Massonia pustulata JACQ. 1791 and *M. longipes* BAKER 1897 (Hyacinthaceae), two frequently misunderstood species – or how *M. pustulata* became depressed

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Abstract: By comparison of morphological and molecular data we found that *Massonia pustulata* and *M. longipes* are two well separated species of their own with no evident relationships to *M. depressa* or *M. echinata*. We provide new data for distinguishing these species. We traced back the history of the perception of these two species in order to disentangle the confusing situation in recent literature.

Zusammenfassung: Durch den Vergleich morphologischer und molekularer Daten wurden die Eigenständigkeiten der beiden Arten *Massonia pustulata* und *M. longipes* festgestellt. Beide Arten zeigen weder zu *M. depressa* noch zu *M. echinata* nähere Bezüge. Neue Daten zur Unterscheidung der beiden Arten werden vorgestellt. Ein historischer Abriss über die unterschiedliche Perzeption dieser Taxa in der jüngeren Literatur soll dazu beitragen, die verworrene Situation zu entschärfen.

Key words: *Massonia pustulata, Massonia longipes,* Hyacinthaceae, South Africa.

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Introduction

The genus *Massonia* was described in 1780 by HOUTTUYN (1780). Up to now, approximately 90 species have been described within this genus and about 60 fit into its recent concept. Among these, there are three species with rather large and densely pustulated leaves: *Massonia pustulata* JACQ. (JACQUIN 1791), *M. scabra* THUNB. (THUNBERG 1800) and *M. longipes* BAKER (BAKER 1897).

Massonia pustulata JACQ.: In the year 1788 Franz BOOS, a gardener of the Schoenbrunn Botanical Garden, returned from his trip to South Africa, Mauritius and Reunion (GUNN & CODD 1981). He brought with him a new species of Hyacinthaceae that he and/or Georg SCHOLL had collected on one of their excursions through the Cape Province of South Africa. JACQUIN (1791) described this plant as *Massonia pustulata* JACQ. and provided a beautiful illustration of the new species in his "Plantarum rariorum Horti Caesarei Schoenbrunnensis" (JACQUIN 1804: t. 454). This illustration (Figs. 1, 6) represents the iconotype of this species. One year earlier KER GAWLER (1803: t. 642) had also provided an illustration of this taxon (Fig. 2). LEIGHTON (1943: t. 915) presented an illustration of a different type of plant as *M. pustulata* (Fig. 5). She stated that the illustration of JACQUIN (1804: t. 454) represents a "somewhat etiolated greenhouse specimen".

Massonia scabra THUNB.: Francis MASSON, the titular person of the genus *Massonia*, on his second trip to South Africa, painted a plant in 1792 (Fig. 3) that THUNBERG (1800) described as *Massonia scabra* THUNB. An illustrative picture of this taxon has also been provided by ANDREWS (1802: t. 220) (Fig. 4). As type for this species a specimen deposited at the UPS-THUNB under the number 7992 has been selected. This specimen has been collected by MASSON without a collection number and with the locality "Cap. b. Spei". *M. scabra* has been regarded as a synonym of *Massonia pustulata* by BAKER (1897: 411)

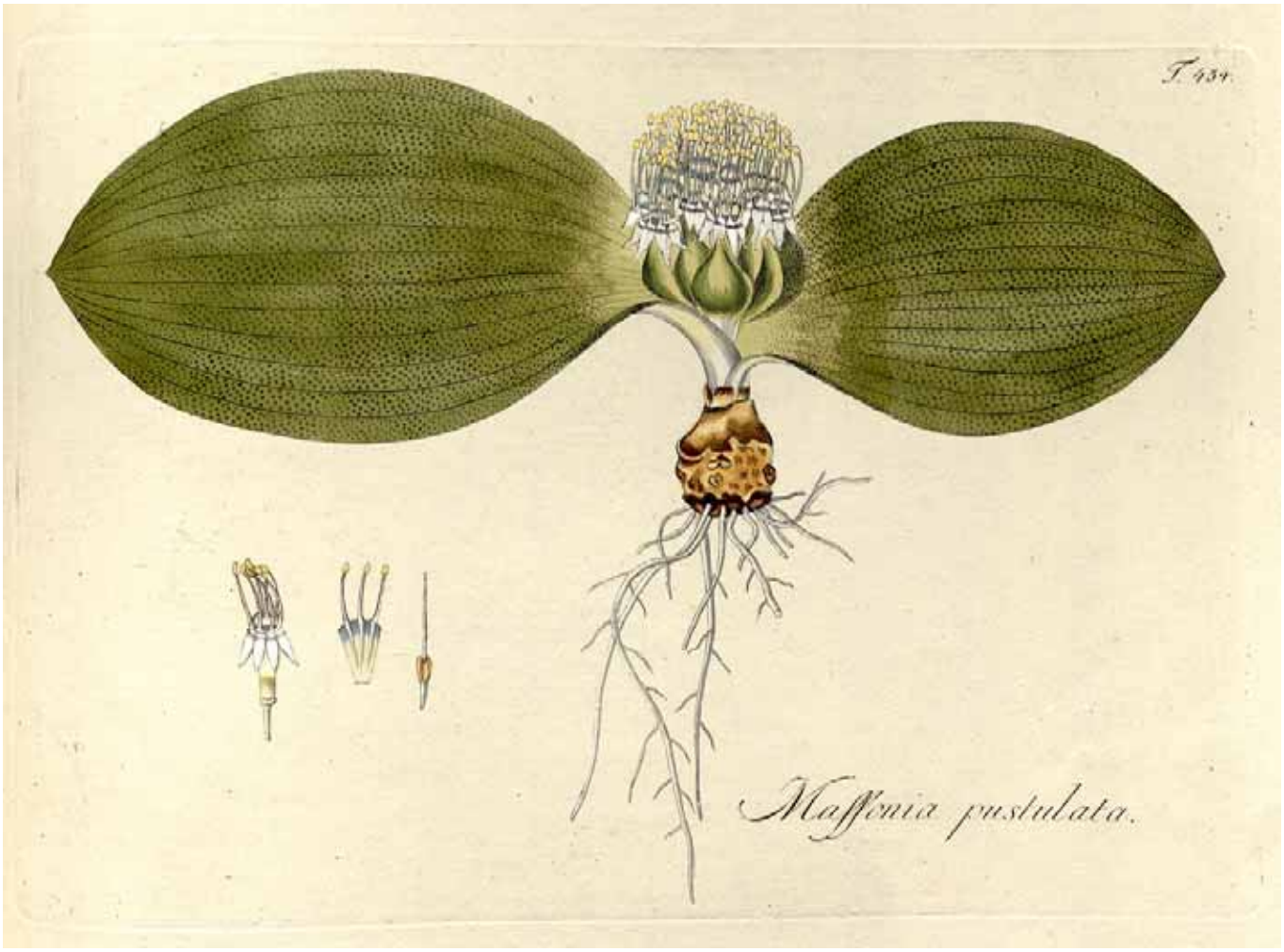

Fig. 1: *Massonia pustulata* — Iconotype. Table 454, JACQUIN (1804).

and MÜLLER-DOBLIES & MÜLLER-DOBLIES (1997: 69) and as a synonym of *Massonia echinata* L.f. by JESSOP (1976: 414) and SUMMERFIELD (2004: 35).

Massonia longipes BAKER: BAKER (1897) in his final treatment of the genus *Massonia* described *M. longipes* BAKER. Type specimen of *M. longipes* is Bolus 5973, a herbarium sheet deposited at the Kew Herbarium (K). JESSOP (1976: 420) treated *M. longipes* as a synonym of *M. angustifolia* L.f. (for a discussion of this taxon see MANNING & VAN DER MERWE 2002). *M. longipes* has been regarded as a synonym of *Massonia pustulata* JACQ. by SUMMERFIELD (2004). It is interesting however, that SUMMERFIELD determined the type specimen "*M. echinata* L.f." (see determination slip at the type specimen – A.M. VAN DER MERWE [the married name of A.M. SUMMERFIELD]; 14.12.2001).

We became aware of a problem with the interpretation of *M. pustulata* and *M. longipes* when we tried to determine two obviously different types of densely pustulated Massonias (Figs. 7, 8). When using the key provided by BAKER (1897: 408) we determined the plants as *M. pustulata* and *M. longipes*. When we used the more recent keys of JESSOP (1976: 408), MÜLLER-DOBLIES & MÜLLER-DOBLIES (1997: 66), MANNING et al. (2002: 450) and SUMMERFIELD (2004: 33) we determined the same plants as *M. depressa* HOUTT. and *M. pustulata*.

In this paper we study the morphology of two different types of Massonias (Figs. 7, 8) with densely pustulated leaves (two populations of each type). We compare the morphology with the first descriptions and type specimens of *M. pustulata*, *M. scabra* and *M. longipes* and with the treatments of these species in literature. Furthermore we provide a preliminary phylogeny of the *trnC-ycf6* region of the chloroplast genome of the genus *Massonia* to determine the position of these four populations within a phylogeny of the genus.

The aim of this study is to clarify the taxonomic situation of the large-leaved, densely pustulated taxa of the genus *Massonia*.

Tab. 1: List of taxa investigated in this study, with voucher and locality information. All vouchers are deposited at LI (Herbarium Biocenter of the Upper Austrian Museums).

Taxon	Voucher	Locality
Massonia depressa HOUTT.	WW03964	ZAF: Leliefontein
-"-	WW03968	ZAF: Kamieskroon 1
-"-	WW03969	ZAF: Kamieskroon 2
Massonia echinata L.f.	WW03970	ZAF: Vanrhyn´s Pass
Massonia longipes BAKER	WW03979	ZAF: Cape Infanta
-"-	WW03983	ZAF: DeHoop, Koppie Allen
Massonia pustulata JACQ.	WW01140	ZAF: Swellendam
-"-	WW03984	ZAF: Napier

Material and Methods

Taxonomic sampling — species sampled in this study are listed in Tab. 1.

Morphological data — morphological measurements of flower parameters were performed on material fixed in FAA from plants cultivated in the greenhouse. Previously, it had been tested on selected specimens that flower morphology of greenhouse-cultivated plants did not differ from flowers collected from natural localities. Measurements of leaf morphology were performed on fresh leaves.

Molecular techniques, DNA data generation — DNA was extracted from leaf tissue using either the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) or the CTAB method (DOYLE & DOYLE 1987) with modifications (PFOSSER et al. 2006). The *trnC*^{GCA}-*ycf6* intergenic region was sequenced for this study. Primers used for amplification were *trnC*^{GCA}F (CCA GTT CRA ATC YGG GTG) (modified from DEMESURE et al. 1995) and *ycf6*R (GCC CAA GCR AGA CTT ACT ATA TCC AT) (SHAW et al. 2005) using standard thermal cycling conditions (95°C, 5 min; 35 cycles of 94°C, 20 sec; 50°C, 30 sec; 72°C, 1 min; final extension at 72°C, 10 min). PCR was performed using Hybaid thermal cyclers in 20 µL volumes with the following reaction components: 2 µL template DNA (10–100 ng), 2X DreamTaq ReadyMix PCR reaction mix (Fermentas) and 0.1 µmol/L each primer. Amplified double-stranded DNA fragments were purified with Exonuclease I and Shrimp alkaline phosphatase (Fermentas) following the protocol of the manufacturer to remove unincorporated nucleotides and excess primers prior to sequencing. Dideoxy sequencing was performed using the purified PCR fragments following the DYEnamicET cycle sequencing protocol (General Healthcare, USA). Both strands were sequenced using the same primers as for amplification. Separation of fragments and base calling was performed on a MegaBace 500 automated sequencer (General Healthcare, USA). On average, less than 1% of data matrix cells were scored as missing data.

Fig. 2: *Massonia pustulata*. Table 642, Ker Gawler (1803).

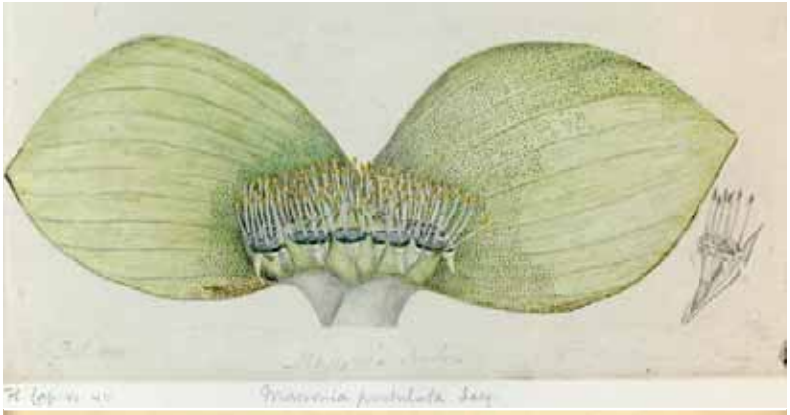

Fig. 3:
Massonia pustulata.
As *M. scabra*, unpublished painting, MASSON (1792).
(Image courtesy of Natural History Museum, London.)

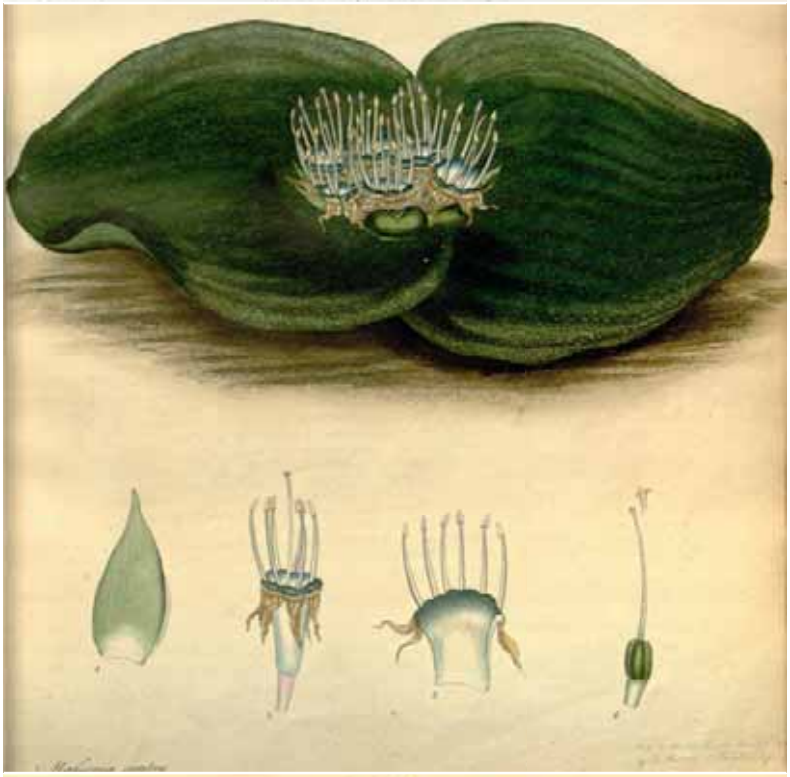

Fig. 4:
Massonia pustulata.
As *M. scabra*, table 220, ANDREWS (1802).

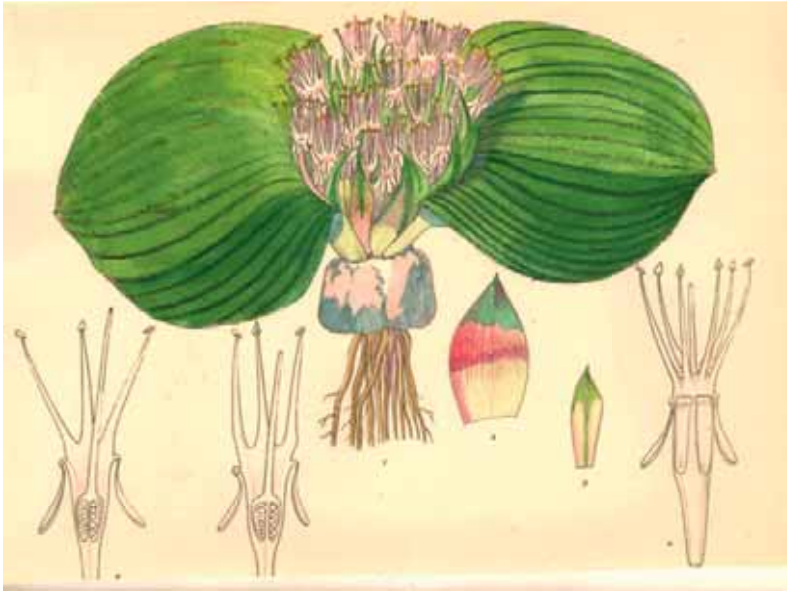

Fig. 5:
Massonia longipes (or *M. setulosa?*).
As *M. pustulata*, table 915, LEIGHTON (1943).

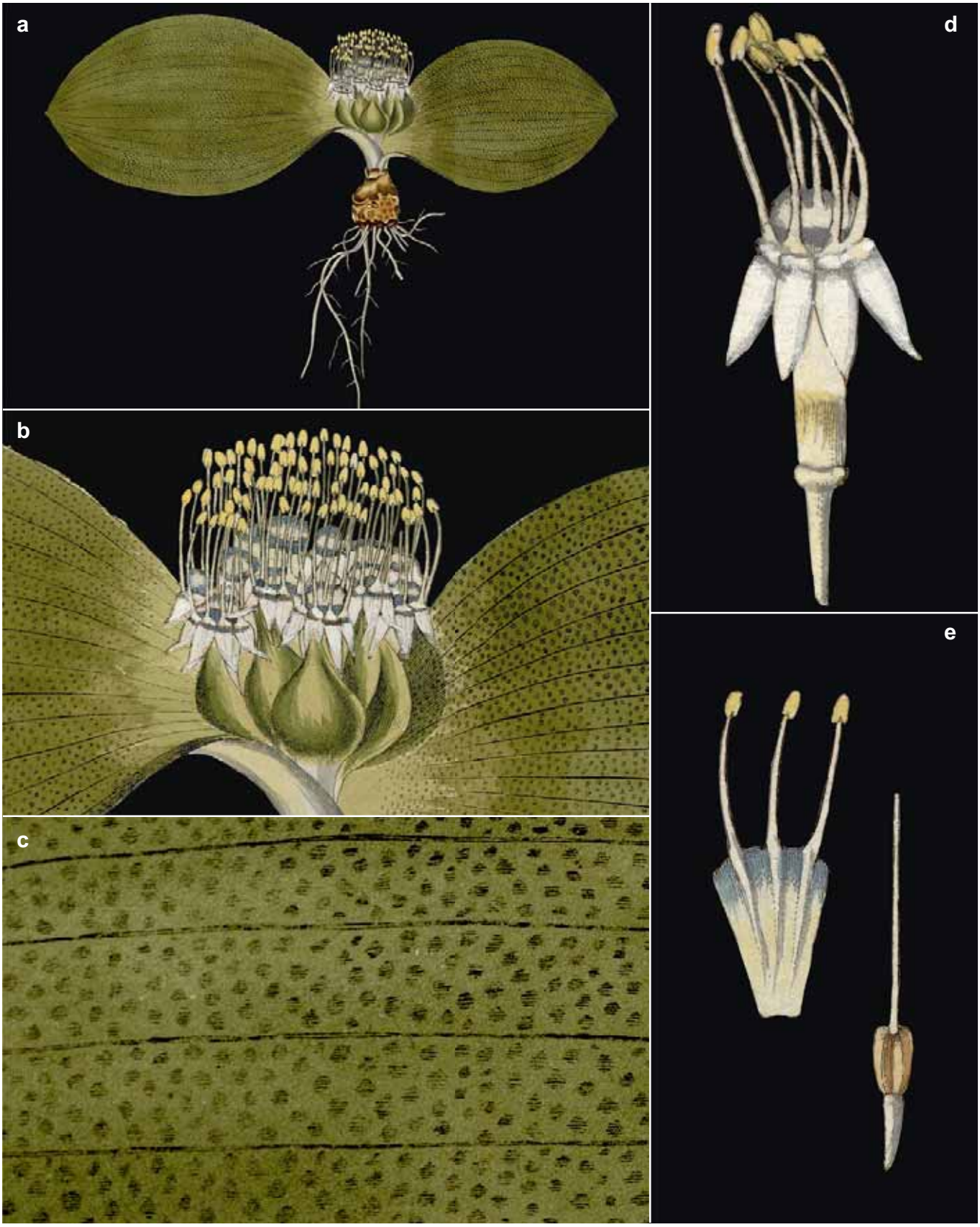

Fig. 6: *Massonia pustulata* — Iconotype, JACQUIN (1804, tab. 454). a) habitus; b) inflorescence; c) leaf surface; d) flower; e) opened flower and pistil. For easier comparison with Figs. 7–10 the illustrations are presented in a similar way. Scaling of the illustrations is not possible as there is no scale bar at the original table.

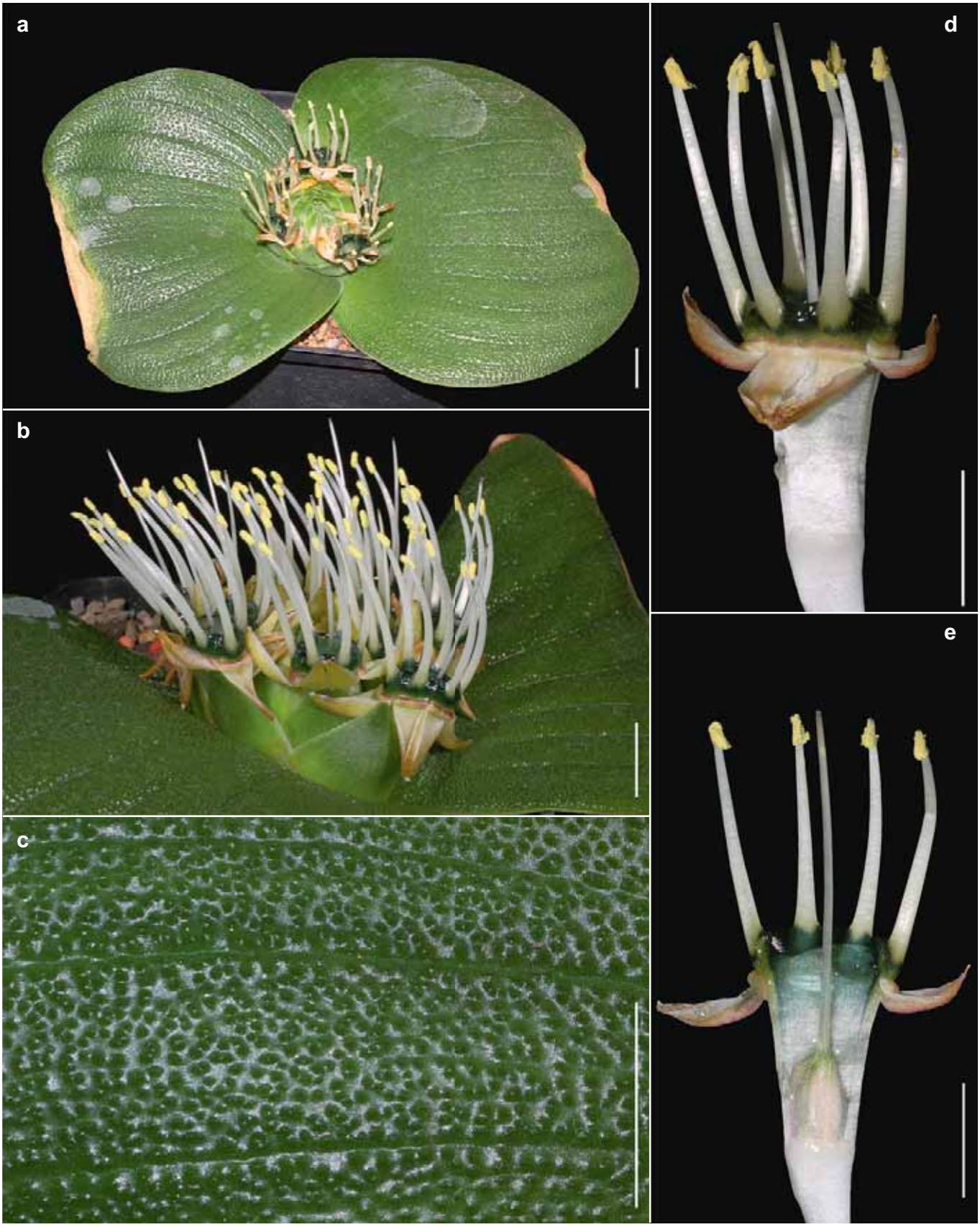

Fig. 7: *Massonia pustulata* (WW 03984). a) habitus; b) inflorescence; c) leaf surface; d) flower; e) opened flower. Scale bar 1 cm.

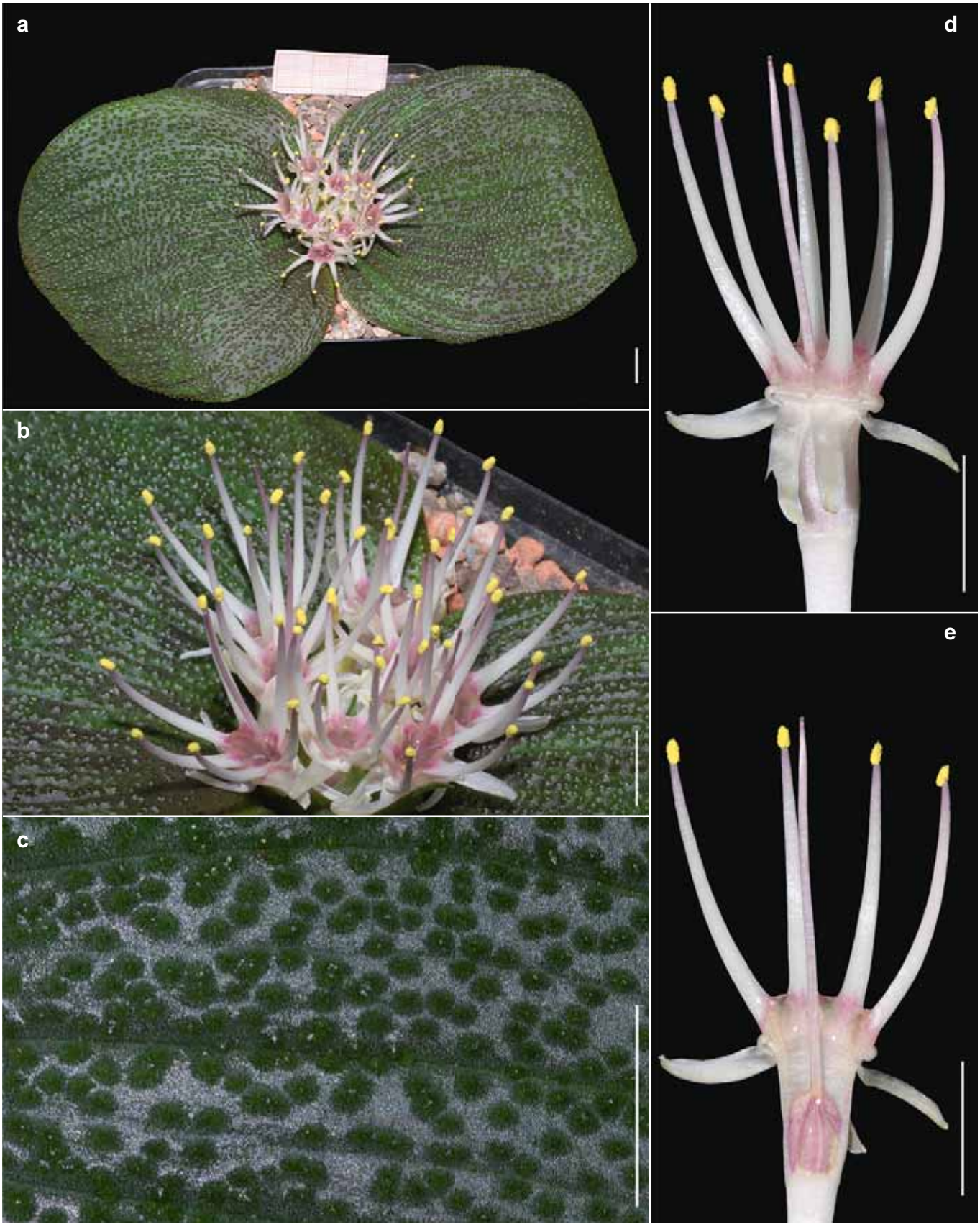

Fig. 8: *Massonia longipes* (WW 03979). a) habitus; b) inflorescence; c) leaf surface; d) flower; e) opened flower. Scale bar 1 cm.

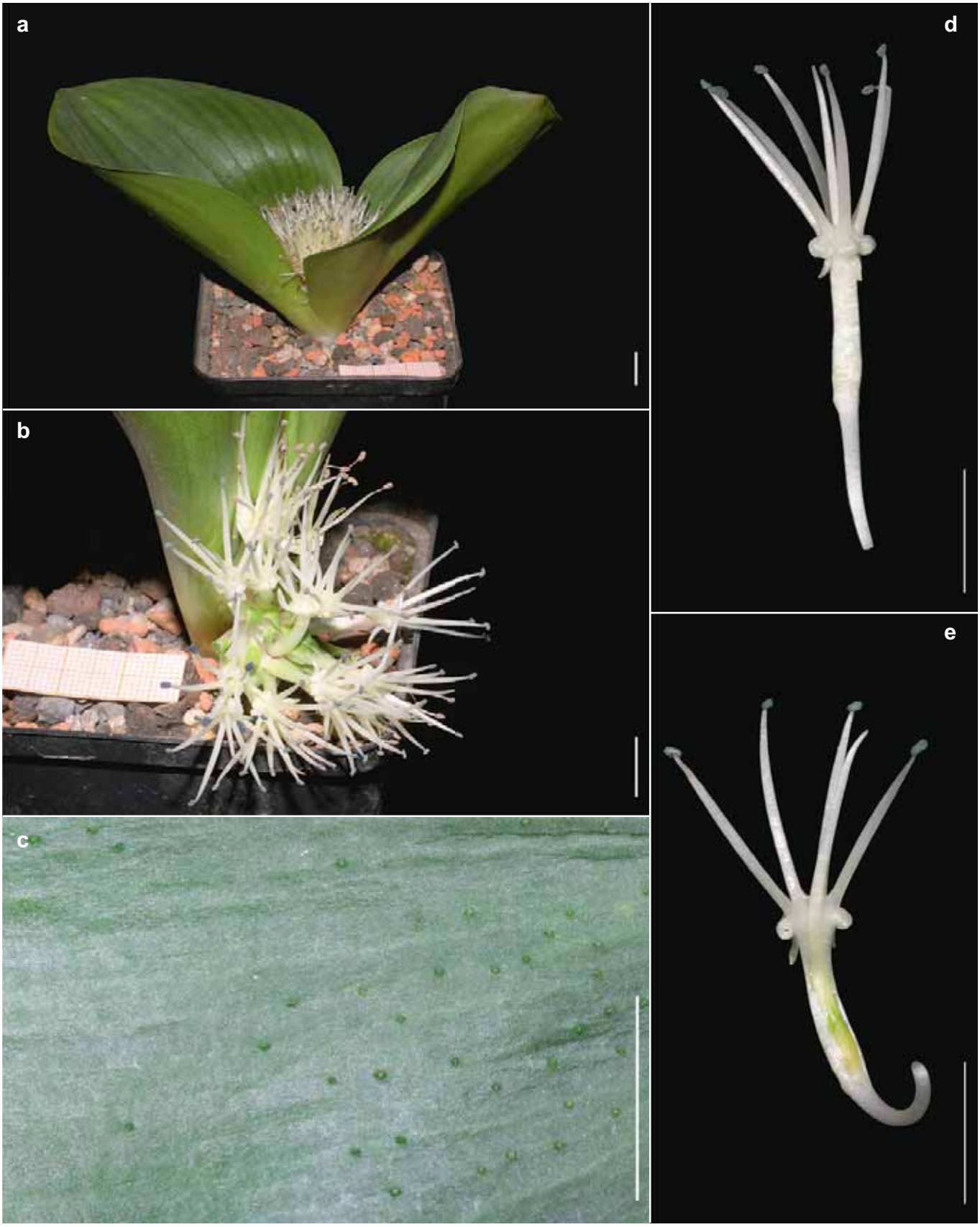

Fig. 9: *Massonia echinata* (WW 03970). a) habitus; b) inflorescence; c) leaf surface; d) flower; e) opened flower. Scale bar 1 cm.

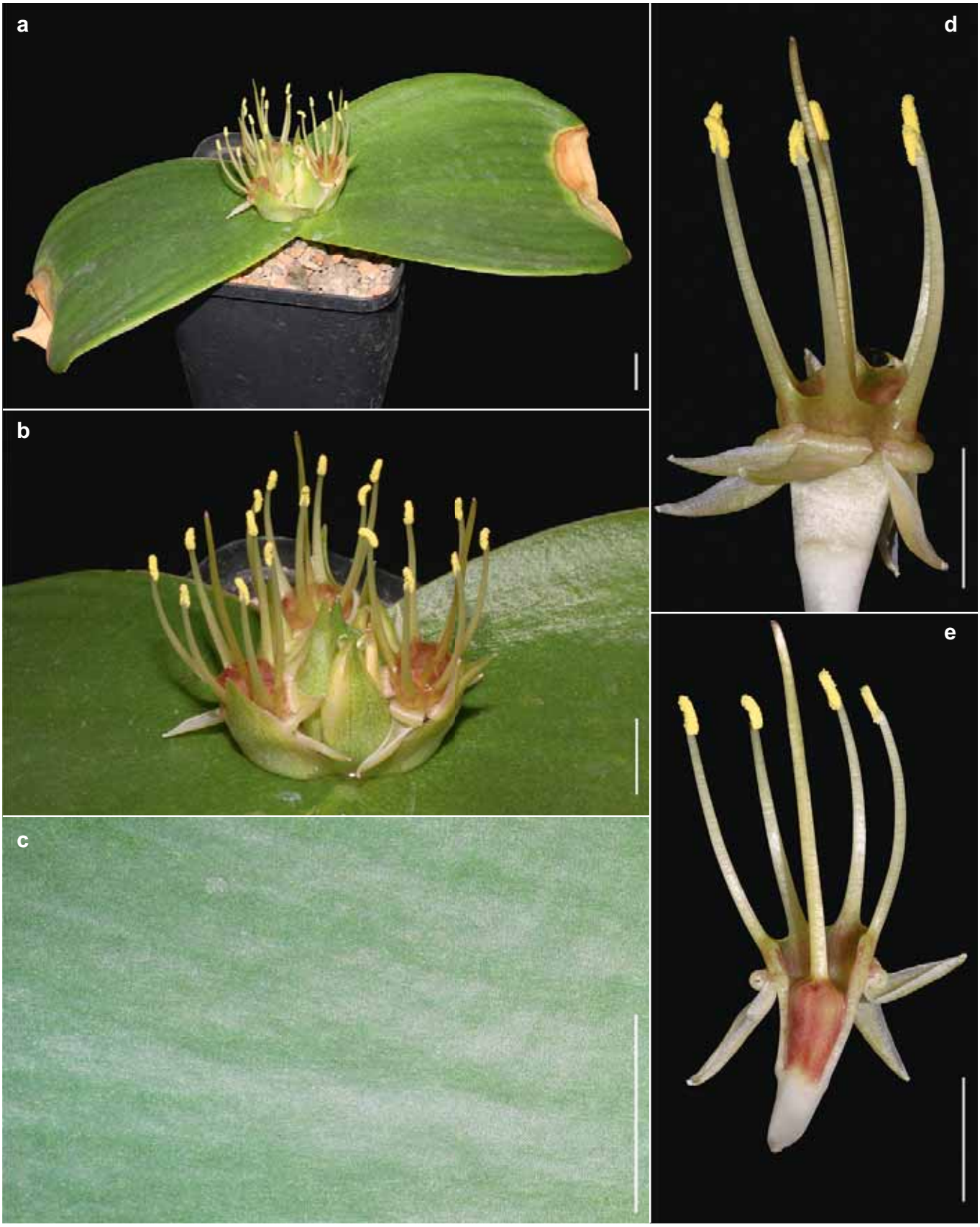

Fig. 10: *Massonia depressa* (WW 03964). a) habitus; b) inflorescence; c) leaf surface; d) flower; e) opened flower. Scale bar 1 cm.

Tab. 2: Comparison of character states of selected characters of four taxa (6 populations) of the genus *Massonia*.

	M pustulata		*M. longipes*		*M. depressa*	*M. echinata*
	(01140) Swellendam	(03984) Napier	(03979) Infanta	(03983) DeHoop	(03968) Kamieskroon	(03970) Vanrhyn´s Pass
Leaf length (cm)	10	11,5	9	12	19	13,5
Leaf width (cm)	12	13	11	12	20	8
Leaf pustulate (y/n)	y	y	y	y	n	n
Pustules number/cm^2	170	115	27	36	0	0
Pustules diameter (mm)	0,5	0,7	1,1	1,1	0	0
Perigon-filament-tube length (mm)	12	15	12	10	5	8-(11)-14
Perigon-filament-tube width (mm)	5x7	9	5	5	10x8	(2)-4
Filament-tube/apex of perigon-filament-tube color	bluish-green	bluish-green	pink	light pink	reddish-brown	white
Filament length (mm)	16	17	17	15	20	(9)-12
Filament-tube length (mm)	3	3	2	2	2	0
Anther length (mm)	3	3	1	2	5	2
Anther color	yellow	yellow	yellow	yellow	yellow	bluish-grey
Ovary length (mm)	6	7	6	5	9	4-(5)-10
Ovary width (mm)	4	4,5	4	3	6	2
Style length (mm)	19	30	22	23	28	14-(15)-20

Indels in the data matrix were coded as additional characters, and tree searches were performed using the nucleotide data together with the indel data. Phylogenetic analysis using the maximum parsimony (MP) method were performed with the computer program PAUP* version 4.0b10 (SWOFFORD 2000). MP analyses were performed either without or with successive character weighting (rescaled consistency index) until tree lengths remained the same in two successive rounds. Most parsimonious trees were obtained by 1000 replicates of random sequence addition using tree bisection-reconnection (TBR) branch swapping under the Fitch criterion (FITCH 1971). Ten thousand fast bootstrap replicates (FELSENSTEIN 1985) were used to assess confidence limits for the resulting tree topologies.

Results and Discussion

Measurements of morphological data are summarized in Tab. 2.

***M. pustulata*:** When morphological data of WW01140 and WW03984 (Fig. 7) are compared to the first descriptions of *M. pustulata*, *M. scabra* and *M. longipes* it is evident, that they show large concordance with the first description and the iconotype of *M. pustulata* (Figs. 1, 6) and with the description and illustrations of *M. scabra* (Figs. 3, 4). This is especially true for the characteristic bluish-green color of the entrance to the filament- and perigon-filament-tube. JACQUIN (1791) stated in the first description: "Nectarium carnosum, ex viridi caerulescens, lucidum." [at that time the filament tube was regarded the nectarium] (see Figs. 1, 2, 3, 4 and 6). We therefore agree with BAKER (1897: 411) and MÜLLER-DOBLIES & MÜLLER-DOBLIES (1997: 69) that *M. scabra* is a synonym of *M. pustulata* and we disagree with JESSOP (1976: 414) and SUMMERFIELD (2004: 35) who regarded *M. scabra* as synonym of *M. echinata* L.F. (see Fig. 9 for an illustration of *M. echinata*).

If these two accessions (WW01140 and WW03984) of *M. pustulata* are determined with the key provided by BAKER (1897: 408) the result is *M. pustulata*. However, if these same two accessions are determined with the more recent keys provided by JESSOP (1976: 408), MÜLLER-DOBLIES & MÜLLER-DOBLIES (1997: 66), MANNING et al. (2002: 450) and SUMMERFIELD (2004: 33) the result is *M. depressa* (see Fig. 10 for an illustration of *M. depressa*). Evidently, there must have been a change in the perception of *M. pustulata*.

When we tried to trace back the history of perception of this taxon we came to the conclusion that most probably LEIGHTON (1943) initiated this change. She presented a plant as *M. pustulata* (Fig. 5) that showed considerable differences to the first description, the iconotype and earlier illustrations of *M. pustulata* (Figs. 1, 2) and *M. scabra* (Figs. 3, 4). Her plant (although the illustration is rather inaccurate – e. g. the sigmoid curve of the tepals has not been depicted) showed a plant that lacked the characteristic bluish-green color of the entrance of the filament- and perigon-filament-tube. Furthermore, the diameter of this entrance is narrow in contrast to the much wider entrance of JACQUIN´s plant. As justification for the differences to JACQUIN´s plant LEIGHTON (1943) stated: "The type of this species [*M. pustulata* JACQ.] was figured by JACQUIN in his Hortus Schoenbrunnensis (t. 454) in 1804. The plant depicted was probably grown in the Emperor of Austria´s garden at Schoenbrunn, and represents a somewhat etiolated greenhouse specimen. We are fortunate, therefore, in having a more normal example of this interesting species for publication here. It was collected … at Cape Infanta in the Swellendam Division …". However, our own studies showed that *M. pustulata* both at its natural habitat and in cultivation did not exhibit any differences in character

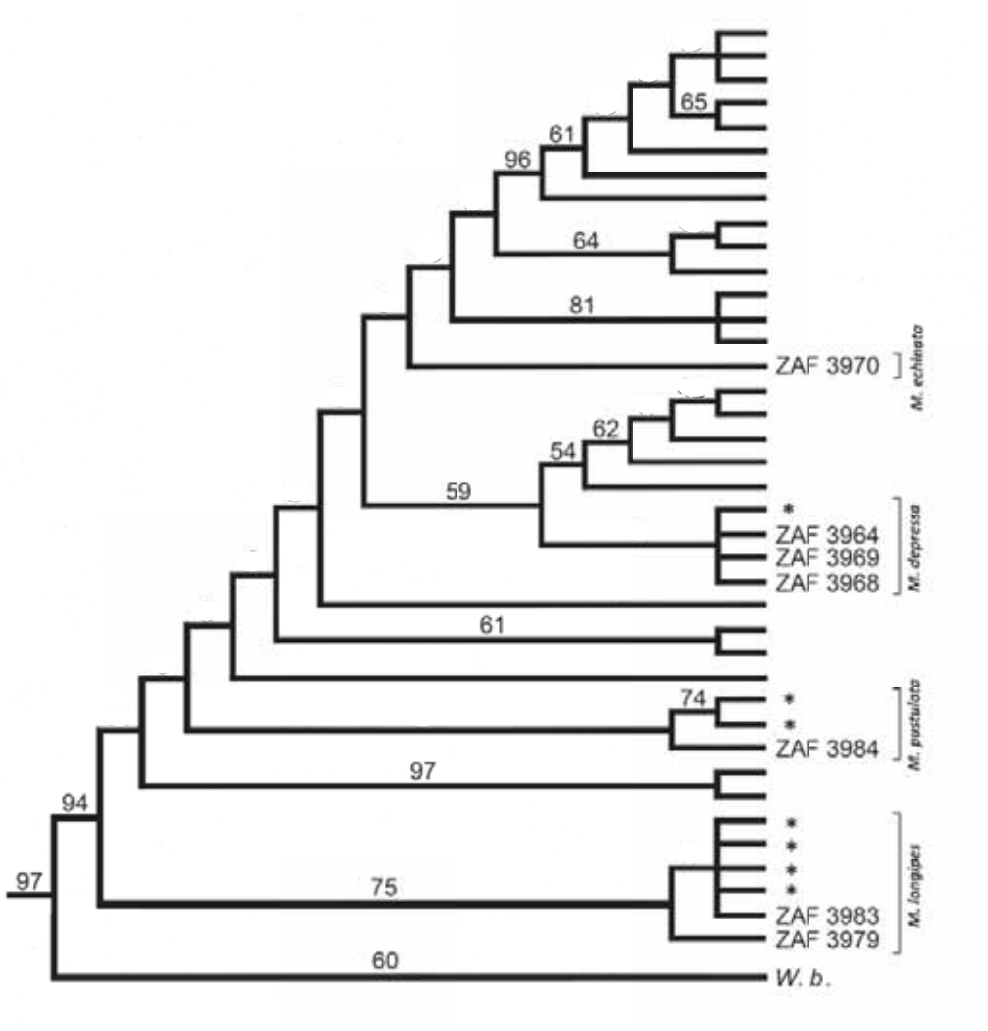

Fig. 11: Phylogenetic reconstruction based on maximum parsimony of members of the genus *Massonia*. A majority-rule consensus tree is shown with bootstrap support values > 50 % (indicated above branches); outgroup: *W. b.*: *Whiteheadia bifolia*; (*, accessions of unknown geographical origin).

states of diameter and coloring of the entrance to the filament- and perigon-filament-tube between wild and greenhouse plants. Instead, what LEIGHTON (1943) described was another species – *M. longipes*. The illustration of her article is inaccurate, so it is hard to decide if it shows *M. longipes* or *M. setulosa* BAKER (a species we found at Cape Infanta besides *M. longipes*).

Most probably it was LEIGHTON´s article that influenced JESSOP (1976) and subsequent authors in their erroneous perception of *M. pustulata*. This resulted in the fact, that in books (e. g. MANNING et al. 2002: 276), journal articles (e. g. SCHLESIES 1995: 18, SUMMERFIELD 2004: 30) and on the Internet (e. g. PBS 2012 – photographs of ITTNER and MACMASTER) almost all illustrations of "*M. pustulata"* are in fact depicting *M. longipes.* On the other hand, plants that represent "JACQUIN´s *M. pustulata*" are treated as *M. depressa* (PBS 2012 – photograph of MACMASTER).

***Massonia longipes*:** In the first description of *M. longipes* BAKER (1897) stated, that the perigon tube is 1/3 in. [8,4 mm] long and that the reflexing segments are slightly shorter than the tube. This is confusing as the filament tube of the type specimen (Bolus 5973, K!) has perigon-filament-tubes of about 12 mm length. The width of the entrance to the filament- and perigon-filament-tube is about 2 mm. The width is 5 mm in our plants although it has to be considered that our measurements were not made from herbarium material. However, the narrow entrance to the filament- and perigon-filament-tube, the anthers of about 1 mm length (3 mm in *M. pustulata*) in connection with the pustulated leaves and the locality near the coast strongly suggest that our accessions WW03979 (Fig. 8) and WW 03983 are conspecific with *M. longipes*.

Diagnoses: *M. pustulata* and *M. longipes* are clearly separated from other species oft the genus *Massonia* like *M. depressa* (Fig. 10) or *M. echinata* (Fig. 9) by their densely pustulated, relatively large leaves. *M. pustulata* (Figs. 1–4, 6, 7) is easily distinguishable from *M. longipes* (Figs. 5, 8) by the characteristic bluish-green color and the larger diameter (7-9 mm) of the entrance of the filament- and perigon-filament-tube (5 mm and

pink color in *M. longipes*). The two species can also easily be distinguished by their leaf surfaces: *M. pustulata* shows 115-170 pustules per square-centimeter (Fig. 7c) whereas *M. longipes* has only 27-36 (or even less at some basal parts of the leaves) (Fig. 8c). The diameter of the pustules is 0,6 mm in *M. pustulata* (Fig. 7c) and 1,1 mm in *M. longipes* (Fig. 8c). A detailed study of leaf surfaces involving scanning electron microscopy is in preparation (WETSCHNIG et al. in preparation). Moreover, *M. pustulata* and *M. longipes* exhibit different flowering times. Under similar light conditions, temperature and watering regimes, *M. longipes* flowers approximately 14 days earlier then *M. pustulata*.

Distribution: Unfortunately, as access to material of South African herbaria is restricted, we are not able to provide complete data on the distribution of the two species. From our limited perspective, however, it seems that *M. longipes* prefers habitats near the coast. In addition to Cape Infanta and Koppie Allen at DeHoop we know populations from Arniston, Cape Agulhas, Gouriqua, Cape Point and Klipfontein. In contrast to these predominantly coastal habitats, *M. pustulata* is found more inland. To our knowledge localities exist only north of Napier and south of Swellendam.

Phylogeny: Although the phylogeny presented in Fig. 11 is rather preliminary both from the number of taxa investigated and from the number of DNA-regions observed, it seems likely that all populations of *M. pustulata* and *M. longipes* fall into clades of their own. Most important, there is no apparent affinity to *M. depressa* or *M. echinata*. Further phylogenetic studies involving a more complete sampling of the genus including additional markers are under way and will be published elsewhere (WETSCHNIG et al., in preparation).

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Literature

ANDREWS H.C. (1802): Table 220, *Massonia scabra*. — Bot. Rep. **4**.

BAKER J.G. (1897): *Liliaceae*: In: Flora Capensis **6**: 436–470.

DEMESURE B., SODZI N., PETIT R. J. (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. — Mol. Ecol. **4**: 129–131.

DOYLE J.J., DOYLE J.L. (1987): A rapid DNA isolation procedure for small amounts of fresh leaf tissue. — Phytochemical Bulletin **19**: 11–15.

FELSENSTEIN J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. — Evolution **39**: 783–791.

FITCH W. M. (1971): Toward defining the course of evolution: minimum change for a specific tree topology. — Systematic Zoology **20**: 406–416.

GUNN M. & CODD L.E. (1981) : Botanical exploration of Southern Africa. — Balkema, Cape Town.

HOUTTUYN M. (1780) : Natuurlyke historie … II, **12**. — Amsterdam.

JACQUIN N.J. (1791): Collectanea … **4**: Vindobonae.

JACQUIN N.J. (1804): Plantarum rariorum horti Caesarei Schoenbrunnensis **4**. — Vindobonae.

JESSOP J.P. (1976): Studies in the bulbous Liliaceae in South Africa 6. The taxonomy of *Massonia* and allied genera. — J. S. Afr. Bot. **42** (4): 401–437.

KER GAWLER J.B. (1803): Table 642, *Massonia pustulata*. — Curtis's Bot. Mag. **17**.

LEIGHTON F.M. (1943): Table 915, *Massonia pustulata*. — Fl. Pl. S. Afr. **23**.

MANNING J., Goldblatt P. & Snijman D. (2002): The color encyclopedia of Cape bulbs. — Timber Press, Portland, Cambridge.

MANNING J. & VAN DER MERWE A.M. (2002): Notes on African plants: Hyacinthaceae. A new combination in *Daubenya*. — Bothalia **32** (1): 63–83.

MÜLLER-DOBLIES U. & MÜLLER-DOBLIES D. (1997): A partial revision of the tribe Massonieae (Hyacinthaceae). — Feddes Repert. **108** (1–2): 49–96.

PFOSSER M., WETSCHNIG W., SPETA F. (2006): *Drimia cryptopoda*, a new combination in Hyacinthaceae from Madagascar. — Linzer Biol. Beitr. **38**: 1731–1739.

PBS (2012): http://www.pacificbulbsociety.org/pbswiki/index.php/Massonia; internet accession date: 2012.11.12.

SHAW J., LICKEY E. B., BECK J. T., FARMER S. B., LIU W., MILLER J., SIRIPUN K. C., WINDER C. T., SCHILLING E. E., SMALL R. L. (2005): The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. — Amer. J. Bot. **92**: 142–166.

SCHLESIES W. (1995): Unbekannte aus Südafrika. — Gartenpraxis **21** (8): 18–19.

SUMMERFIELD A. (2004). A synopsis of the biosystematic study of the seven minor genera of the Hyacinthaceae. — Bulbs **6** (1): 24–36.

SWOFFORD D. L. (2000): PAUP* Phylogenetic analysis using parsimony (* and other methods), vers. 4. Sinauer Associates, Sunderland, Massachusetts.

THUNBERG C.P. (1800): Prodromus plantarum Capensium … **2**: Appendix. — Upsaliae.

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