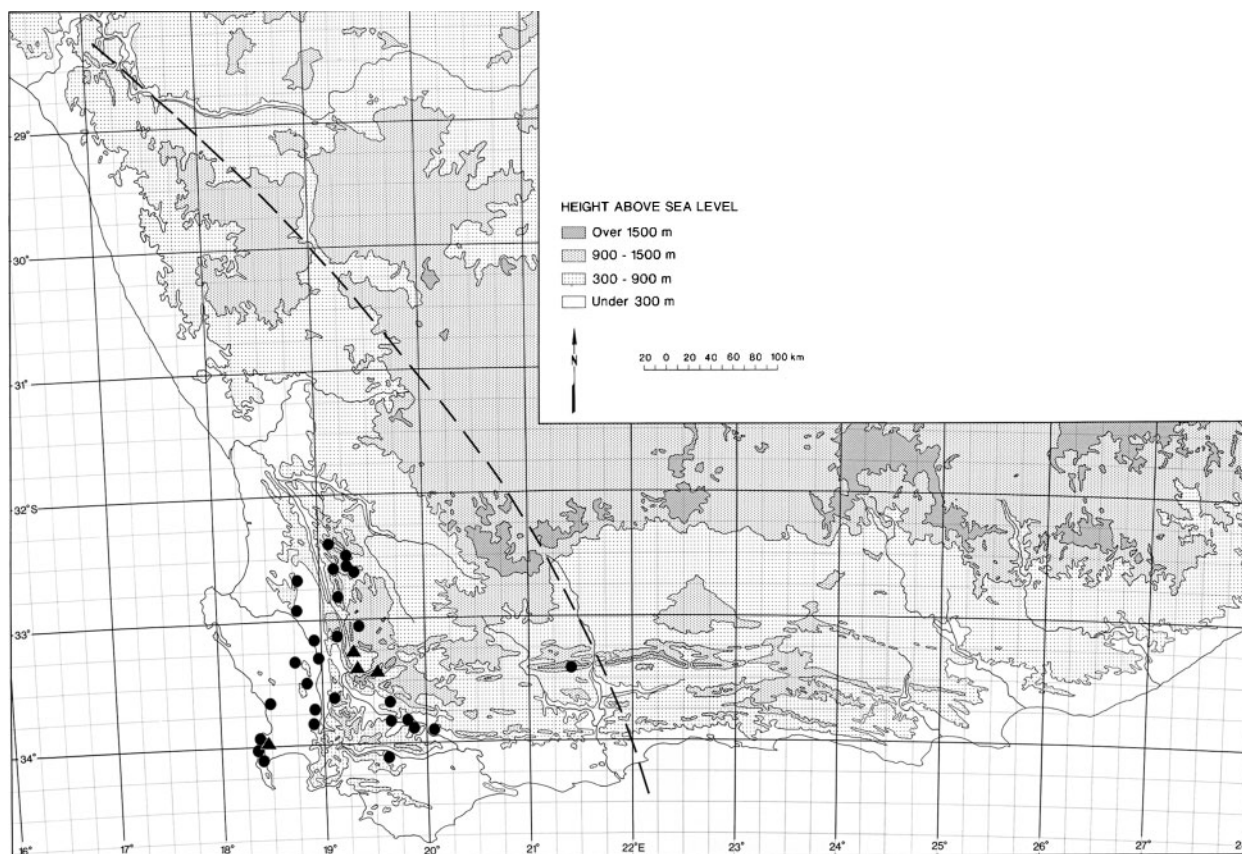
The distribution of *Triquetrella*, like that of a number of other bryophyte species and genera, is xero-thermophilic (Frey & Kürschner, 1988a, b), coinciding largely with areas of Mediterranean climate. The origin of such disjunctions has been the subject of some considerable interest, with the debate centred mainly on whether the disjunctions reflect recent dispersal or fragmentation of ancient ranges (for example, see Axelrod, 1973; Schofield, 1988; Fritsch, 2001; Shaw, Werner & Ros, 2003). Frey and Kürschner (1988a, b) have included plants exhibiting such disjunctions in their xerothermic Pangean element thought to have originated in continental areas of Pangaea in Permo-Triassic times (about 220 mya). Disjunctions between European and western North American areas of Mediterranean climate have similarly been attributed to fragmentation of Madrean-Tethyan distributions about 20–25 mya (Axelrod, 1973). Thus, under such vicariance explanations, taxa exhibiting multi-centric distributions among areas of

Mediterranean climate would have to be ancient and, in the case of bryophyte species or morphologically poorly differentiated genera like *Triquetrella*, this implies long periods of evolutionary stasis (Crum, 1972).

Similarity in form is mirrored in the lack of molecular differentiation amongst species of the core *Triquetrella* group. This is particularly remarkable given that the DNA loci sampled here are used frequently for analyses at the population and individual level in most plant groups including mosses (e.g. Sang *et al.*, 1997; Hedderson & Nowell, 2006). The low levels of molecular and morphological divergence observed in this study argue against vicariance hypotheses and are consistent with recent establishment of the range of *Triquetrella*. Although the absence of suitable fossil data precludes direct calibration of the phylogenetic tree, application of molecular clocks can be useful and provide rough estimates of divergence times, even when certain assumptions of such methods are violated (see Bromham & Penny, 2003). Based on published estimates for the regions sampled here (e.g. Bakker, Olsen & Stam, 1995; McDaniel & Shaw, 2003) divergence of the core *Triquetrella* clade began between 0.08 and 0.4 million years ago, whilst the split between *T. mxinwana* and *T. paradoxa* dates to 0.5–2.7 mya. Support for these dates comes from the strong association (Fig. 2) of *Triquetrella mxinwana* with the winter rainfall area of South Africa. This mega-niche, and much of the plant diversity within it, is thought to have existed only for the last two to five million years (Hendy, 1982; Deacon, 1983; Klak, Reeves & Hedderson, 2004), a chronology highly consistent with our estimates.

Although our dating estimates may be problematic, for example we have made no attempt to account for phenomena such as rate variation across the tree, they highlight the fact that Xerothermic-Pangean or Madrean-Tethyan, or indeed any other vicariance hypotheses that we are aware of, would require extremely unrealistic rates of substitution in all the markers evaluated. We would also point out that, given the position of Pottiaceae, and *Triquetrella* in particular, in recent DNA-based phylogenetic trees, attributing ages of more than a few million years to the genus would require pushing the origin of mosses to more than one billion years BP (T. A. Hedderson, unpublished), an unlikely scenario given currently accepted times for the origin of land plants. Rather, the current distribution of *Triquetrella* appears to be of Pliocene-Pleistocene age and therefore the result of dispersal. Studies of various organisms exhibiting broadly similar patterns of distribution have reached the same conclusion (Coleman *et al.*, 2003; Shaw *et al.*, 2003; Tremetsberger *et al.*, 2005).

A dispersalist hypothesis for *Triquetrella* may seem paradoxical given the rarity of sporophytes in the genus. However, the gametophytes fragment easily and seem to be the source of most new populations in at least the two South African species. In this region *T. tristicha* colonizes disturbed areas, such as road cuttings, very rapidly. At least some species produce gemmae (Casas



**Figure 2.** Map of southern and western South Africa showing the known distribution of *Triquetrella mxinwana*. Triangles indicate populations with sporophytes, and the dotted line indicates the approximate limit of the winter rainfall area, including some regions where rain may also fall in extreme late summer.

*et al.*, 1993) and these may also serve a dispersal role. Furthermore, species of the genus are very drought tolerant (Moore, Luff & Hallam, 1982) and gametophytes survive in a desiccated state for long periods. The combination of enhanced aridity and prevalent high winds that characterized some periods of the Pleiocene–Pleistocene (Stuut *et al.*, 2002; Dupont *et al.*, 2006; Chase & Thomas, 2007) may have rendered these particularly favourable for dispersal of *Triquetrella* and perhaps other mosses. Of relevance here is the recent demonstration by Muñoz *et al.* (2004) that patterns of wind intensity and direction better explain floristic similarity in a range of plant groups among sub-Antarctic islands than does geographic proximity. An alternative, but perhaps less likely, explanation is that during wetter periods, sporophytes might be more commonly produced. Certainly for the two South African species, the few known fruiting populations show great inter-annual variation in sporophyte production that appears to correlate strongly with the amount and timing of rainfall.

Whatever the dispersal mechanism(s), it would be of interest to establish the ancestral area for the group as well as the frequency, direction and more precise timing of the implied dispersal events. The positions of the African and Australasian taxa in the phylogeny are consistent with a southern hemisphere origin for the genus. Although winter

rainfall areas of Mediterranean climate are established by latitude-determined patterns of airflow, land corridors may previously have connected such areas of distinctive evolutionary challenge. The identical four-locus haplotypes shared by sampled individuals of the southern *T. tristicha* and the Mediterranean *T. arapilensis* suggest very recent movement between Southern Africa and the Mediterranean region. This may have occurred during periods of altered climate, such as the hypothermic phases of the late Quaternary (Petit *et al.*, 1999; Prentice & Jolly, 2000), perhaps via an eastern African arid corridor (Balinsky, 1962; Coleman *et al.*, 2003). A similar situation exists with the *T. californica*–*T. patagonica* pair where, again, the identical haplotypes found in the two regions suggest recent (perhaps Holocene) dispersal. Given the low levels of divergence among the taxa, coalescent analyses of denser population samples could be used to test these hypotheses directly.

#### Taxonomic Treatment

***Triquetrella paradoxa*** (I.G.Stone & G.A.M.Scott) Hedd. & R.H.Zander, comb. nov.

Basionym: *Leptodontium paradoxum* I. G. Stone & G. A. M. Scott, J. Bryol. 11: 701. 1981.

**Type:** Australia, Victoria, Mt Alexander, *I.G. Stone* 14550, 16 November 1978 (Isotype BM!).

Representative specimens examined:

**AUSTRALIA:** **New South Wales**, S of Goulburn, Crisp's Creek, *Vitt* 27377 (MO); **South Australia**, Mt Barker, *H. Streimann* 54794 (MO); **Victoria**, Flat Rock, Grampians National Park, *H. Streimann* 55251 (Musci Australasiae Exsiccati 569) (MO); **Western Australia**, Boyagin Rock, *H. Streimann* 54182 (Musci Australasiae Exsiccati 423) (MO).

**Triquetrella mxinwana** Hedd. & R.H.Zander, **sp. nov.** (Fig. 3)

Plantae dense caespitosae. Caulis in sectione transversali rotundato-triangularis, filio centrali nullo, hyaloderme nulla, pilis axillaribus cellulis basalibus 1–2, parietibus tenuiter crassis. Folia tri-seriata, in statu madido late patentia vel patenti-recurva, ovato-triangularia, lamina superna carinata, marginibus in 2/3 usque 3/4 parte infera folii recurvis; margines basales foliaries longe decurrentes; costa percurrens, epiderme ventrali nulla, filo hydroideo nullo; cellulae supernae laminales rotundato-rhombicae vel quadratae, leniter triangulae, papillis parvis humilibus bifidus per lumen 4–6; cellulae basales parum distinctae. Perichaetia terminalia, foliis interioribus ovatis vel ellipticis, valde setam vaginantia. Perigonia lateralia, ex axillis folii orta. Seta 0.9–1.5 cm longa. Capsula 1.0–1.8 mm longa, elliptica, annulo e cellulis vesiculosus in 3 series dispositis composito; dentes peristomii 16, usque ad basem fissi, obtuse subulati, hyaline vel pallide lutei, maximam partem leaves vel dentibus paucis pro parte leniter spiraliter striates. Calyptra laevis. Sporae 12–20  $\mu\text{m}$  diametro. Color laminae in KOH reagens luteus vel aurantiacus.

**Type:** South Africa, Western Cape Province, E. side of Skuurweberg, Fairfield Farm, 3319AD, *Hedderson* 15208, 27 August 2003 (holotype at BOL, isotype at MO).

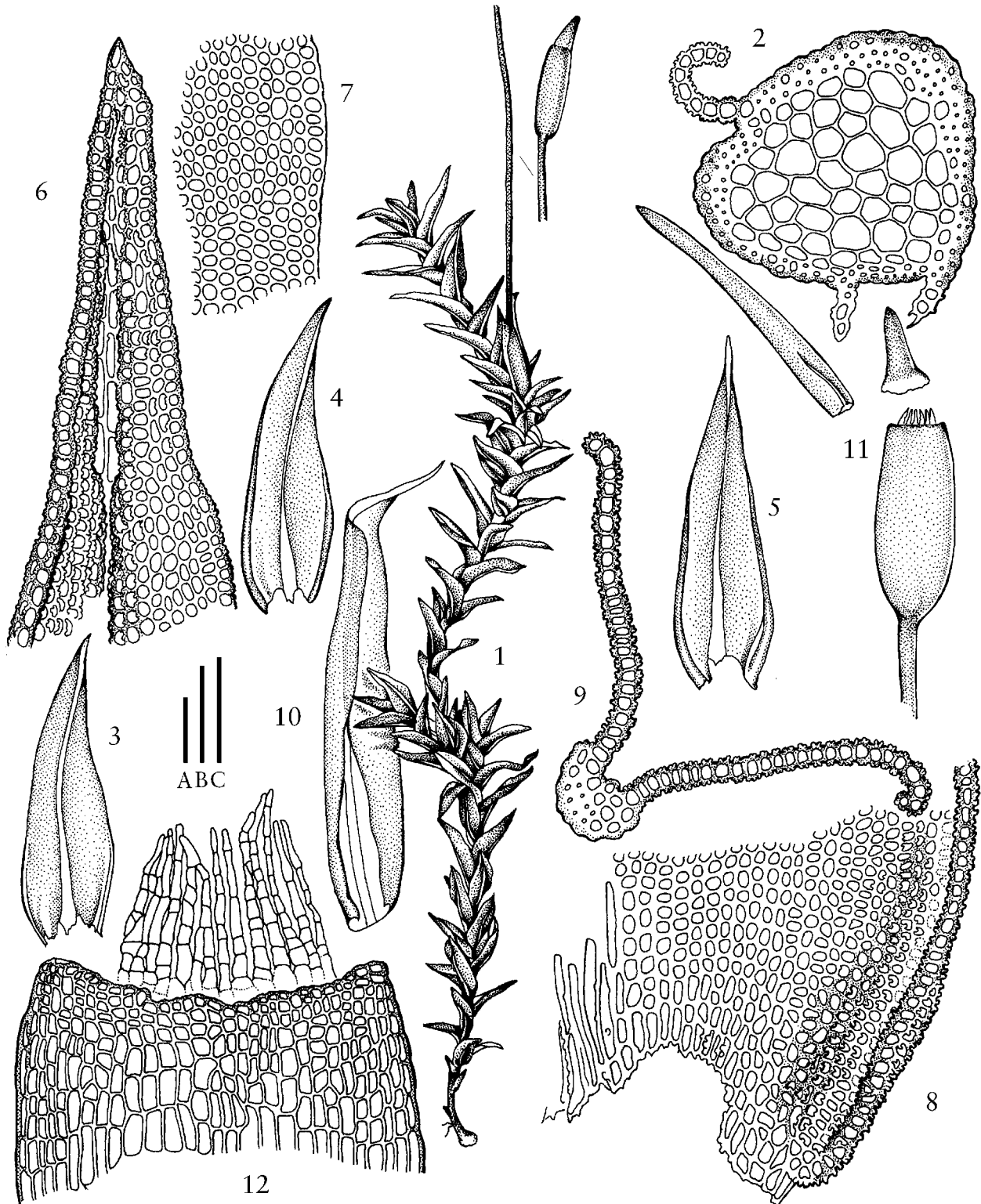
Plants growing in dense mats, yellowish or blackish green above, brown or blackish brown below. Stems branching occasionally, to 2 cm in length, occasionally papillose, transverse section rounded-triangular to five-sided, central strand absent, sclerodermis present, of 1–3 layers of stereid cells, hyalodermis absent; axillary hairs 6–9 cells in length with cells weakly bulging, basal 1–2 cells very weakly thick-walled; sparsely radiculose below. Leaves in 3 distinct rows, the rows straight or weakly spiralling counter-clockwise, appressed-incurved to weakly spreading when dry, widely spreading to spreading-recurved when moist, ovate-triangular, 1.0–1.4(–1.7) mm in length, upper lamina keeled, narrowly channelled along costa, margins recurved in lower 2/3 to 3/4 of leaf, entire; apex narrowly acute to short-acuminate; base ovate, basal leaf margins broadly long-decurrent; costa percurrent, superficial cells elongate and smooth or weakly papillose ventrally, rhombic-quadrate near apex and smooth or papillose dorsally, 2(–4) rows of cells across costa ventrally at midleaf, costal transverse section ovate to semicircular, ventral epidermis absent, dorsal stereid band usually absent, guide cells 2–4 in 1 layer, hydroid strand absent, dorsal epidermis differentiated as thick-walled cells with semicircular lumens;

upper laminal cells rounded-rhombic to quadrate, 8.4–10.5  $\mu\text{m}$  in width, 1 : 1, occasionally elongate transversely, walls thickened, weakly trigonous, superficially weakly convex on both sides; papillae small, low, bifid, 4–6 per lumen; basal cells differentiated weakly as a single row of slightly elongate cells at leaf insertion. Dioicous. Perichaetia terminal but often appearing lateral because of sub-perichaetial innovation, inner leaves ovate to elliptic, to 2.2 mm in length, strongly sheathing seta, apex short-acuminate and reflexed, lower cells rhombic, upper cells narrowly rectangular to rhombic, thick-walled throughout. Perigonia lateral, borne in leaf axils; bracts ovate to nearly circular. Seta 0.9–1.5 cm in length, 1 per perichaetium, yellow, twisted clockwise above. Capsule 1.0–1.8 mm in length, yellowish brown, elliptical, annulus of ca 3 rows of smaller, vesiculose cells; peristome teeth 16, cleft to base, blunt, subulate, transparent or pale yellowish, mostly smooth or some teeth weakly spirally striate in part, 110–130  $\mu\text{m}$  in length, with ca 4–6 articulations, straight, basal membrane absent; operculum bluntly conic, ca 0.5 mm in length, cells straight. Calyptra smooth, ca 2.2 mm in length. Spores ellipsoidal to spherical, 12–20  $\mu\text{m}$  in diameter, yellowish brown, finely papillose. Laminar KOH colour reaction yellow to orange.

The species epithet is derived from the isiXhosa adjectival -mxinwa, meaning narrow, and refers to the narrow leaves of this species compared with its congeners. It is correctly pronounced in isiXhosa as 'm(side-affricative)inwana', and in Latin it is pronounced 'm'ksinwana'; but, as usual, botanical Latin is acceptably pronounced according to one's native tongue.

Additional specimens examined (all at BOL unless otherwise indicated):

**SOUTH AFRICA: Western Cape Province.** Citrusdal Area, Cederberg, Algeria Forestry Station, 3219AC, *Hedderson* 13078. Citrusdal Area, Cederberg. Apex Peak, 3219CA, *Hedderson* 13620, 13653. Koue Bokkeveld Mountains, Twee Riviere, Suikerbossie Farm, 3219CB, *Hedderson* 13766. Oliphant's River Mountains, Berghof Farm, on margin of Groot Winterhoek Reserve, 3219CC, *Hedderson* 14025. Helderfontein Farm, ca 45 km N. of Cape Town, 3318CB *Townsend* 82/289 (MO). Cape Peninsula, Table Mountain, between Window Gorge and Newland's Ravine, 3318CD, *Hedderson* 13813. Cape Peninsula, Table Mountain, North of Window Gorge, 3318CD, *Hedderson* 14986, c.fr. Table Mountain, Disa Ravine, 3318CD, *Sim s.n.* (PRE). Cape Peninsula, southern slopes of Lion's Head, 3318CD, *Pillans* 4088 (BOL, MO). Cape Peninsula, Camps Bay, Kloof Nek, road to the Round House, 3318CD, *Townsend* 82/58 (MO). Cape Peninsula, Table Mountain, Orange Kloof, 3318CD *Hedderson* 13539 (BOL, MO). Paarl, Paarl Rock, 3318DB, *Garside* 6621. Stellenbosch, Platklip, 3318DD *Perold* 463 (MO). Ceres Area, Op-die-Berg, 3319AB *Morley* 271 (MO). Worcester Division, Ceres Area, Theronsberg Pass, 3319AD, *Linder s.n.* BOL no. 32366, c.fr. Ceres Area, Waaihoek Berge, Trail through Waterval and Blokspring Kloofs, 3319AD, *Hedderson* 14426. Du



**Figure 3.** *Triquetrella mxinwana*: 1, habit; 2, transverse section of stem; 3–5, leaves; 6, leaf apex; 7, cells at mid-leaf; 8, leaf base; 9, transverse section at mid-leaf; 10, perichetial leaf; 11, capsule with operculum and calyptra; 12, peristome. Scale bars: A (1)=1 mm, B (3–5, 10, 11)=0.5 mm, C (2, 6–9, 12)=50  $\mu$ m.

Toits Kloof Pass, 3319CA, *Hedderson 13266*. Koo Valley, Simonsberg Farm, 3319DA, *Hedderson 15463*. Villiersdorp Area, Jonaskop, ca 35 km S. of Worcester, 3319DC *Townsend 821279* (MO). Robertson Area, Dassie's Hoek

Nature Reserve, 3319DD, *Hedderson 15557*. Robertson, Klaas Vogdts West., Heuningberg, 3320CC, *Hedderson 15523*. Seven Weeks Port, N.E. of Ladismith, 3321AD, *Magill 6153* (PRE). Cape Peninsula, Silvermine Nature

Reserve, 3418AB, *Hedderson 11698*. Rivieronderend Mountains, McGregor Area, Boesman's Kloof, 3419BA, *Hedderson 15242*.

**Habitat:** The species is found in fynbos, succulent karoo and renosterveld vegetation (vegetation types follow Low, 1996). Most collections are from shales and granites, or clay soils derived from these substrates, and populations on sandstone soils in fynbos are mostly in transitional zones where there is admixture of either shale or granite. At higher altitudes, however, it occurs directly on sandstone, or in 'zuurvlaakte' on deep, pure, quartzitic sands; 100–1400 m a.s.l.

*Triquetrella mxinwana* fits well into the complex of characters described above as characterizing the genus. Its peristome is quite unlike that of *Leptodontium* but characteristic of *Triquetrella* (see Zander, 1993). *Triquetrella mxinwana* differs most saliently from the majority of congeners by its low, bifid laminal papillae several occurring over each lumen. Furthermore, it is smaller, with stems to 2 cm, not 7 cm, in length; the central strand is never present; leaves are entire, never weakly serrulate above; the seta is 0.9–1.0 cm long while the seta of other species is generally 1.5–2.3 cm in length; and the theca is shorter, 1.0–1.2 mm as opposed to 1.6–2.1 mm in length.

In gametophytic traits it is similar to *T. paradoxa*, and like that species the stem section is occasionally 5-sided rather than 3-sided (as pointed out by Stone & Scott, 1981). However, the two differ in a substantial number of traits as follows: plants of *T. mxinwana* as a whole are half the size, with leaves having about half the number of cells across the leaf and stems half the length; leaves are 1.9–2.8 times longer than broad (versus 2.8–3.5 times); elongate cells at the leaf base are few or absent (versus 3–5 rows of strongly elongate cells); distal laminal cells are larger, 8.4–10.5  $\mu\text{m}$ , with thick walls and somewhat irregular lumens (versus 6.0–8.5  $\mu\text{m}$ , thin walls and smoothly rounded lumens); papillae are larger and more prominent (versus small and difficult to make out).

It is most readily (and unambiguously) distinguished from *T. tristicha*, the only other South African species, by the papilla character. With experience the two can also be separated reliably in the field since whilst the new species is shiny, *T. tristicha* is usually matte in appearance with a glaucous overtone to the yellowish colour. In addition the leaves of the latter are clearly ovate compared with the more distinctly triangular to ovate-lanceolate leaves of *T. mxinwana*.

Catcheside (1980) indicated that sporophytes are rare for this genus in South Australia, and Brotherus (1924, 1925) found it mostly sterile on a global level. The other South African species commonly has perigonia but only rarely fruits (Magill, 1981); sporophytes have been seen in only two populations. Both perichaetia and perigonia are present in *T. mxinwana* and a few specimens have sporophytes. Most of the sporulating specimens are from a small area of the Warm Bokkeveld, where some populations may fruit quite prolifically (as at the type locality, where there were hundreds of sporophytes), but a

single sporophyte was also found in a population from the Cape Peninsula (Fig. 2).

*Triquetrella mxinwana* is currently known only from the winter rainfall area of the Western Cape Province (Fig. 2), but within this range it is rather common in certain habitats. For example, a recent study of remnant renosterveld vegetation in the Swartland region (Hedderson, Watson & Rugengamanzi, unpublished) found it in about half of the surveyed fragments. It has been collected numerous times over the past century, and several specimens were found at BOL, MO and PRE marked as an unidentifiable species of *Triquetrella*, often including notes calling attention to the salient distinguishing feature, the small laminal papillae that occur several per lumen. Many specimens of *T. mxinwana* were identified by T. R. Sim as *T. strictissima* Rehm. ex Müll.Hal. However, examination of type material of that species (BM, H-Br) shows clearly that it is a synonym of *T. tristicha* as indicated by Magill (1981).

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TAXONOMIC ADDITIONS AND CHANGES: *Triquetrella paradoxa* (I.G.Stone & G.A.M.Scott) Hedd. & R.H.Zander comb. nov. (syn. *Leptodontium paradoxum* I.G.Stone & G.A.M.Scott); *Triquetrella mxinwana* Hedd. & R.H.Zander sp. nov.

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