Going Bananas in Papua New Guinea: A preliminary study of starch granule morphotypes in Musaceae fruit

Research

Carol J. Lentfer

Abstract

Starch granules can be well preserved in a variety of archaeological contexts, for example, in residues and sediments. Therefore, starch analysis has potential to provide another means of tracking the exploitation, dispersal and domestication of *Musa* bananas and *Ensete*. Starch granule morphotypes from fruits of *Ensete glaucum* and wild and cultivated Australimusa and Eumusa bananas were analyzed in this preliminary study. Numerous starch granule morphotypes were present in every sample analyzed. One hundred and nine morphotypes, representing 38 morphotype groups (variants) were described. Of these, several are specific to the samples analyzed and others occurred in more than one sample. They can be used to discriminate between different genera, sections, species and cultivars. Raphides were also numerous in wild Australimusa bananas. Although additional studies are required to determine levels of specificity, this preliminary study shows that starch analysis (and raphide presence and abundance) can be used in a similar way to phytolith analysis in the identification of Musaceae and has extremely good potential as a tool for tracing the prehistory of bananas in the archaeological record.

Introduction

Starch granules can be well preserved in a variety of archaeological contexts, in residues on stone tools, pottery, other artefacts, ecofacts, dental calculus and in sediments and coprolites (e.g., Barton & Paz 2007, Crowther 2005, Englyst *et al*. 1992, Fullagar *et al*. 2006, Horrocks *et al*. 2004, Lentfer *et al*. 2002, Parr & Carter 2003, Piperno *et al*. 2004, Therin *et al*. 1999, Zarillo *et al*. 2008). Therefore, similar to phytoliths, starch granules promise to provide another way of tracking the prehistory of banana exploitation, dispersal and domestication. Given that bananas and *Ensete* are major starch-rich staples in many regions of the world, including Papua New Guinea (De Langhe *et al*. 2009), the study of Musaceae starch granule morphotypes has been an important component of the modern comparative starch reference collection, currently being developed by Lentfer for the Southeast Asian and Pacific region (Lentfer 2009a). This paper presents results from preliminary analyses of Musaceae starch granules from *Ensete* and wild and cultivated examples of Australimusa and Eumusa bananas collected from Papua New Guinea. All plant parts contain starch and are in the process of being analyzed; the starch from fruit pulp is the focus of this paper.

Methods

Fruit from 13 accessions (see Table 1) are included in this analysis. Fruit, collected in the field, was stored in 70% ethyl alcohol and subsequently dried at temperatures at or below 35^{o} Celsius to avoid gelatinization of starch. Sub-samples of fruit pulp were prepared for microscopic analysis by grinding with a pestle and mortar with distilled water. The ground residue was stored in vials with 70%

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Table 1. Starch granule morphotypes found in fruit pulp of Musaceae. Descriptives consist of descriptors for ten attributes each separated by a slash (Type/Size/2D Shape/3DShape/Facet/Texture/Lamellae/Hilum Position/Hilum Type/Fissure). Refer to Table 2 for descriptor categories. Size category and hilum type attributes were excluded for variant classifications. Bold morphotypes occur in more than one sample.

Species or Cultivar (vernacular name/ language)	Sample	Abundance/ Morphotype /Variant	Descriptive	Raphide abundance
Ensete glaucum (Roxb.) Cheesman (**Gudu gudu**/Haigwai)	MB1	ab M Eg1(el.ov) (V1)	s/l/el.ov/el.ov/0/s/+/he/sv/0	rare (<20)
		ab M Eg2(h?el.ov) (V1)	s/l/el.ov/el.ov/0/s/+/he/?/0	
		ab M Eg3(el.ov.) (V1)	s/s/el.ov/el.ov/0/s/+/he/sv/0	
		ab M Eg4 (h?el.ov) (V1)	s/s/el.ov/el.ov/0/s/+/he/?/0	
		c M **6(h?ov) (V2)**	s/m/ov/ov/0/s/+/e/?/0	
		c M **1(h?ov) (V2)**	s/s/ov/ov/0/s/+/e/?/0	
Australimusa Section				
M. maclayi F. Muell. ex Mikl.-Maclay ssp. *ailulai* Arg. (**Bihibihiya**/Haigwai)	MB3	c **M 4(h?ov) (V3)**	s/m/ov/ov/0/s/+/he/?/0	v. abundant (>100)
		c M Mm1(ov) (V3)	s/m/ov/ov/0/s/+/he/lv/0	
		c M Mm2(ov) (V3)	s/m/ov/ov/0/s/+/he/sv/0	
		c **M 1(h?ov) (V2)**	s/s/ov/ov/0/s/+/e/?/0	
		c M Mm3(ov) (V2)	s/s/ov/ov/0/s/+/e/lv/0	
		c **M 2(ov) (V2)**	s/s/ov/ov/0/s/+/e/sv/0	
		c **M 3(h?ov) (V3)**	s/s/ov/ov/0/s/+/he/?/0	
		c M Mm4(ov) (V3)	s/s/ov/ov/0/s/+/he/lv/0	
		c **M 5(ov) (V3)**	s/s/ov/ov/0/s/+/he/sv/0	
		c Mr Mm5(ov) (V2)	s/m/ov/ov/0/s/+/e/sv/0	
		c M Mm6 (ov) (V2)	s/m/ov/ov/0/s/+/e/lv/0	
		c **M 6(h?ov) (V2)**	s/m/ov/ov/0/s/+/e/?/0	
M. peekelii Lauterb. ssp. *angustigemma* (Simmonds) Arg. (**Dor**/Em)	M5	ab M Mp1(h?subsph) (V4)	s/s/sr/sub.sph/0/s/+/e/?/0	v. abundant (>100)
		ab **M 1(h?ov) (V2)**	s/s/ov/ov/0/s/+/e/?/0	
		ab M Mp1(h?subsph) (V4)	s/m/sr/sub.sph/0/s/+/e/?/0	
		ab **M 6(h?ov) (V2)**	s/m/ov/ov/0/s/+/e/?/0	
		ab M Mp3(h?subsph) (V4)	s/vs/sr/sub.sph/0/s/+/e/?/0	
		ab M Mp4(h?ov) (V2)	s/vs/ov/ov/0/s/+/e/?/0	
Fe`i (**Utafan**/Malik)	NI14	ab **M 1(h?ov) (V2)**	s/s/ov/ov/0/s/+/e/?/0	rare (<20)
		ab **M 2(ov) (V2)**	s/s/ov/ov/0/s/+/e/sv/0	
		ab **M 3(h?ov) (V3)**	s/s/ov/ov/0/s/+/he/?/0	
		ab **M 5(ov) (V3)**	s/s/ov/ov/0/s/+/he/sv/0	
		ab M F1(h?sub.ov) (V5)	s/s/sub.ov/sub.ov/0/s/+/e/?/0	
		ab M F2(sub.ov) (V5)	s/s/sub.ov/sub.ov/0/s/+/e/sv/0	
		ab M F3(h?sub.ov) (V6)	s/s/sub.ov/sub.ov/0/s/+/he/?/0	
		ab M F4(sub.ov) (V6)	s/s/sub.ov/sub.ov/0/s/+/he/sv/0	
		r **M 7(h?irr) (V7)**	s/m/irr/irr/0/s/+/c/?/0	
		r M F5(h?irr) (V8)	s/m/bell/bell/0/s/+/c/?/0	
		r **M 8(h?irr) (V7)**	s/l/irr/irr/0/s/+/c/?/0	
		r M F6(h?bell) (V8)	s/l/bell/bell/0/s/+/c/?/0	

Species or Cultivar (vernacular name/ language)	Sample	Abundance/ Morphotype /Variant	Descriptive	Raphide abundance
Eumusa Section				
M. acuminata Colla ssp. *banksii* (F. Muell.) Simmonds (**Yesing**/Mino)	ES6	c **M 12(h?subsph) (V9)**	s/s/sr/sub.sph/0/s/0/e/?/0	rare (<20)
		c M Mab1(h?subsph) (V10)	s/s/sr/sub.sph/f/s/0/e/?/0	
		c M Mab2(h?subsph) (V9)	s/m/sr/sub.sph/0/s/0/e/?/0	
		c M Mab3(h?subsph) (V10)	s/m/sr/sub.sph/f/s/0/e/?/0	
		c M 13(h?el.ov) (V11)	s/vs/el.ov/el.ov/0/s/0/he/?/0	
		c M 14(h?el.ov) (V11)	s/s/el.ov/el.ov/0/s/0/he/?/0	
		c **M 15(h?el.ov) (V11)**	s/m/el.ov/el.ov/0/s/0/he/?/0	
	M7	vab **M 9(h?ov) (V12)**	s/s/ov/ov/0/s/0/e/?/0	absent
		vab M Mab7(h?el.ov) (V13)	s/m/el.ov/el.ov/0/s/0/c/?/0	
M. acuminata ssp. *banksii* (**Yesing**/Mino)	ES11	vab **M 9(h?ov) (V12)**	s/s/ov/ov/0/s/0/e/?/0	absent
		vab M Mab8(h?ov) (V14)	s/m/ov/ov/0/s/0/he/?/0	
M. acuminata ssp. *banksii* (**Wil banana**/pisin)	WNB10	ab **M 3(h?ov) (V3)**	s/s/ov/ov/0/s/+/he/?/0	rare (<20)
		ab M Mab9(h?irr.ov) (V15)	s/s/irr.ov/irr.ov/0/s/+/he/?/0	
		ab **M 4(h?ov) (V3)**	s/m/ov/ov/0/s/+/he/?/0	
		ab M Mab10(h?irr.ov) (V15)	s/m/irr.ov/irr.ov/0/s/+/he/?/0	
		vr M Mab11(h?irr) (V16)	s/m/irr/irr/0/s/+/e/?/0	
		r **M 7(h?irr) (V7)**	s/m/irr/irr/0/s/+/c/?/0	
		r M Mab12(h?irr) (V16)	s/l/irr/irr/0/s/+/e/?/0	
		r **M 8(h?irr) (V7)**	s/l/irr/irr/0/s/+/c/?/0	
		vr M Mab13(h?el.irr) (V17)	c/s/el.irr/el.irr/0/s/0/c/?/0	
		vr M Mab 14(h?ov) (V18)	c/s/ov/ov/0/s/0/c/?/0	
AA (**Pitu**/Kuanua)	ENB9	r M AA1(h?ov) (V12)	s/vs/ov/ov/0/s/0/e/?/0	absent
		r **M 9(h?ov) (V12)**	s/s/ov/ov/0/s/0/e/?/0	
		r M AA2(rect.ov) (V19)	s/vs/rect.ov/rect.ov/0/s/0/e/irr/0	
		r M AA2(rect.ov) (V19)	s/s/rect.ov/rect.ov/0/s/0/e/irr/	
		r M AA4(h?tri.ov) (V20)	s/vs/tri.ov/tri.ov/0/s/0/e/?/0	
		r M AA5(h?tri.ov) (V20)	s/s/tri.ov/tri.ov/0/s/0/e/?/0	
		r **M 10(h?kid) (V21)**	s/vs/kid/kid/0/s/0/e/?/0	
		r **M 11(h?kid) (V21)**	s/s/kid/kid/0/s/0/e/?/0	
		r M AA6(h?sr) (V9)	s/vs/sr/sub.sph/0/s/0/e/?/0	
		r **M 12(h?sr) (V9)**	s/s/sr/sub.sph/0/s/0/e/?/0	
AA? (Maya/Bakovi)	WNB5	ab M AA?1(ov) (V12)	s/vs/ov/ov/0/s/0/e/sv/0	absent
		ab M AA?2(ov) (V12)	s/s/ov/ov/0/s/0/e/sv/0	
		ab M AA?3(subsph) (V9)	s/vs/sr/sub.sph/0/s/0/e/sv/0	
		ab M AA?4(subsph) (V9)	s/s/sr/sub.sph/0/s/0/e/sv/0	
		ab M AA?5(sph) (V22)	s/vs/r/sph/0/s/0/e/sv/0	
		ab M AA?6(sph) (V22)	s/s/r/sph/0/s/0/e/sv/0	
		ab M AA?7(el.ov) (V23)	s/vs/el.ov/el.ov/0/s/0/e/sv/0	
		ab M AA?8(el.ov) (V23)	s/s/el.ov/el.ov/0/s/0/e/sv/0	
		r **M 15(h?el.ov) (V11)**	s/m/el.ov/el.ov/0/s/0/he/?/0	

Species or Cultivar (vernacular name/ language)	Sample	Abundance/ Morphotype /Variant	Descriptive	Raphide abundance
AAA (-/-)	F/03/13	vr M AAA1(ov) (V18)	s/s/ov/ov/0/s/0/c/sv/0	rare (<20)
		vr **M 9(h?ov) (V12)**	s/s/ov/ov/0/s/0/e/?/0	
		vr M AAA2(h?irr) (V24)	s/s/irr/irr/mff/s/0/c/?/0	
		vr M AAA3(h?plgl) (V25)	s/s/plgl/plhdl/mff/s/0/c/?/0	
		vr M AAA4(h?kid) (V26)	s/s/kid/kid/0/wr/0/c/?/sw	
		vr M AAA5(h?el.ov) (V27)	s/l/el.ov/el.ov/0/wr/+/he/?/0	
		vr M AAA6(h?tri.irr) (V28)	s/m/tri.irr/tri.irr/0/s/0/he/?/0	
		vr M AAA7(h?subsph) (V29)	s/l/sr/sub.sph/0/s/+/c/?/0	
ABB (**Wan kina**/Pisin)	NI4	ab M ABB1(h?rect.ov) (V30)	s/l/rect.ov/rect.ov/0/s/+/c/?/0	absent
		ab M ABB2(h?ell.irr.ov) (V31)	s/l/el.irr.ov/el.irr.ov/0/s/+/he/?/0	
		c **M 17(h?unilob) (V 32)**	s/m/unilob/unilob/0/s/+/he/?/0	
		ab **M 18(h?unilob) (V 32)**	s/l/unilob/unilob/0/s/+/he/?/0	
		ab M ABB3(h?unilob) (V 32)	s/vl/unilob/unilob/0/s/+/he/?/0	
		ab M ABB4(h?unilob) (V 33)	s/l/unilob/unilob/0/s/+/he/?/oi	
		ab **M 16(h?el.ov) (V11)**	s/l/el.ov/el.ov/0/s/0/he/?/0	
		ab M ABB5(h?irr) (V16)	s/vl/irr/irr/0/s/+/e/?/0	
		r M ABB6(h?tri.ov) (V34)	s/m/tri.ov/tri.ov/0/s/+/he/?/0	
		r M ABB7(h?subsph) (V35)	s/m/sr/sub.sph/0/wr/0/e/?/0	
		r M ABB8(h?irr.ov) (V36)	s/m/irr.ov/irr.ov/0/wr/+/he/?/0	
		r M ABB9(h?) (V22)	s/m/r/sph/0/s/0/e/?/0	
ABB? (**Tamane buro**/Bakovi)	WNB4	c M ABB?1(h?subsph) (V37)	s/vs/sr/sub.sph/0/s/0/c/?/0	absent
		c M ABB?2(h?subsph) (V37)	s/s/sr/sub.sph/0/s/0/c/?/0	
		c M ABB?3(h?subsph) (V37)	s/m/sr/sub.sph/0/s/0/c/?/0	
		c M ABB?4(h?subsph) (V37)	s/l/sr/sub.sph/0/s/0/c/?/0	
		ab M ABB?5(h?el.ov) (V23)	s/vs/el.ov/el.ov/0/s/0/e/?/0	
		ab M ABB?6(h?el.ov) (V23)	s/s/el.ov/el.ov/0/s/0/e/?/0	
		ab M ABB?7(h?el.ov) (V23)	s/m/el.ov/el.ov/0/s/0/e/?/0	
		ab M ABB?8(h?el.ov) (V23)	s/l/el.ov/el.ov/0/s/0/e/?/0	
		ab M 13(h?el.ov) (V11)	s/vs/el.ov/el.ov/0/s/0/he/?/0	
		ab M 14(h?el.ov) (V11)	s/s/el.ov/el.ov/0/s/0/he/?/0	
		ab M 15(h?el.ov) (V11)	s/m/el.ov/el.ov/0/s/0/he/?/0	
		ab M 16(h?el.ov) (V11)	s/l/el.ov/el.ov/0/s/0/he/?/0	
		c **M 10(h?kid) (V21)**	s/vs/kid/kid/0/s/0/e/?/0	
		c **M 11(h?kid) (V21)**	s/s/kid/kid/0/s/0/e/?/0	
		c M ABB?9(h?kid) (V21)	s/m/kid/kid/0/s/0/e/?/0	
		c M ABB?10(h?kid) (V21)	s/l/kid/kid/0/s/0/e/?/0	
		c M ABB?11(h?unilob) (V32)	s/vs/unilob/unilob/0/s/+/he/?/0	
		c M ABB?12(h?unilob) (V32)	s/s/unilob/unilob/0/s/+/he/?/0	
		c **M 17(h?unilob) (V32)**	s/m/unilob/unilob/0/s/+/he/?/0	
		c **M 18(h?unilob) (V32)**	s/l/unilob/unilob/0/s/+/he/?/0	
		c M ABB?13(h?irr.ov) (V38)	s/vs/irr.ov/irr.ov/0/s/0/e/?/0	

Species or Cultivar (vernacular name/ language)	Sample	Abundance/ Morphotype /Variant	Descriptive	Raphide abundance
		c M ABB?14(h?irr.ov) (V38)	s/s/irr.ov/irr.ov/0/s/0/e/?/0	
		c M ABB?15(h?irr.ov) (V38)	s/m/irr.ov/irr.ov/0/s/0/e/?/0	
		c M ABB?16(h?irr.ov) (V38)	s/l/irr.ov/irr.ov/0/s/0/e/?/0	

ethyl alcohol. Sub-samples of 100 µl of ground residue were mounted onto microscope slides and examined in a wet mount of distilled water to allow rotation of granules.

Slide residues were analyzed using light microscopy at x400, x500 and x1000 magnification. Presence, absence and abundance of starch and raphides were noted and slides were fully scanned. Descriptives for all starch morphotypes encountered were recorded according to the list of attributes in Table 2. Where a morphotype occurred in only one sample (i.e., it was sample-specific), it was labelled and numbered according to that sample. Morphotypes occurring in multiple samples were assigned a unique number. All morphotypes were further defined by three dimensional shape. Where hilum type could not be identified, this is indicated on Table 1 as h?. Morphotypes were then grouped into categories based on all morphological attributes listed in Table 2 with the exception of starch granule size. It was also necessary to exclude hilum type since this could not be successfully defined under light microscopy for the majority of cases. For the purpose of this preliminary study, the resultant grouped morphotype categories (see Table 1) are defined as variants. Photographs of each variant were taken with microscope-dedicated digital cameras using non-polarized and polarized light. Descriptives were transferred to an electronic database to facilitate searches and comparative analyses. The microscope slides and sub-samples in vials are presently stored in laboratories in the School of Social Science at the University of Queensland. Following the initial qualitative analyses presented here, quantitative measurements of length and width of starch granules will be taken for future morphometric analyses to further define diagnostic values.

Results

Starch granules were present in all samples analyzed. They were rare in the triploid Eumusa AAA and the diploid AA cultivars, but common to very abundant in all other samples. Raphides were very abundant only in the wild Australimusa bananas (Figure 1) and rare

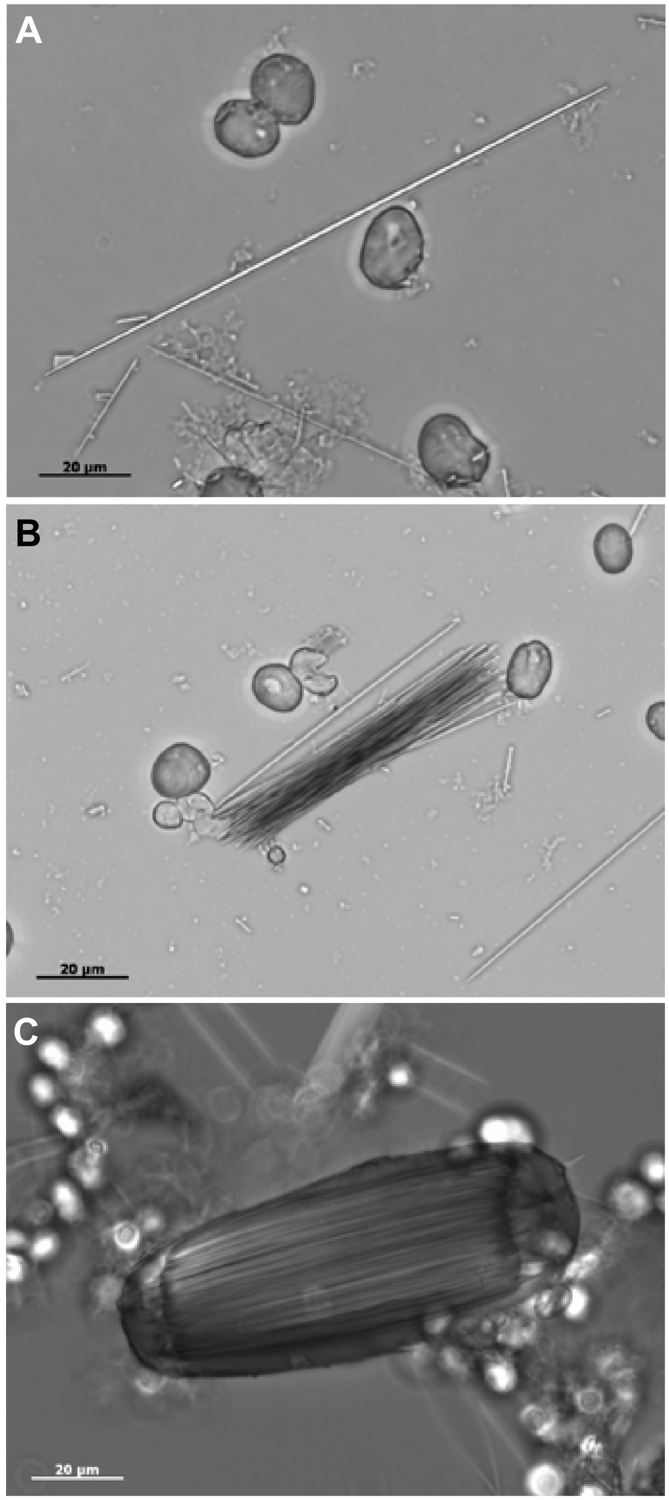

Figure 1. **A**-**B**: Raphide and raphide bundle found in fruit of *M. maclayi* ssp. *ailulai* (accession no. MB3). **C**: Raphide bundle within specialized idioblast cell in the fruit of *M. peekelii* ssp. *angustigemma* (accession no. M5).

Table 2. List of attributes used for banana starch description (see Lentfer 2009a).

1. Abundance	
absent (0)	a
rare (<20 granules)	r
common (>20-50)	c
abundant (>50-100)	ab
v. abundant (>100)	vab

2. Type	
simple	s
compound	c
semi-compound	sc

3. Size (max dim)	
<5 μm	vs
>5-10 μm	s
>10-20 μm	m
>20-50 μm	l
>50 μm	vl

4. 2D shape	
subround	sr
round	r
ovate	ov
sub-ovate	sub. ov
triangular ovate	tri. ov
rectangular ovate	rect. ov
elongate ovate	el. ov
irregular ovate	irr. ov
irregular triangular	irr. tri. ov
irregular triangular ovate	irr. tri. ov
elongate irregular ovate	el. irr. ov
polygonal	plygl
irregular	irr
elongate irregular	el. irr
crescent	cr
kidney	kid
elongate kidney	el. kid
triangular	tri
square	sq
bell	bell
unilobate	unilob
irregular square	irr. sq

5. 3D shape	
hemispherical	hsph
elongate hemisph.	el. hsph
spherical	sph
subspherical	subsph
ovoid	ov
sub-ovoid	sub. ov
triangular ovoid	tri. ov
rectangular ovoid	rect. ov
elongate ovate	el. ov
irregular ovoid	irr. ov
irregular triangular ovoid	irr. tri. ov
elongate irregular ovoid	el. irr. ov
globose	gl
polyhedral	plyhdl
quadrilateral	qu
irregular	irr
elongate irregular	el. irr
globose elongate	gl. el
kidney	kid
elongate kidney	el. kid
cone	cone
disc	di
bell	bell
unilobate	unilob
elongate irregular	el. irr
globose irregular	gl. irr.
triangular irregular	tri. irr
triangular prism	tri. pr
irregular cone	irr. co
irregular spherical	irr. sph

6. Protrusion	
present	+
absent	0

7. Facet	
none observed	0
flat	fl
concave	cc
multifaceted flat	mff
multifaceted concave	mfc

8. Texture	
wrinkle	wr
smooth	s
rough	r
ridged	rdg
unknown	0

9. Lamellae	
present	+
absent	0

10. Hilum - Position	
eccentric	e
centric	c
hyper-eccentric	he
unknown	0

11. Hilum - Type	
large vacuole	lv
small vacuole	sv
crystal	cr
slot	sl
irregular	irr
papilla	pap
unclear	0

12. Hilum - Fissure	
none	0
stellate	stel
simple straight	ss
open slot	os
open irreg.	oi
tri	tri
disc	d
simple winged	sw
irregular	irr
open	o

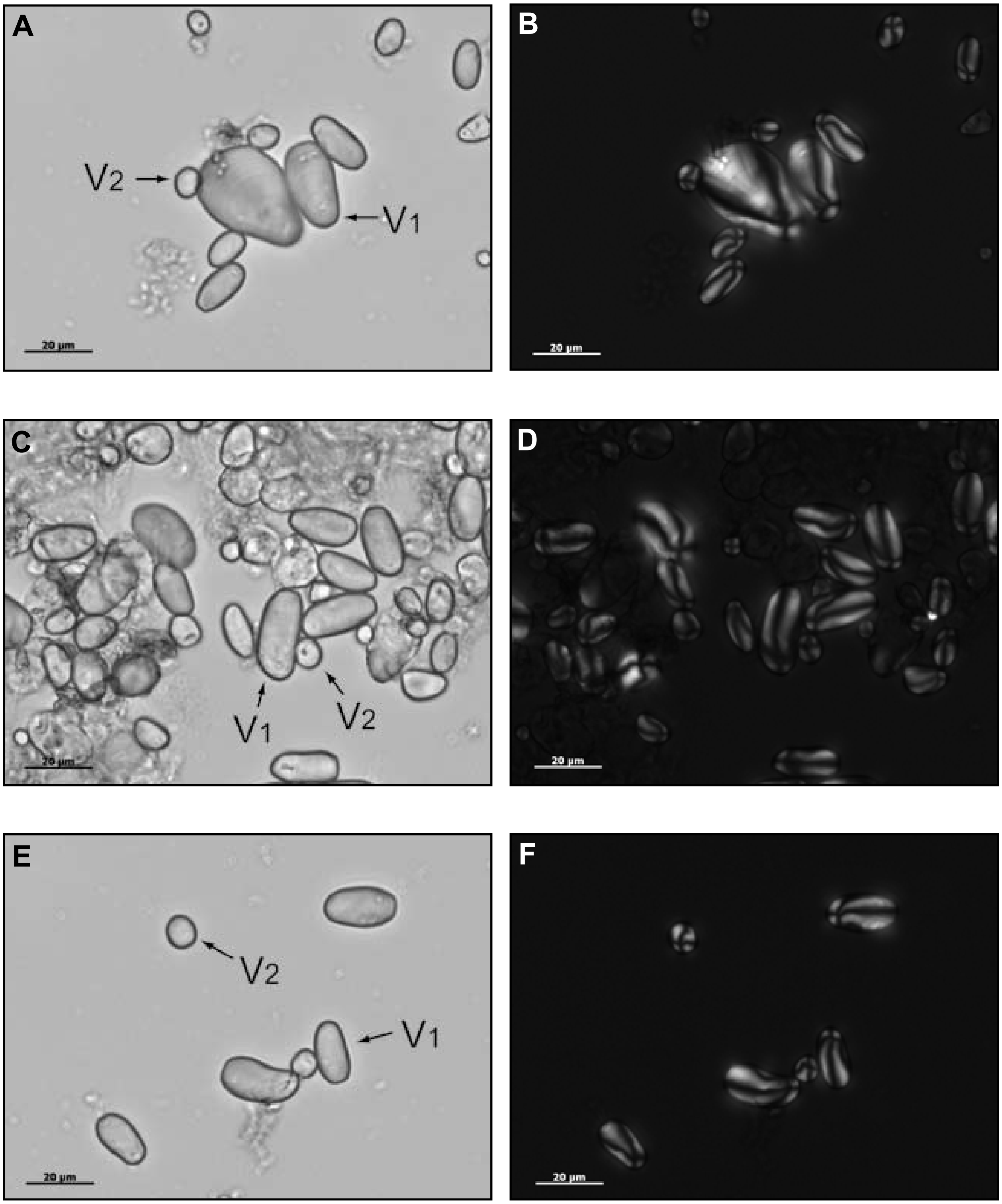

Figure 2. Examples of starch granule morphotypes found in *Ensete glaucum* (accession no. MB1). Images taken with cross polarized light (**B**, **D**, **F**) show extinction crosses through eccentric (off-center) and hyper-eccentric (close to one end of the starch granule and almost polar) hilums. The elongate ovoid morphotypes with hyper-eccentric hilums are abundant and dominate the starch granule assemblages shown in **A**-**F**; these are classified as Variant 1 morphotypes and are specific to *Ensete glaucum*. The small and medium-sized ovoid starch granules with eccentric hilums (Variant 2 morphotypes) are also found in Australimusa bananas.

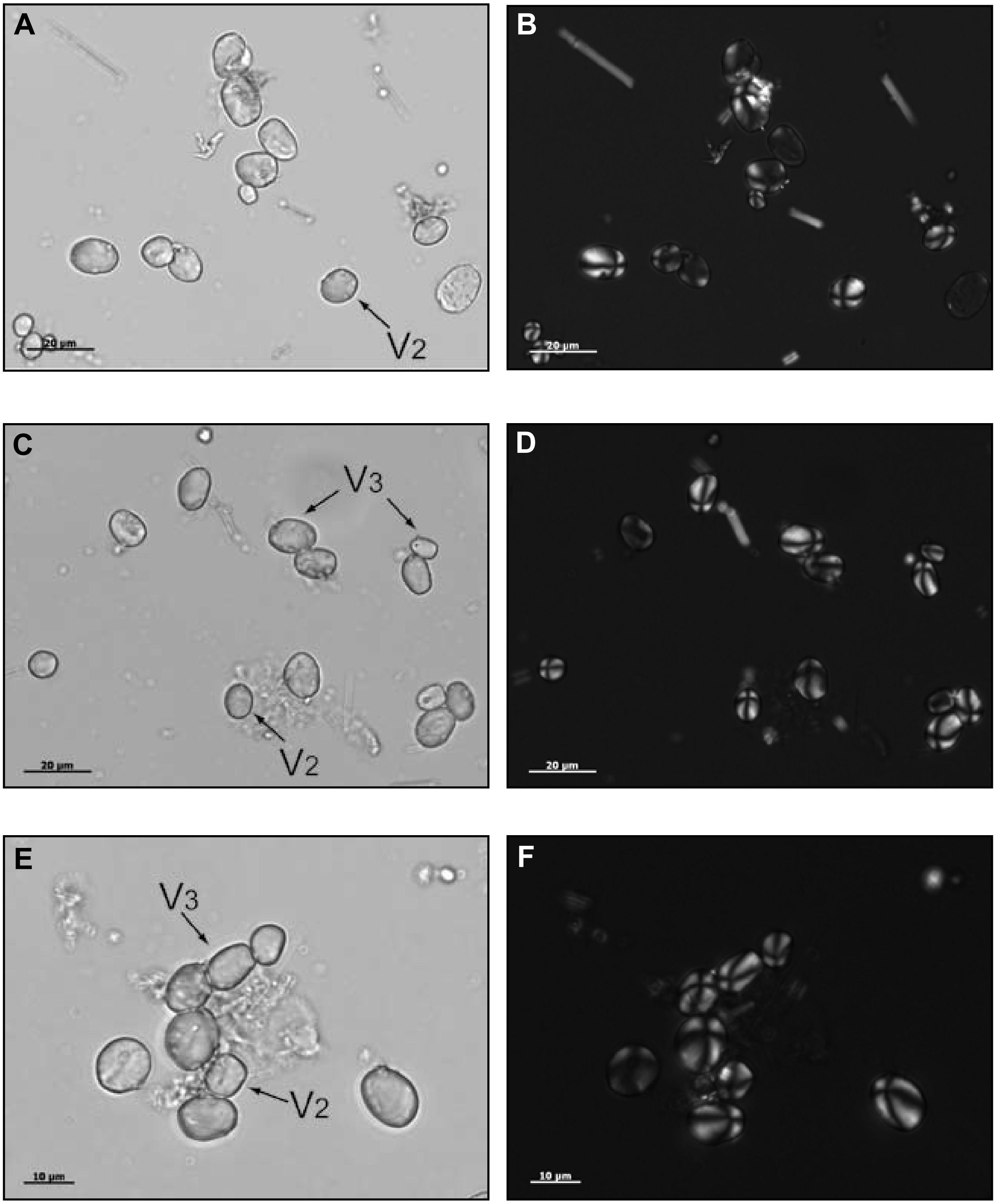

Figure 3. Examples of starch granule morphotypes found in *Musa maclayi* ssp. *ailulai* (accession no. MB3). Images taken with cross polarized light (**B**, **D**, **F**) show extinction crosses through eccentric and hyper-eccentric hilums. The small to medium morphotypes are all ovoid with hyper-eccentric (Variant 3) and eccentric (Variant 2) hilums.

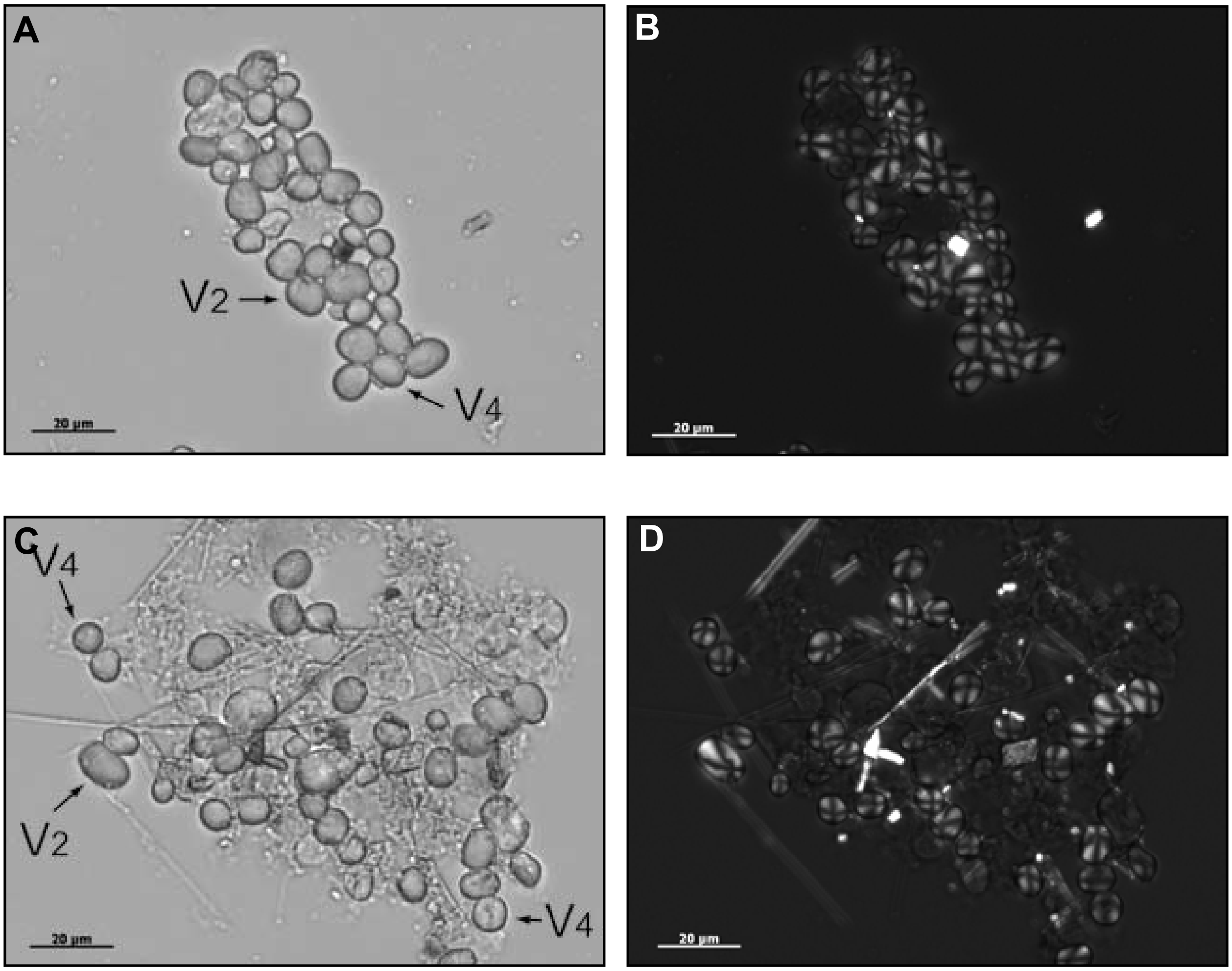

Figure 4. Examples of starch granule morphotypes found in *M. peekelii* ssp. *angustigemma* (accession no. M5). Images taken with cross polarized light show extinction crosses through eccentric hilums (**B**, **D**). All granules are very small to medium. The ovoid morphotypes belong to the Variant 2 group. The sub-spherical morphotypes specific to *M. peekelii* belong to the Variant 4 group.

to absent in *Ensete glaucum* and all other samples. Notably, the majority of samples, including *E. glaucum*, had a range of large to medium sized starch granules (see size categories in Table 2), but the AA and AA? diploids were characterized by very small to small starch granules. Compound granules were found only in *M. acuminata* ssp. *banksii*, sample WNB 10, but were very rare.

One of the most striking outcomes is the number and range of starch granule morphotypes identified in the sample assemblage. A total of 109 different morphotypes were described (Table 1, and see Figures 2 to 11). Of these, 91 were sample-specific. The remaining 18, however, were found in more than one sample. Nevertheless, these were still useful for discriminating between genera, section, species and cultivars. For instance, Morphotype 1 was common to abundant in *E. glaucum* and all the Australimusa samples, both wild and cultivated. It was not found in any of the Eumusa samples. Morphotypes 2 and 5 were found only in Australimusa, the wild species *M. maclayi* and the cultivar **Fe`i**, and, like Morphotype 1, they were not found in Eumusa. Morphotypes 3, 4, 7 and 8 were found in both Australimusa and Eumusa samples. Morphotype 6 was found in *E. glaucum* and the wild Australimusa bananas, *M. maclayi* and *M. peekelii*. Morphotypes 9 to 18 were found only in Eumusa bananas. Morphotype 12 occurred in wild and cultivated AA diploid bananas, Morphotype 9 occurred only in AA diploid and AAA triploids, and Morphotypes 16, 17 and 18 occurred only in the *M. acuminata* x *M. balbisiana* Colla triploids ABB and ABB?.

Exclusion of the size attribute yielded a much reduced morphotype set of 38 variants (see Table 1). This resulted in a higher level of redundancy in comparison to the ungrouped morphotypes (11 out of the 38 variants occurred in more than one sample), but discrimination between genera, sections, species and/or cultivars was still possible (see Figure 12). For example, *E. glaucum* was differ-

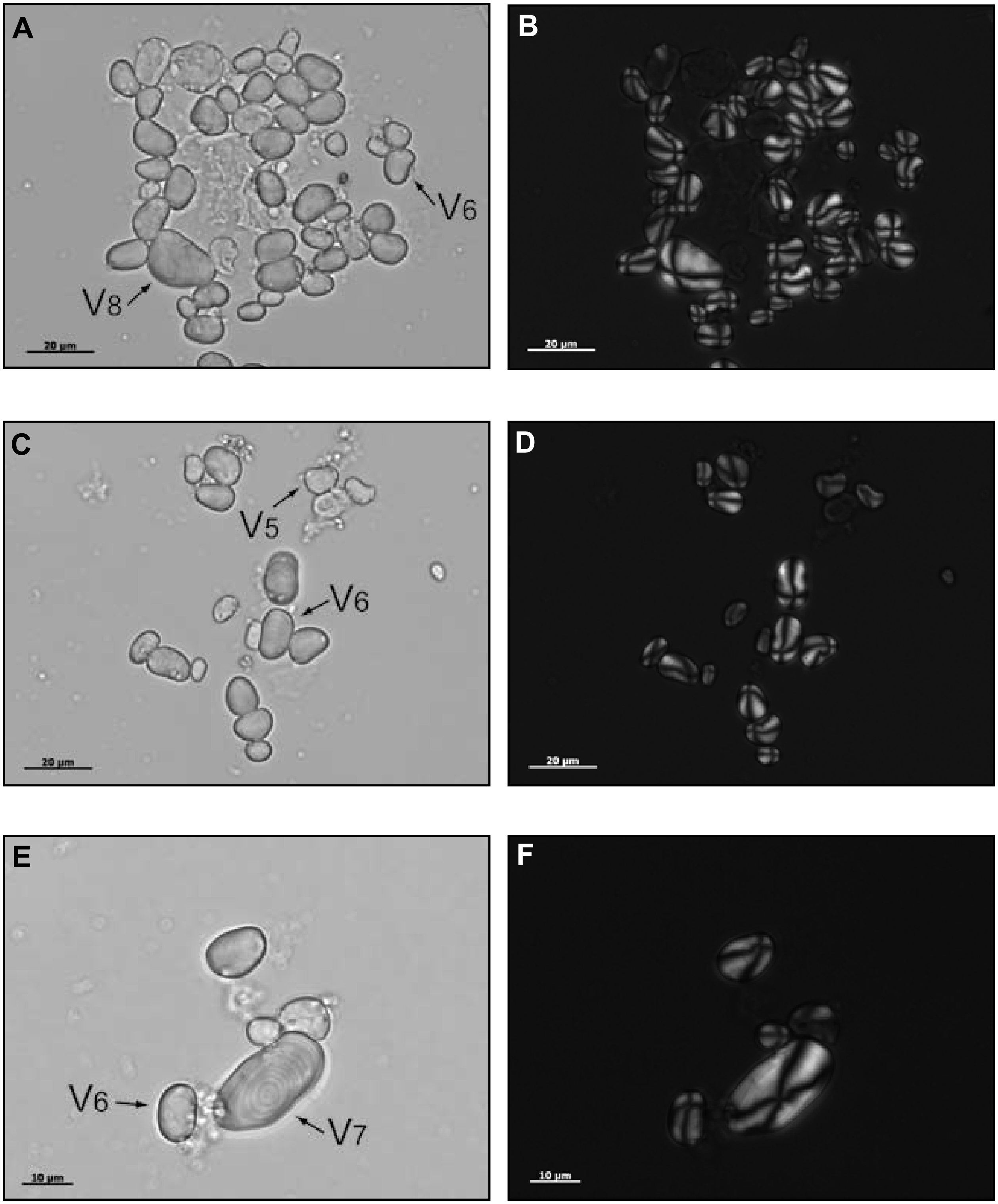

Figure 5. Examples of starch granule morphotypes found in **Fe`i** (accession no. ANI14). Images taken with cross polarized light (**B**, **D**, **F**) show extinction crosses through hyper-eccentric, eccentric and centric hilums. The subovoid morphotypes with eccentric hilums (Variant 5), hyper-eccentric hilums (Variant 6) and the bell-shaped morphotypes with centric hilums (Variant 8) are specific to **Fe`i**. Irregular morphotypes in the Variant 7 group (not shown here) are also found in the wild Eumusa species, *M. acuminata* ssp. *banksii*. The ovoid morphotypes classified as Variants 2 and 3 are found in the wild Australimusa species *M. maclayi* and *Ensete*.

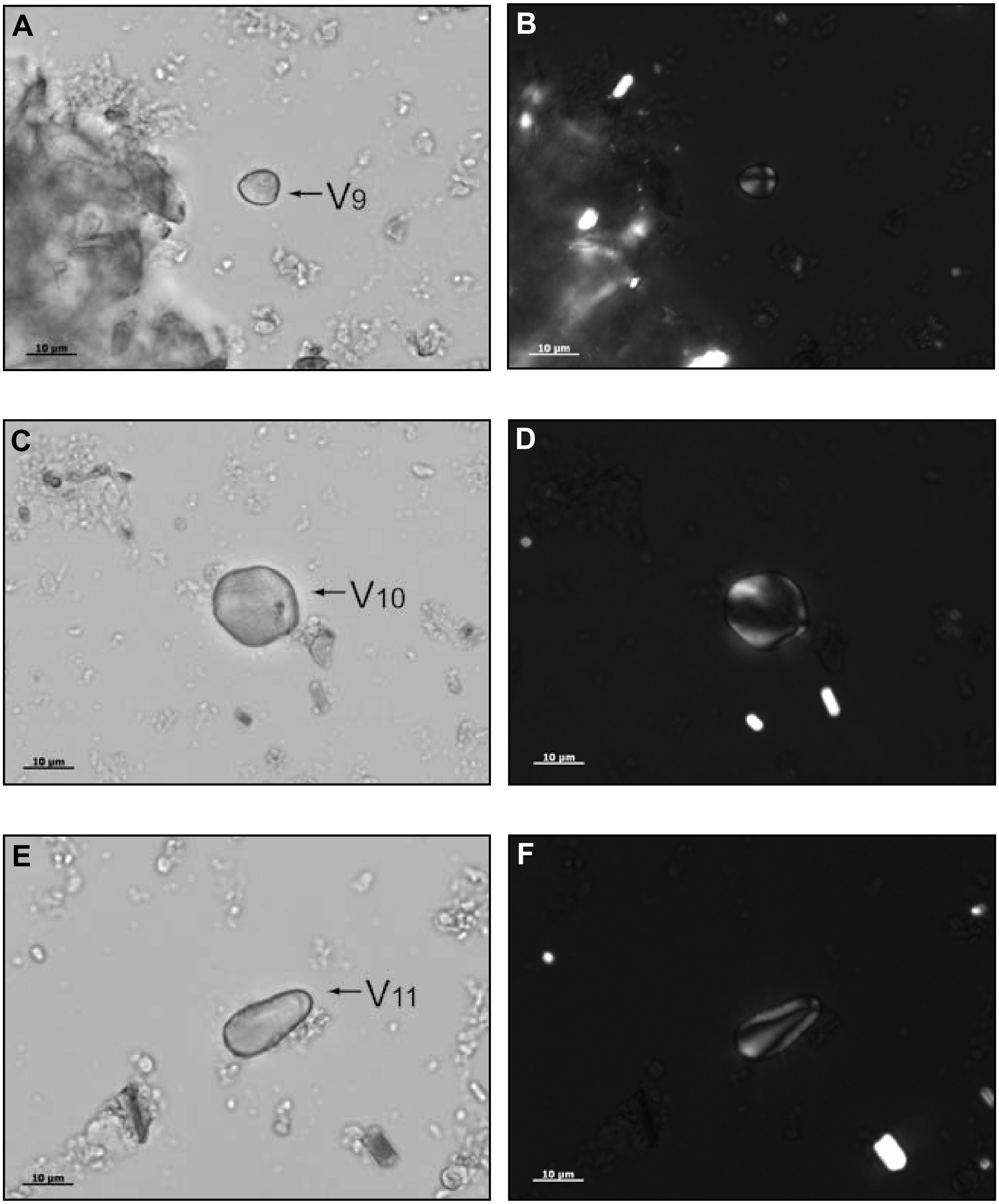

Figure 6. Examples of starch granule morphotypes found in *M. acuminata* ssp. *banksii* (**A**-**F**: accession no. ES6). The small subspherical morphotypes with eccentric hilums (Variant 9: **A**-**B**) and the ovoid morphotypes with eccentric hilums (Variant 12: see examples in **G**-**H**) also occur in the cultivated AA diploid (ENB9) and AA? diploid (WNB9). The elongate ovoid morphotypes with hyper-eccentric hilums (**E**-**F**: Variant 11) also occur in the AA? diploid sample. Other morphotypes were only found in *M. acuminata* ssp. *banksii* samples. The facetted subspherical morphotype (Variant 10: **C**-**D**) was recorded from the ES6 sample only. (continued on following page)

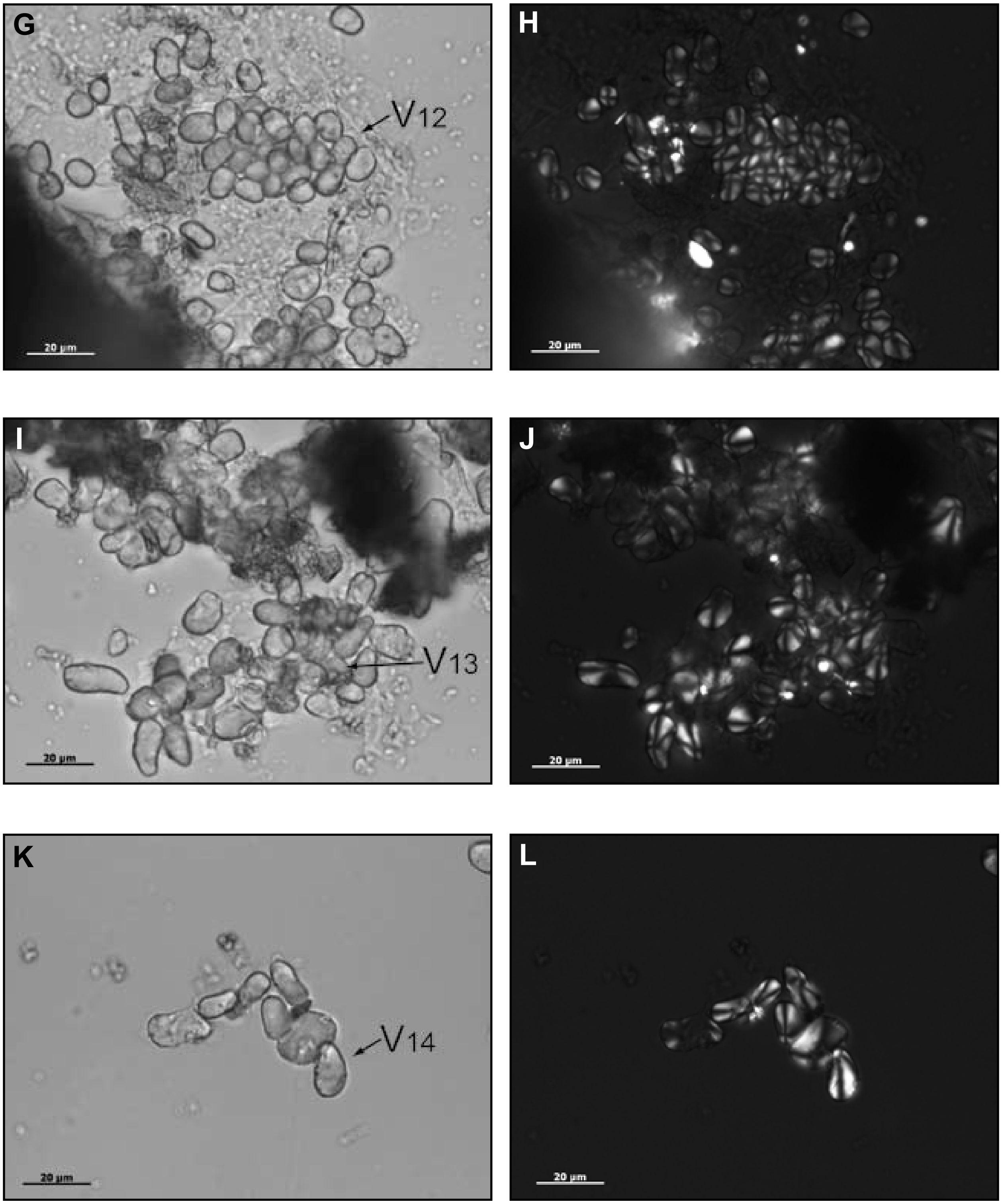

Figure 6 cont. Examples of starch granule morphotypes found in *M. acuminata* ssp. *banksii* (**G**-**J**: accession no. M7; **K**-**L**: accession no. ES11:). The elongate ovoid morphotype with an eccentric hilum (Variant 13: see examples in **G**-**H**) was recorded from M7. The ovoid morphotype with a hyper-eccentric hilum with no lamellae (Variant 14: see examples in **K**-**L**) was recorded from ES11. (continued on following page)

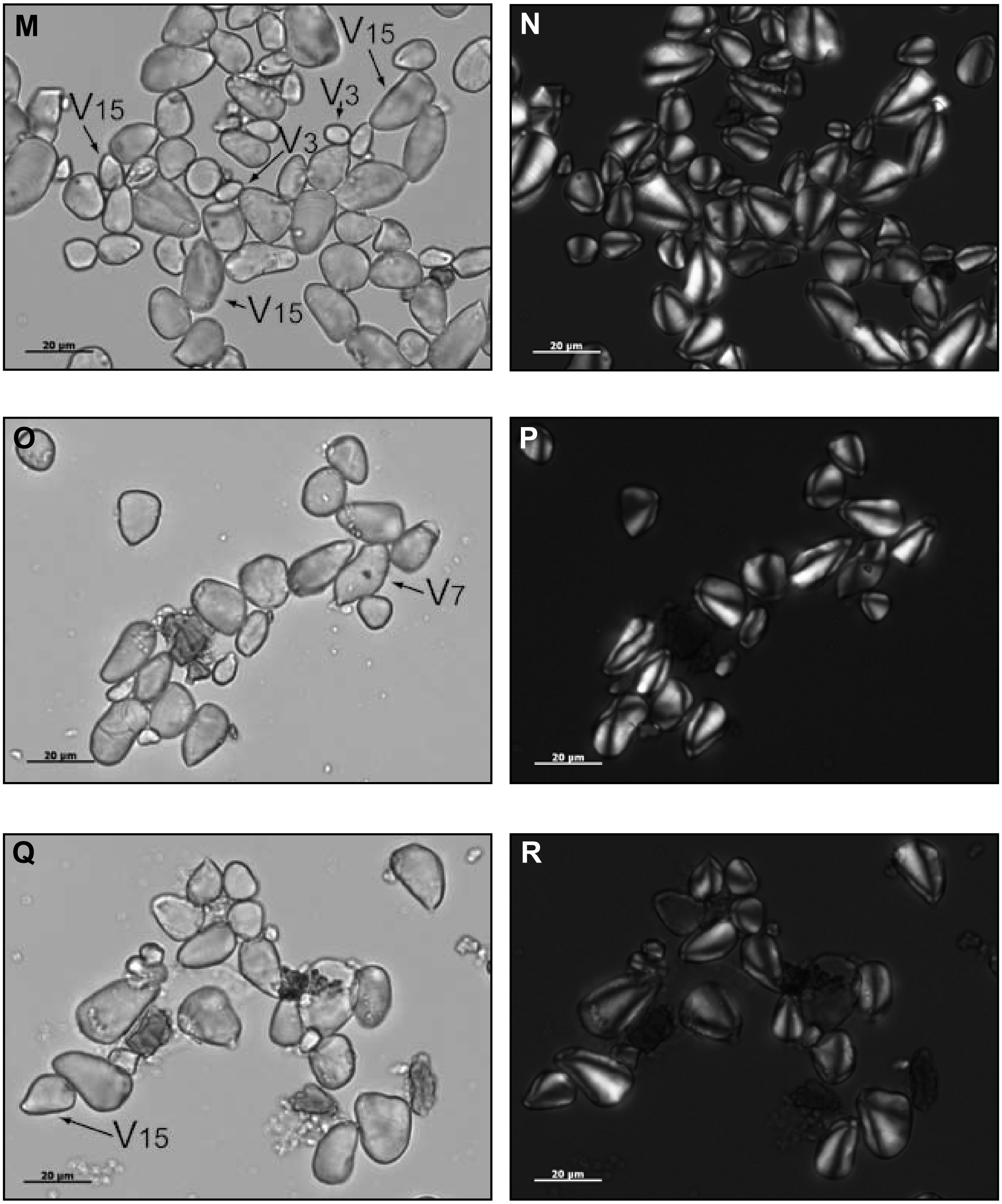

Figure 6 cont. Examples of starch granule morphotypes found in *M. acuminata* ssp. *banksii* (**M**-**R**: accession no. WNB10). The irregular (Variant 16), irregular ovoid (Variant 15), elongate irregular (Variant 17) morphotypes and ovoid morphotypes with centric hilums (Variant 18) were found only in sample WNB10 (see examples of Variant 15 in **M**-**R**; images of the rarer variants are not available).

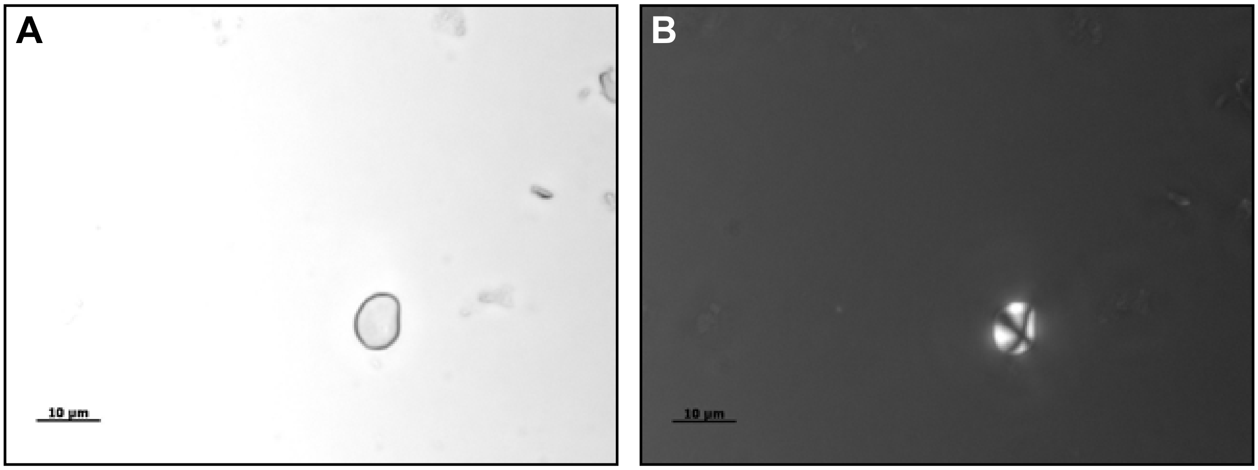

Figure 7. Example of subspherical morphotype (Variant 9) from the AA diploid (accession no. ENB9) (N.B. No images showing morphotypes representative of Variants 19, 20 and 21 are presently available).

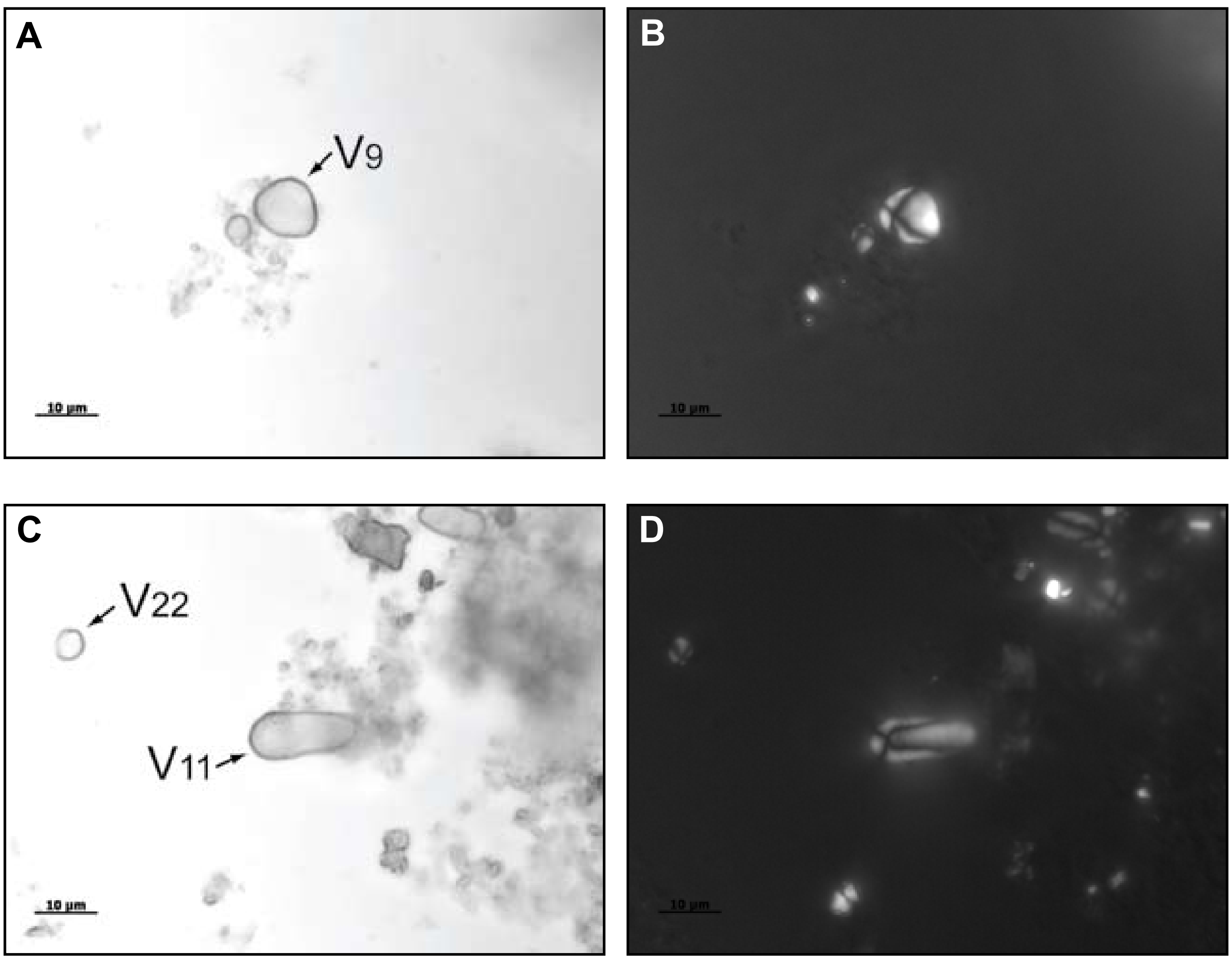

Figure 8. Examples of spherical (Variant 22), subspherical (Variant 9) and elongate ovoid (Variant 11) starch granule morphotypes found in the AA? diploid (accession no. WNB5).

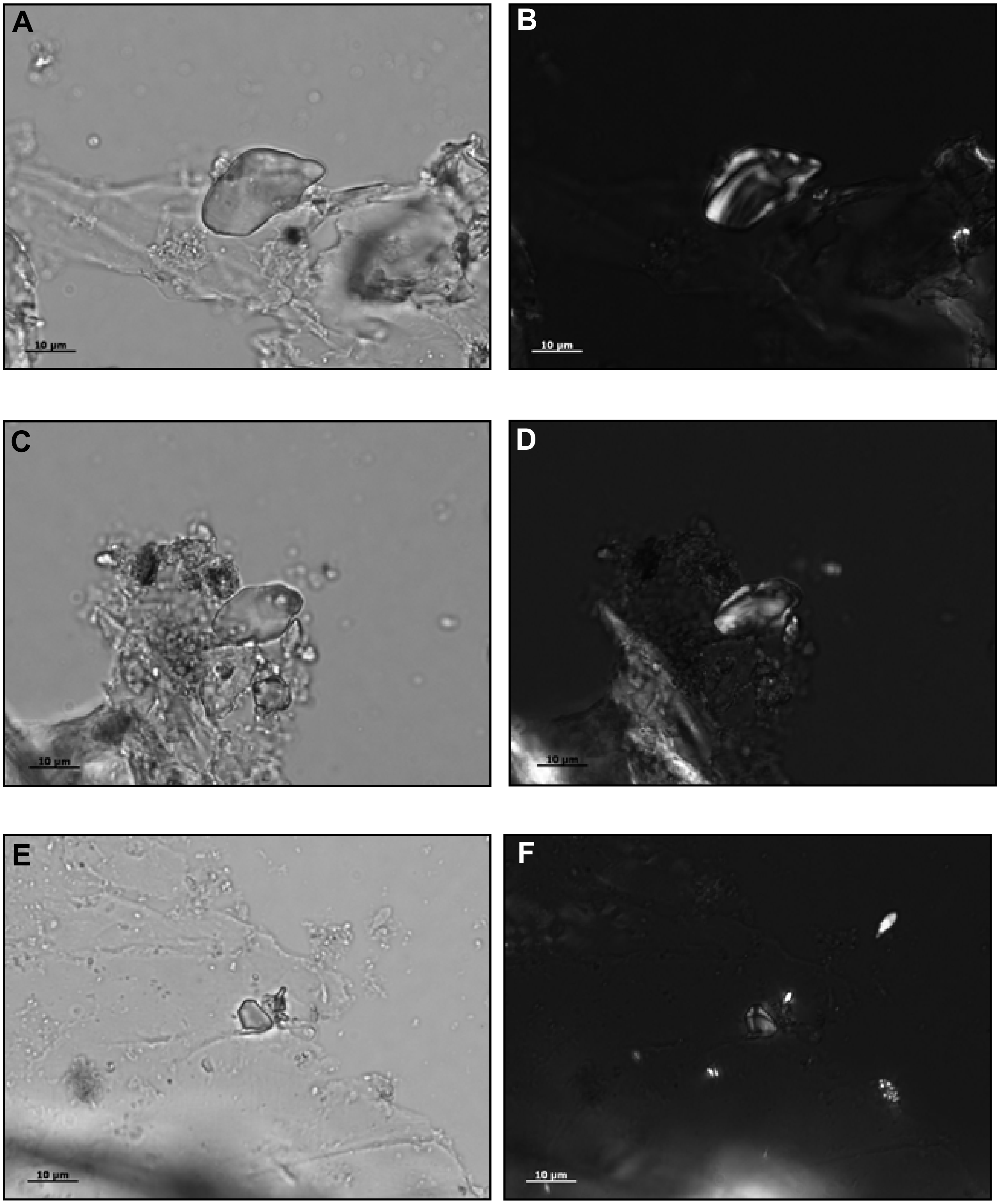

Figure 9. Examples of starch granule morphotypes found in the AAA triploid (accession no. F/03/13): multifaceted irregular morphotypes (Variant 24: **A**-**D**); multifaceted polyhedral morphotypes (Variant 25: **E**-**F**). (continued on following page)

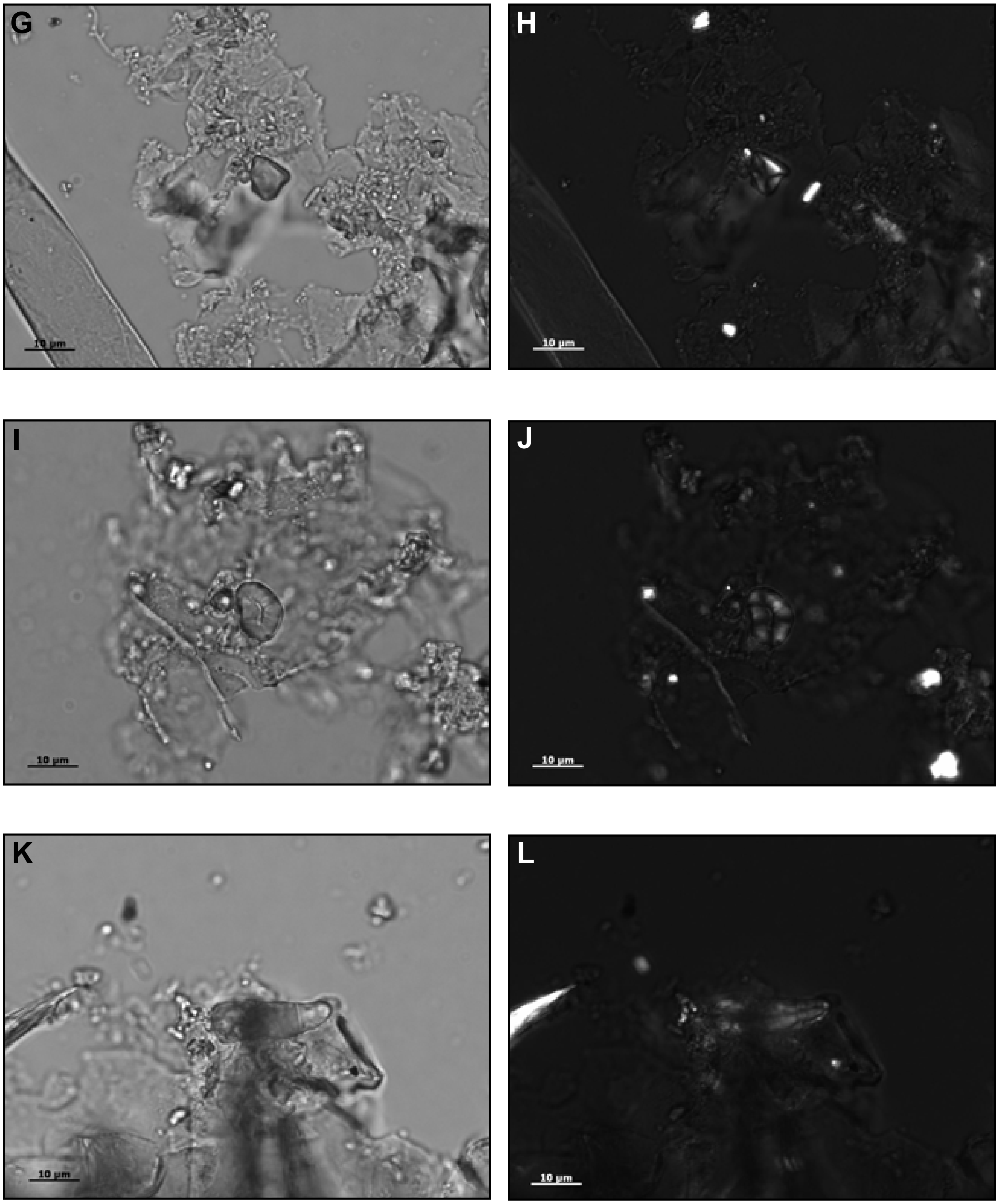

Figure 9 cont. Examples of starch granule morphotypes found in the AAA triploid (accession no. F/03/13): multifaceted polyhedral morphotypes (Variant 25: **G**-**H**); kidney shaped morphotype with wrinkled texture (Variant 26: **I**-**J**); elongate ovoid morphotype with wrinkled texture (Variant 27: **K**-**L**). (continued on following page)

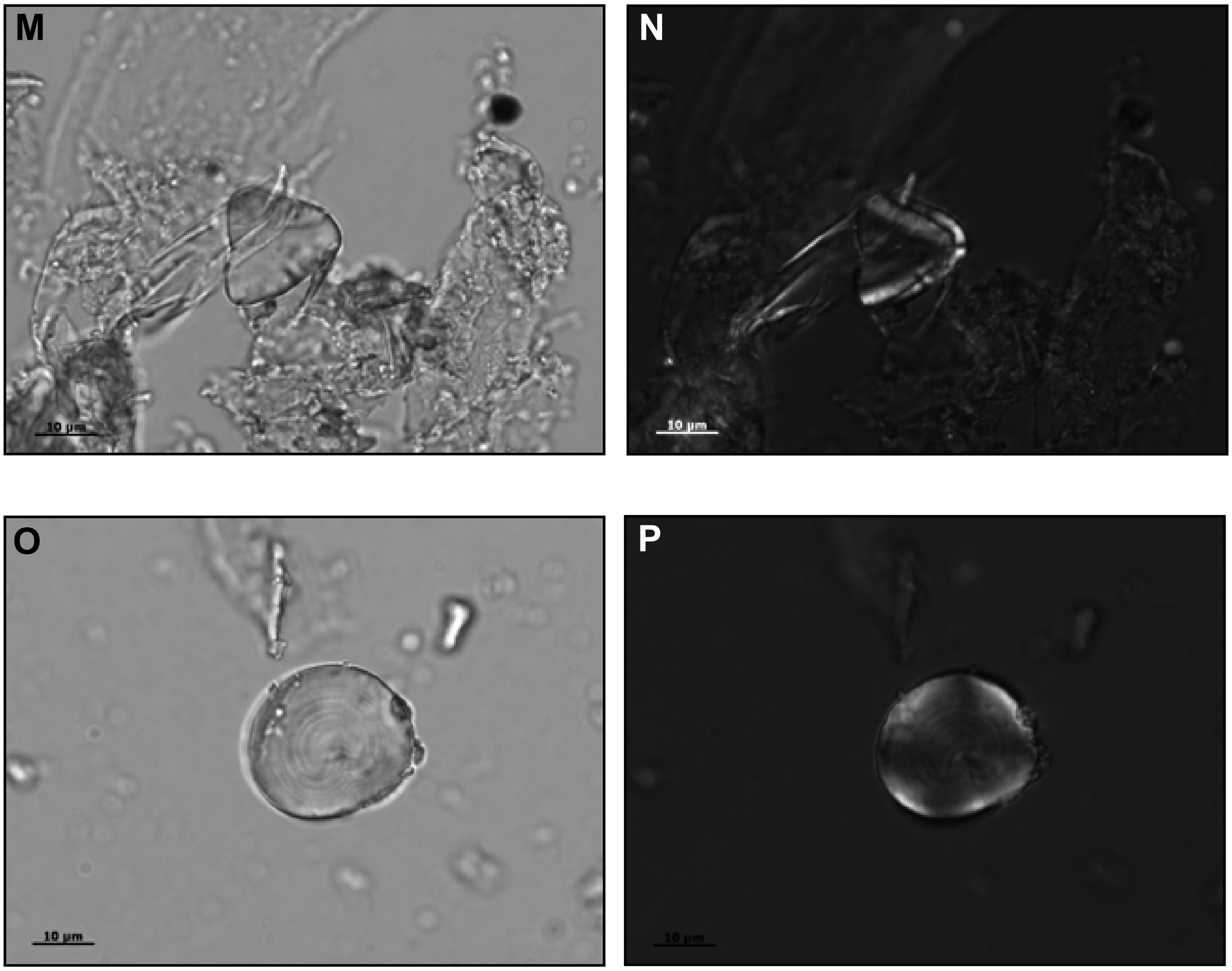

Figure 9 cont. Examples of starch granule morphotypes found in the AAA triploid (accession no. F/03/13): triangular irregular morphotype (Variant 28: **M**-**N**); and, subspherical morphotype with centric hilum (Variant 29: **O**-**P**).

entiated from the *Musa* samples by Variant 1. Variants 4, 5, 6, 7 and 8 were confined to Australimusa, and Variants 9 to 38 were confined to Eumusa. Within the Australimusa section, Variant 3 occurred in the wild species, *M. maclayi* and the **Fe`i** cultivar. Variants 5, 6, 7 and 8 also occurred in **Fe`i**, and Variant 4 was confined to the other wild species *M. peekelii*. Within the Eumusa section, AA diploids, including the wild species *M. acuminata* ssp. *banksii* were discriminated from the triploids by the presence of Variants 9, 10, 13, 14, 15, 17, 19 and 20. Variants 10, 13, 14, 15 and 17 were confined to *M. acuminata* ssp. *banksii*, and Variants 19 and 20 were confined to the AA diploid cultivar. Variants 24 to 29 were confined to the AAA triploid cultivar and Variants 30 to 38 were confined to the triploid *acuminata* x *balbisiana* cultivars. Variant 11 was common to wild *M. acuminata* ssp. *banksii* and *acuminata* cultivars, including the *acuminata* x *balbisiana* cultivars; Variant 12 was common to the wild and cultivated AA diploids and AAA triploid cultivar; and, Variant 16 was common to the wild *M. acuminata* ssp. *banksii* and the ABB triploid cultivar.

Discussion and conclusion

These preliminary qualitative analyses of Musaceae fruit starch granules provide a very good indication that banana fruit starch has considerable potential for differentiation to generic, section, species and cultivar levels. Some sets of morphotypes (variants) and individual morphotypes are useful for discriminating between genera and sections, but several others are useful for discrimination at a higher resolution, differentiating between species and cultivars. It is significant that some individual morphotypes and variants are shared between cultivars and wild species. For example, Morphotypes 2 and 5 occur only in the wild species, *M. maclayi*, and the domesticate **Fe`i**, and Morphotypes 12 and 9 occur only in the *acuminata* domesticates and the wild species, *M. acuminata* ssp. *banksii*, from

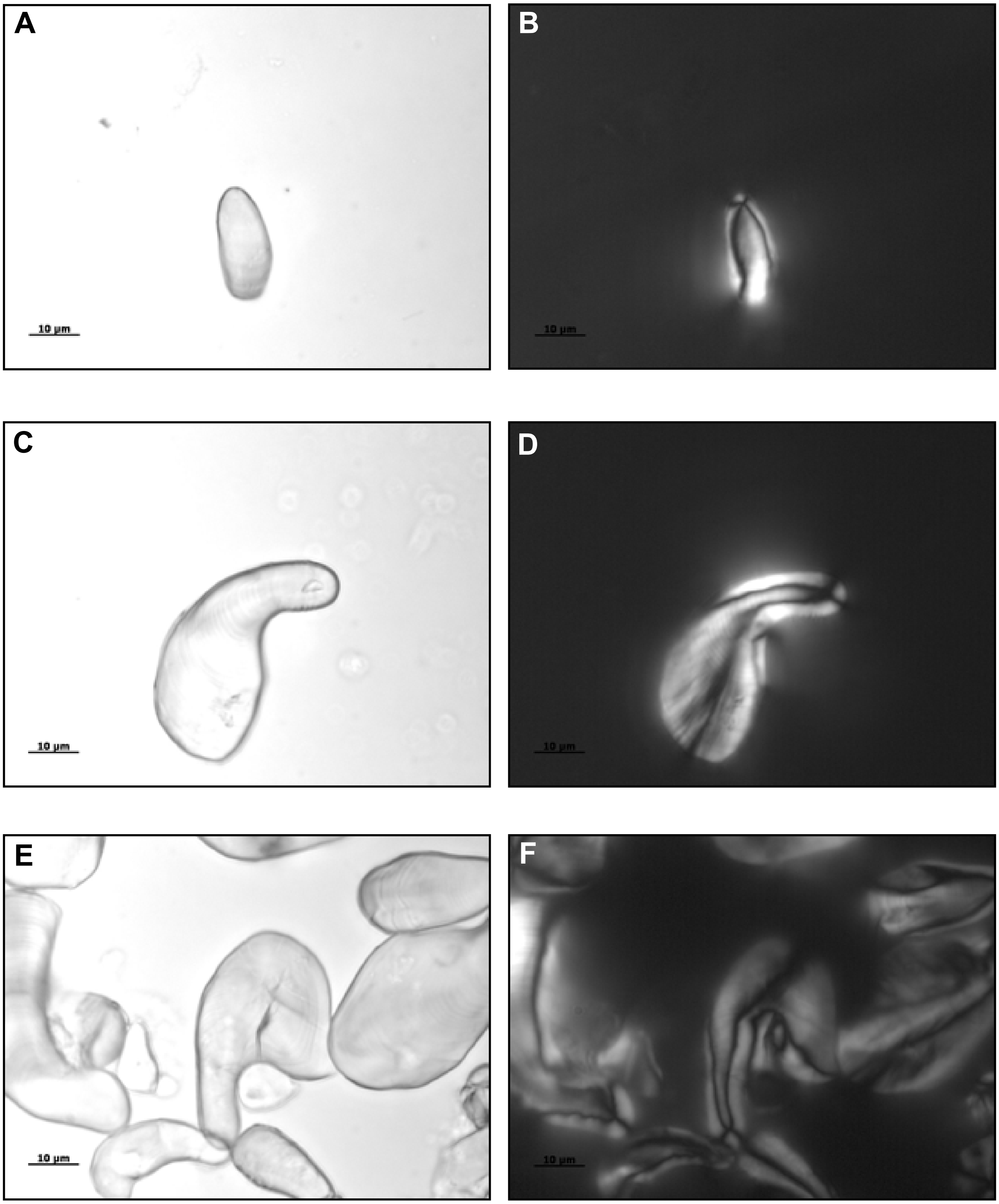

Figure 10. Examples of starch granule morphotypes found in the ABB triploid (accession no. NI14): elongate ovoid morphotype (Variant 23: **A**-**B**); unilobate (Variant 32: **C**-**F**); and, elongate irregular ovoid morphotypes (Variant 31: **E**-**F**). Unilobate morphotypes are also abundant in the ABB? sample WNB4. Irregular ovoid morphotype with a wrinkled texture (Variant 35: **G**-**H**). Triangular ovoid morphotype (Variant 33: **I**-**J**).

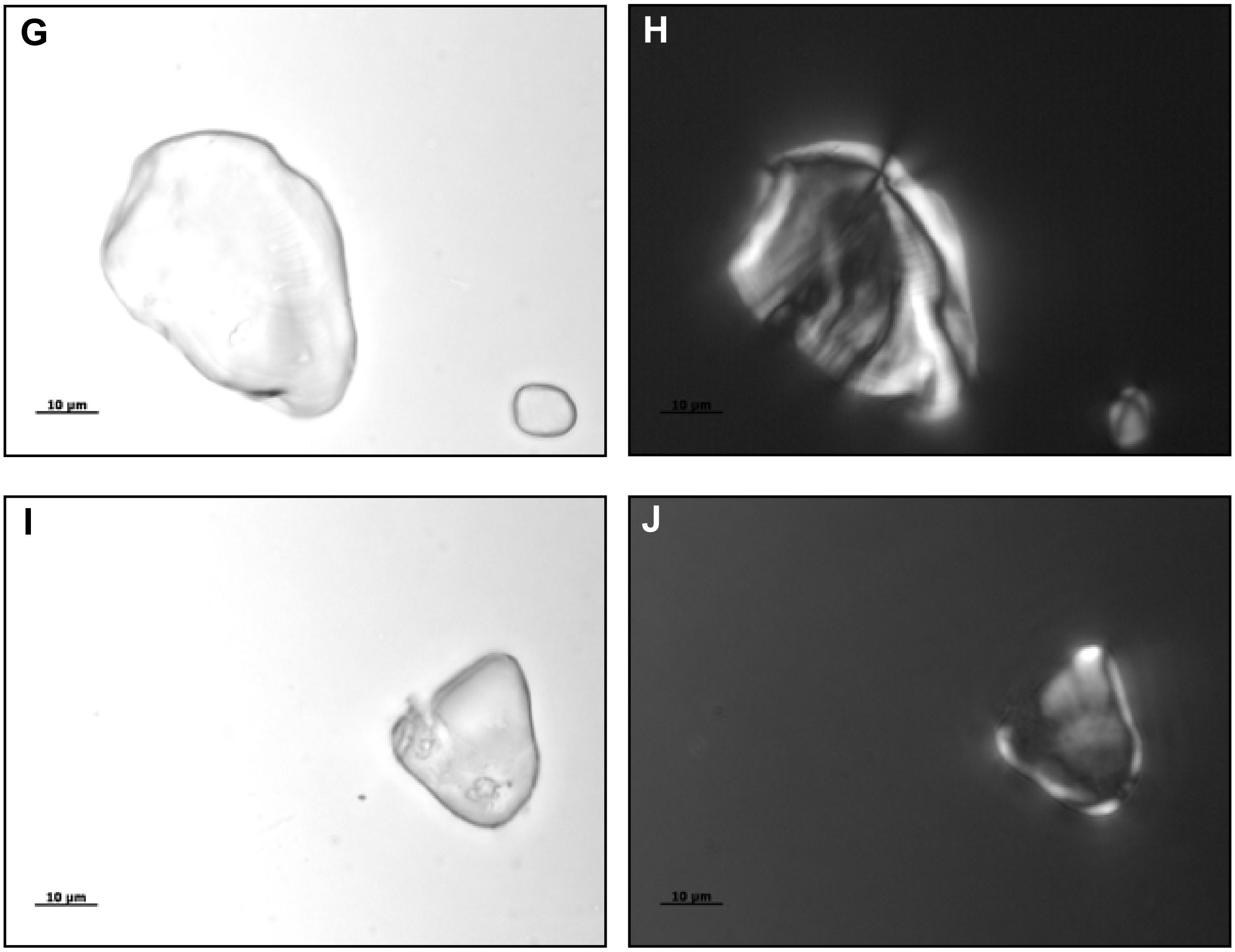

Figure 10 cont. Examples of starch granule morphotypes found in the ABB triploid (accession no. NI14): elongate ovoid morphotype (Variant 23: **A**-**B**); unilobate (Variant 32: **C**-**F**); and, elongate irregular ovoid morphotypes (Variant 31: **E**-**F**). Unilobate morphotypes are also abundant in the ABB? sample WNB4. Irregular ovoid morphotype with a wrinkled texture (Variant 35: **G**-**H**). Triangular ovoid morphotype (Variant 33: **I**-**J**).

which they were most likely derived (e.g., De Langue & De Maret 1999, Lebot 1999, Perrier *et al*. 2009). Hence, as with phytoliths (see Vrydaghs *et al*. 2009), it is possible that some starch granule morphotypes may be linked to alleles of wild progenitors and could possibly be used to determine linkages with domesticated derivatives.

The two classification systems using a) morphotypes, and b) variants, are both useful, and both have advantages. The morphotype system with the inclusion of the size category has the capacity for higher levels of discrimination. However, analysis using this system is complicated by the large number and range of morphotypes for comparison. In contrast, the variant system, with fewer cases for comparison, has the means to facilitate analytical procedure at the same time as providing an effective means for discrimination of Musaceae at every level. Therefore, it might be advantageous for researchers to use the variant system in the first instance, and then proceed to the more definitive morphotype system including size categories, if more detailed analysis at higher resolution is required. Additionally, more rigorous morphometric analysis may result in even higher resolution for identification. It should be noted, however, that results at this stage are not conclusive, since only a small number of Musaceae samples have, as yet, been analyzed using the qualitative method described here. Work in progress includes analyses of additional examples of the species and cultivars examined here, additional species and cultivars not represented here, as well as analyses of plant organs including, roots, leaves, bracts, petioles, peduncles, seeds, pseudostems and corms. Additionally, to determine levels of specificity, Musaceae starch morphotype assemblages will be compared with assemblages from other plants (see Lentfer 2009a). Nevertheless, using the assemblages of diagnostic starch granules identified in this study, albeit preliminary, it should be possible to at least identify Musaceae presence and possibly differentiate some species and cul-

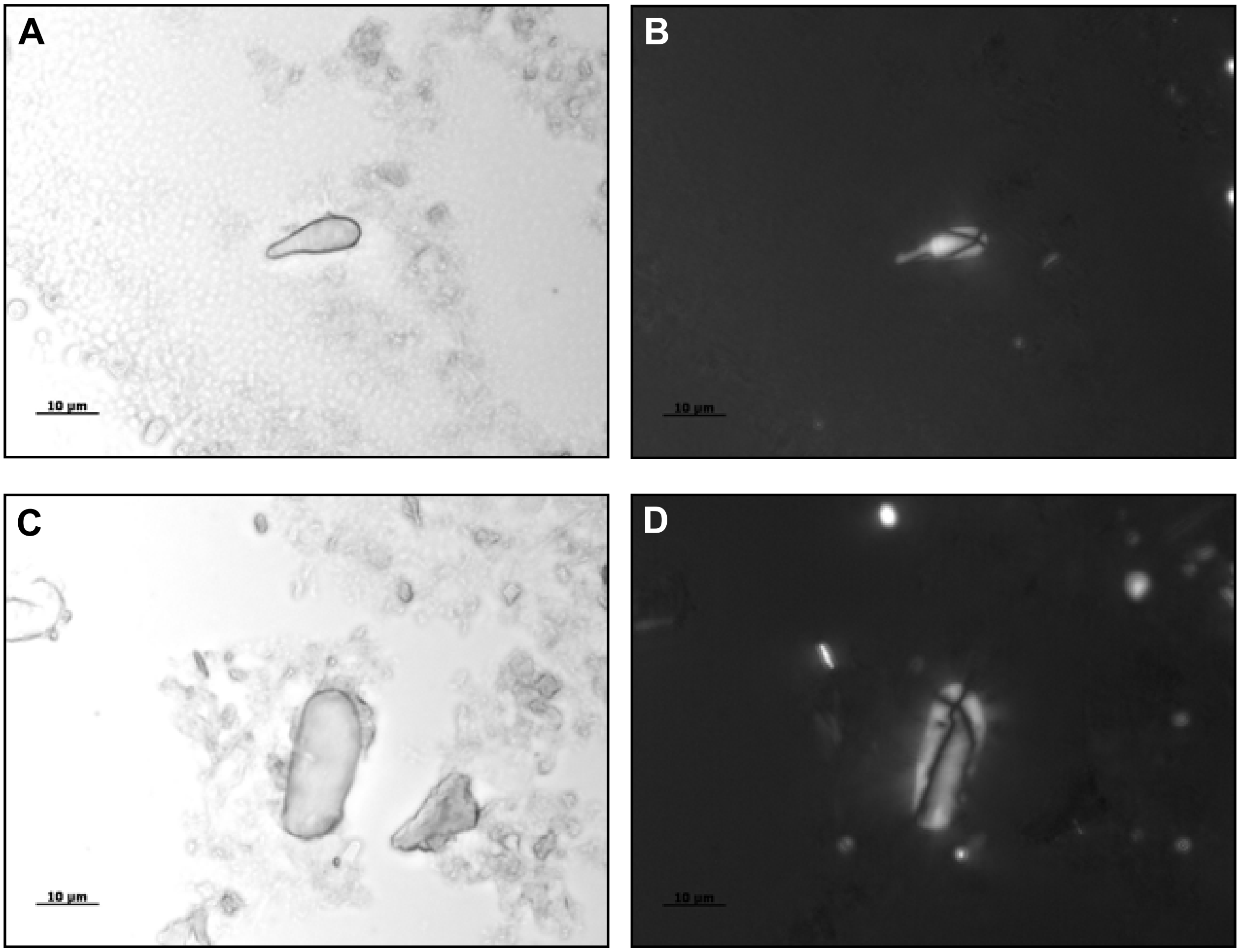

Figure 11. Examples of starch granule morphotypes found in the ABB? triploid (accession no. WNB4): unilobate morphotype (Variant 11: **A**-**B**) and elongate ovoid morphotype (Variant 32: **C**-**D**).

tivars in archaeological assemblages. Although, it might not be possible to make reliable identifications from a single starch granule, especially given the numbers and range of starch granule morphotypes present in Musaceae, the co-occurrence of a number of diagnostic granules and morphotypes would increase reliability. Also, the co-occurrence of starch morphotypes with raphides constitutes another means for identification, and is especially relevant for discriminating wild Australimusa species.

In conclusion, this preliminary analysis has successfully used qualitative starch granule analysis to differentiate within and between genera, sections, species and cultivars of Musaceae. Hence, it shows that starch granule analysis has the promise of being another valuable tool for palaeobotanical analysis, complementary to other microfossil studies including raphides and phytoliths (Lentfer 2009b, Vrydaghs *et al*. 2009). Therefore, while further development is necessary to clarify the diagnostic reliability of traits and levels of specificity, there is undoubtedly good potential for starch analysis to facilitate more robust interpretations of archaeobotanical assemblages and for tracking the history of banana dispersal and domestication, not only in Papua New Guinea, but throughout all the regions important to banana prehistory.

Acknowledgements

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Figure 12. Variants present in Musaceae samples. *Musa acuminata* ssp. *banksii* samples are combined. *Ensete* (green), Australimusa (yellow) and Eumusa (orange).

Variants	*Ensete glaucum*	*Musa maclayi*	*Musa peekeii*	**Fe`i**	*Musa acuminata ssp banksii*	AA	AA?	AAA	ABB	ABB?
1	X									
2	X	X	X	X						
3		X		X	X					
4			X							
5				X						
6				X						
7				X						
8				X						
9					X	X	X			
10					X					
11					X		X		X	X
12					X	X	X	X		
13					X					
14					X					
15					X					
16					X				X	
17					X					
18					X			X		
19						X				
20						X				
21						X				X
22							X		X	
23							X			X
24								X		
25								X		
26								X		
27								X		
28								X		
29								X		
30									X	
31									X	
32									X	X
33									X	
34									X	
35									X	
36									X	
37										X
38										X

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