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Research article

Endosperm starch grains of *Andropogon, Arthraxon hispidus*, and *Hyparrhenia rufa* (Andropogoneae, Panicoideae, Poaceae)

Granos de almidón del endospermo de *Andropogon, Arthraxon hispidus,* e *Hyparrhenia rufa* (Andropogoneae, Panicoideae, Poaceae)

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Abstract:

Background and Aims: Grasses have five different types of endosperm starch grain morphology. Even though there is high diversity within the family, the morphology of the starch grains is generally represented by one or two species. Some genera, such as *Andropogon* (Andropogoneae), were reported to have at least three types of starch grains. However, most of the reviewed species were transferred to other genera. Therefore, the question whether the genus has one or more types of starch grain morphology remains unanswered.

Methods: Between four and eight mature caryopses were removed from specimens deposited in the herbarium IEB for most species, as well as from plants monitored in the field until they had mature caryopses. The caryopses were attached on a plate with a drop of white adhesive Resistol® or resin and then sectioned with a razor blade. Sections were stained with a drop of diluted Lugol´s solution, microscopically observed and photographed at several magnifications.

Key results: All *Andropogon* species observed have only one type of starch grain morphology, the *Andropogon*-type. In all species the simple starch grains are much more abundant than the compound ones, except in *A. tenuispatheus* where the ratio is inverted. The other two reviewed species, *Arthraxon hispidus* and *Hyparrhenia rufa* have *Andropogon*-type and *Panicum*-type starch grains, respectively.

Conclusions: It is confirmed that, so far, all *Andropogon* species observed have one type (*Andropogon*-type) of endosperm starch grain morphology. There is variation in the size, size distribution and shape of the starch grains among the species. *Andropogon gayanus* is the only reviewed species with large starch grains reaching 28 µm, whereas those in the other species measure up to 15 µm in diameter.

Key words: *Andropogon fastigiatus*, *Andropogon virginicus* complex, granules, hilum, *Miscanthus*-type, *Triticum*-type.

Resumen:

Antecedentes y Objetivos: Las gramíneas tienen cinco tipos diferentes de morfología de granos de almidón del endospermo; sin embargo, debido a la alta diversidad dentro de la familia, la morfología de los granos de almidón generalmente está representada por una o dos especies. Para géneros como *Andropogon* (Andropogoneae), se ha reportado que tiene al menos tres tipos de granos de endospermo; sin embargo, la mayoría de las especies que fueron revisadas han sido transferidas a otros géneros. Por lo tanto, la pregunta de si el género tiene uno o más tipos de morfología de los granos de almidón del endospermo, aún permanece sin contestar.

Métodos: Se removieron entre cuatro y ocho cariópsides maduras de especímenes depositados en el herbario IEB y de algunas especies que fueron monitoreadas en el campo hasta que tuvieran cariópsides maduras. Las cariópsides fueron fijadas en una placa con una gota de adhesivo blanco Resistol® o resina. Se hicieron los cortes con una navaja de rasurar muy delgada. Los cortes se tiñeron con una gota diluida de solución Lugol, se observaron al microscopio y se tomaron fotografías a diferentes amplificaciones.

Resultados clave: Todas las especies de *Andropogon* tienen solo un tipo de morfología de granos de almidón, el tipo-*Andropogon.* En todas las especies los granos de almidón simples son más abundantes que los compuestos, excepto *A. tenuispatheus* donde sucede lo contrario. Las otras dos especies revisadas, *Arthraxon hispidus* e *Hyparrhenia rufa* tienen tipo-*Andropogon* y tipo-*Panicum*, respectivamente.

Conclusiones: Hasta este momento, se confirma que todas las especies revisadas tienen un solo tipo de morfología (tipo-*Andropogon*) de granos de almidón del endospermo. Existe variación en el tamaño, distribución de tamaños y formas de granos de almidón entre las especies. *Andropogon gayanus* es la única especie que tiene granos de almidón grandes hasta 28 µm, mientras que en las otras especies estos pueden medir hasta 15 µm de diámetro.

Palabras clave: *Andropogon fastigiatus*, complejo *Andropogon virginicus*, gránulos, hilio, tipo-*Miscanthus*, tipo-*Triticum*.

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Introduction

Grass starch grains have been studied from the phylogenetic point of view, as well as because of their importance as a source of food from all edible grains and cereals (Sarwar et al., 2013). There are several classifications, some of them very detailed (Reichert, 1913) and others simplified, depending on the group (Tateoka, 1954, 1962; GPWG, 2001; Matsushima et al., 2013; Matsushima, 2015). The first classifications were inclusive for all plants with at least 17 different types of starch grains from approximately 137 genera and 346 species of grasses (Reichert, 1913). Tateoka (1954) observed 37 and 172 Pooideae genera and species, respectively, and Tateoka (1962), in a broader review, described four types of starch grains for 244 genera and 766 species throughout the family. Tateoka´s (1962) classification includes whether the grains are simple or compound, with only one or two sizes and the number and size of the granules in the compound grains.

The current classification of starch grains in Poaceae is based on five types of morphologies, based on Tateoka (1962), and modified by the GPWG (2001). Type 1 or *Triticum*-type was designated for simple starch grains with shapes ranging from broadly elliptic, elliptic-round to rarely reniform, from 4-6 µm to medium-large size between 30-40 µm. This type of starch grain is common in the Pooideae subfamily. There are taxa with homogenous or unimodal sizes and heterogeneous or bimodal size starch grains. Type 2 or *Panicum*-type includes simple, round, polygonal to rarely rectangular starch grains, commonly 4-6 µm, but some genera with large grains measuring between 30-40 µm. This type of starch grains is common in Panicoideae, as well as in some taxa from other subfamilies such as Chloridoideae, Bambusoideae, Pooideae and Micrairoideae (Tateoka, 1962).

Type 3 (*Miscanthus*-type) or *Andropogon*-type is a mixture of simple and compound starch grains, where the first are round to polygonal (like *Panicum*-type) and between 15-40 µm in diameter. Commonly, compound grains consist of 2-4 granules, which are usually slightly smaller than simple grains. Depending on the ratio between simple and compound starch grains, the most common is where the simple starch grains are much more numerous, a second ratio is when both simple and compound starch grains are somewhat the same in some species, and a third ratio, perhaps the most uncommon, where the compound starch grains are numerous. This type of starch grain is common in Andropogoneae, some Chloridoideae and Arundinoideae taxa. Finally, Type 4 or *Festuca*-type is recognized in species where most of the grains are compound, each with few to many tiny granules between 2-6 µm in diameter (Judziewicz and Soderstrom, 1989; Bultosa et al., 2002; Yun and Kawagoe, 2010; Ai et al., 2016).

GPWG (2001) segregated the fifth type calling it *Brachyelytrum*-type, where all the starch grains are only large, between 30-40 µm diameter, without the smaller grains typical of *Panicum*-type. This difference had also already been noticed by Tateoka (1962).

Tateoka (1954) reviewed six species of andropogonoid genera, half of them with *Panicum*-type and the other half with compound grains. The author did not explain if the last three species had both simple and compound grains, but the illustration shows compound one with 2-4 and 13 granules, likely the *Andropogon*-type. Later, in a review of 49 species from 28 genera of the tribe Andropogoneae (unfortunately no names for the species were given), Tateoka (1962) found three types of grains. Fourteen genera have exclusively the most common *Panicum*-type, four *Andropogon*-type, six with *Festuca*-type, three with *Panicum*-type and *Andropogon*-type, and two with *Panicum*-type and *Festuca*-type. At the species level, 30 have *Panicum*-type, ten *Festuca*-type, and only eight species *Andropogon*-type (Table 1).

The starch grains of *Andropogon* L. are known only from two out of the 100-120 species recognized in the genus (Campbell, 2003; Nagahama and Norrmann, 2012). Previously, Reichert (1913) described the starch grains of 14 species assigned to *Andropogon*, from which only two were within the genus and the remaining 12 belonged to other genera. These two species, *Andropogon leucostachyus* Kunth and *Andropogon virginicus* L. have *Andropogon*-type starch grains (Reichert, 1913). Summarizing Reichert´s (1913) and Tateoka´s (1962) findings, it means that *Andropogon* has three types of starch grain morphology, *Panicum*-type, *Andropogon*-type, and *Festuca*-type, having in mind that Tateoka´s (1962) studied species remain nameless. Perhaps this variation or uncertainty led Watson

Table 1: List of genera and number of species of the tribe Andropogoneae with known starch grain types (taken from Tateoka (1962)). Boldface indicates genera with more than one type of starch grains.

	Species number		
Genus	***Panicum*-type**	***Andropogon*-type**	***Festuca*-type**
**Andropogon* L.	**7**		**1**
Apluda L.			1
**Arthraxon* P. Beauv.	**1**	**1**	
Bothriochloa Kuntze	1		
**Chrysopogon* Trin.	**1**	**1**	
Cleistachne Benth.	1		
Coix L.	2		
Cymbopogon Spreng.		1	
Dichanthium Willimet	1		
Dimeria R. Br.	1		
Eremochloa Buse	2		
Erianthus Michx.			1
Hemarthria R. Br.	1		
Heteropogon Pers.	1		
Hyparrhenia Andersson ex E. Fourn.	1		
**Ischaemum* L.	**1**		**3**
Manisuris L.			1
**Microstegium* Nees	**1**	**1**	
Miscanthus Andersson		2	
Mnesithea Hack.			1
Phacelurus Griseb.			1
Pogonatherum P. Beauv.			1
Sorghastrum Nash	1		
Sorghum Moench	2		
Spodiopogon Trin.	3		
Themeda Forssk.		2	
Zea L.	1		

et al. (1992) to leave out this information in the generic description of *Andropogon.* A different variation within a genus was observed by Matsushima et al. (2013): bimodal sizes of simple starch grains in *Bromus catharticus* Vahl. However, the genus typically has *Triticum*-type unimodal starch grains. Several other authors had reported only one size simple starch grains for the genus *Bromus* L. (Reichert, 1913; Tateoka, 1962; Musaubach et al., 2013).

Regarding *Arthraxon* P. Beauv. and *Hyparrhenia* Andersson ex E. Fourn., the former was reported to have two different types of starch grains (no species names given), *Panicum*-type and *Andropogon*-type (Tateoka, 1962) and the latter with *Panicum*-type grains (Reichert, 1913; Tateoka, 1962).

The objective of this research was to verify the variation of the starch grains among several species of *Andropogon* and to report the type of grains of *Arthraxon hispidus* (Thunb.) Makino and *Hyparrhenia rufa* (Nees) Stapf.

Materials and Methods

Grass specimens from the herbarium IEB (Centro Regional del Bajío, Instituto de Ecología, A.C.) were reviewed to see if they have developed caryopses, and those species that were nearby Pátzcuaro were monitored and collected when the caryopses were well developed. All collected vouchers were deposited in the herbarium IEB and duplicates will be sent out to the MEXU Herbarium (Instituto de Biología, Universidad Nacional Autónoma de México) and the herbarium EBUM (Universidad Michoacana de San Nicolás de Hidalgo) (acronyms according to Thiers, 2020). Four to eight caryopses were removed from each voucher. Caryopses of *Arthraxon hispidus* and *Hyparrhenia rufa* were also included to verify the morphology of their starch grains. Due to the tiny size of the caryopses, they were attached to a plate with a drop of white adhesive Resistol® or epoxy resin. Once the glue had dried, the caryopses were sectioned with a very thin razor blade. Starch grains were stained with 5% stock Lugol´s solution (Potassium iodine) diluted 1:5 to 1:20 (Reichert, 1913; McNair, 1930). Stained sections were observed using a light microscope (Primo Star, Carl Zeiss, New York, USA), and an IPhone 11 (Apple, California, USA) to take photographs at several magnifications. The grains were measured using an eyepiece micrometer, calibrated with a stage micrometer. All measurements were made to include from the smallest to the largest grain; when possible, in the whole stained section, when the compound grains were scarce, all of them were measured. Photos were edited with GIMP v. 10.12.22 (Kimball et al., 1995-2021). Relative starch grain packing based on the proximity of the grains is characterized by the following traits: loosely packed where the grains are widely separated from each other, closely packed where the grains are close to each other, but not causing pressure, and tightly packed where the grains tightly touched each other, causing flattened facets due to pressure. Relative abundance is defined by the number of compound or simple grains: rarely present (1), low abundance (2-5), moderate abundance (6-30), and very high abundance (6-30).

The morphology of the starch grains is a constant character within a given species as its biosynthesis and review of several individuals has been probed (Matsushima et al., 2013; Tetlow and Emes, 2017). Therefore an individual can represent the whole species. Below is the list of the specimen vouchers from which caryopses were extracted:

Andropogon fastigiatus Sw.: MEXICO. Michoacán, municipio La Huacana, ca. 0.5 km NE of Los Ranchos at western base of cerro El Barril, 18°42'20''N, 102°00'30''W, shady ravine in thornscrub, 300 m, 09.XI.2002, *V. W. Steinmann 2973* (IEB).

Andropogon gayanus Kunth: MEXICO. Michoacán, municipio Huiramba, camino Morelia - Pátzcuaro, cerca del restaurante Campestre Los Pinos, 19°32'15''N, 101°32'00''O, pastizal secundario, 2101 m, 17.XI.2020, *J. G. Sánchez-Ken 1091* (IEB, MEXU).

Andropogon gerardi Vitman: MEXICO. Michoacán, municipio Tzintzuntzan, km 9 de la carretera Tzintzuntzan - Pátzcuaro, 19°36'43''N, 101°34'36''O, pastizal secundario, 2121 m, 28.X.2020, *J. G. Sánchez-Ken 1082* (IEB).

Andropogon liebmannii Hack. var. *liebmannii*: MEXICO. Veracruz, municipio Benigno Mendoza, Mecayapan, 18°18'55''N, 94°46'35''O, ladera, 295 m, 11.VII.1994, *G. Castillo C. et al. 12484* (IEB).

Andropogon pringlei Scribn. & Merr.: MEXICO. Michoacán, municipio Maravatío, La Nopalera, 12 km al SE de Maravatío, sobre la carretera a Tlalpujahua, 19°49'50''N, 100°21'34''O, vestigios de bosque tropical caducifolio, 2100 m, 10.X.1991, *J. Rzedowski 50991* (IEB, MEXU).

Andropogon selloanus (Hack.) Hack.: MEXICO. Tabasco, municipio Huimanguillo, km 27 de la desviación de Huimanguillo a Francisco Ruedas, 17°47'26''N, 93°26'14''O, sabanas, 16 m, 15.VII.1988, *M. A. Magaña et al. 2056* (IEB).

Andropogon tenuispatheus (Nash) Nash: MEXICO. Michoacán, municipio Morelia, antes de llegar a Morelia, carretera Pátzcuaro - Morelia, 19°39'28''N, 101°14'26''O, pastizal secundario en zona inundable, 1898 m, 10.XI.2020, *J. G. Sánchez-Ken et al. 1089* (IEB, MEXU).

Andropogon virginicus L. var. *virginicus*: MEXICO. Veracruz, municipio Xalapa, carretera Xalapa - Coatepec, 19°27'N, 98°56'O, bosque caducifolio con *Liquidambar* y encinar, 1150 m, 7.X.1980, *T. Mejía Saulés 359* (IEB, XAL).

Arthraxon hispidus (Thunb.) Makino: MEXICO. Michoacán, municipio Ziracuaretiro, Malpaís de San Andrés Corú, 19°27'31''N, 101°56'47''O, bosque de pino-encino, 1685 m, 12.XI.2012, *D. Valentín M. 460* (IEB).

Hyparrhenia rufa (Nees) Stapf: MEXICO. Michoacán, municipio Morelia, carretera Pátzcuaro - Morelia, a 1.8 km al SW de La Estancia a un lado de la carretera, 19°34'34''N, 101°18'50''O, pastizal secundario, 2018 m, 21.X.2020, *J. G. Sánchez-Ken 1077* (IEB).

Results

Descriptions of the caryopses and starch grains of eight *Andropogon* species, *Arthraxon hispidus* and *Hyparrhenia rufa* are summarized in Table 2. The following list provides a detailed description of the caryopses and starch grains for each species.

Andropogon fastigiatus (*Andropogon*-type): caryopses fusiform, 3-3.4 × 1-1.2 mm wide, embryo 2-2.3 mm long, hilum punctiform, slightly triangular, 0.5-0.6 mm long (Fig. 1A); simple starch grains irregularly elliptic, triangular to somewhat polygonal with flat facets due to pressure (Fig. 2A), 5-11(-13) µm diameter, size distribution even to uneven towards the aleurone layer, tightly packed; compound starch grains 11-15 µm diameter, with (2-)3(-5) granules, moderate abundance; granules 5-8 µm diameter, circular to squarish, facets flat towards the center, rounded externally (Fig. 3A).

Andropogon gayanus (*Andropogon*-type): caryopses obovate to ovate, 3.4-3.5 × 1.2-1.3 mm wide, embryo 2-2.1 mm long, hilum punctiform, circular, 0.4-0.5 mm long (Fig. 1B); simple starch grains circular, irregularly circular, squarish to polygonal with flat facets due to pressure (Fig. 2B), (5-)8-25(-28) µm diameter, size distribution uneven towards the aleurone layer, closely packed; compound starch grains 7-18 µm diameter, with 3 granules, low abundance; granules (3-)7-10 µm diameter, circular to slightly angular at the union, facets flat towards the union, rounded externally (Fig. 3B).

Table 2: Summary of the characters of the starch grains among the species observed in this study. Types of starch grains: 2=*Panicum*-type, 3=*Andropogon*-type, "3"=inverted *Andropogon*-type.

Species	Type	Simple grains diameter (µm)	Grains packing	Compound grains diameter (µm)	Abundancy	Granules number	Granules diameter (µm)
Andropogon fastigiatus Sw.	3	5-11(-13)	tightly	11-15	moderate	(2-)3(-5)	5-8
Andropogon gayanus Kunth	3	(5-)8-25(-28)	closely	6-15	low	3	(3-)7-10
Andropogon gerardi Vitman	3	5-13(-14)	loosely	10-13	moderate	2-5(-6)	4-6
Andropogon liebmannii Hack. var. *liebmannii*	3	(2-)4-10(-12)	loosely	8-13	low	2-4	4-6
Andropogon pringlei Scribn. & Merr.	3	5-13(-15)	closely	13-14	moderate	2-4	4-6
Andropogon selloanus (Hack.) Hack.	3	(3-)5-11	loosely	7-12	low	2-3	5-6
Andropogon tenuispatheus (Nash) Nash	"3"	3-10	closely	(6-)7-15	very high	(2-)3-9	4-6
Andropogon virginicus L. var. *virginicus*	3	4-12(-14)	loosely	10-13	low	2-3	3-7
Arthraxon hispidus (Thunb.) Makino	3	(3-)5-10(-12)	loosely	9-13	low	3	4-9
Hyparrhenia rufa Stapf	2	9-18(-20)	tightly	-	-	-	-

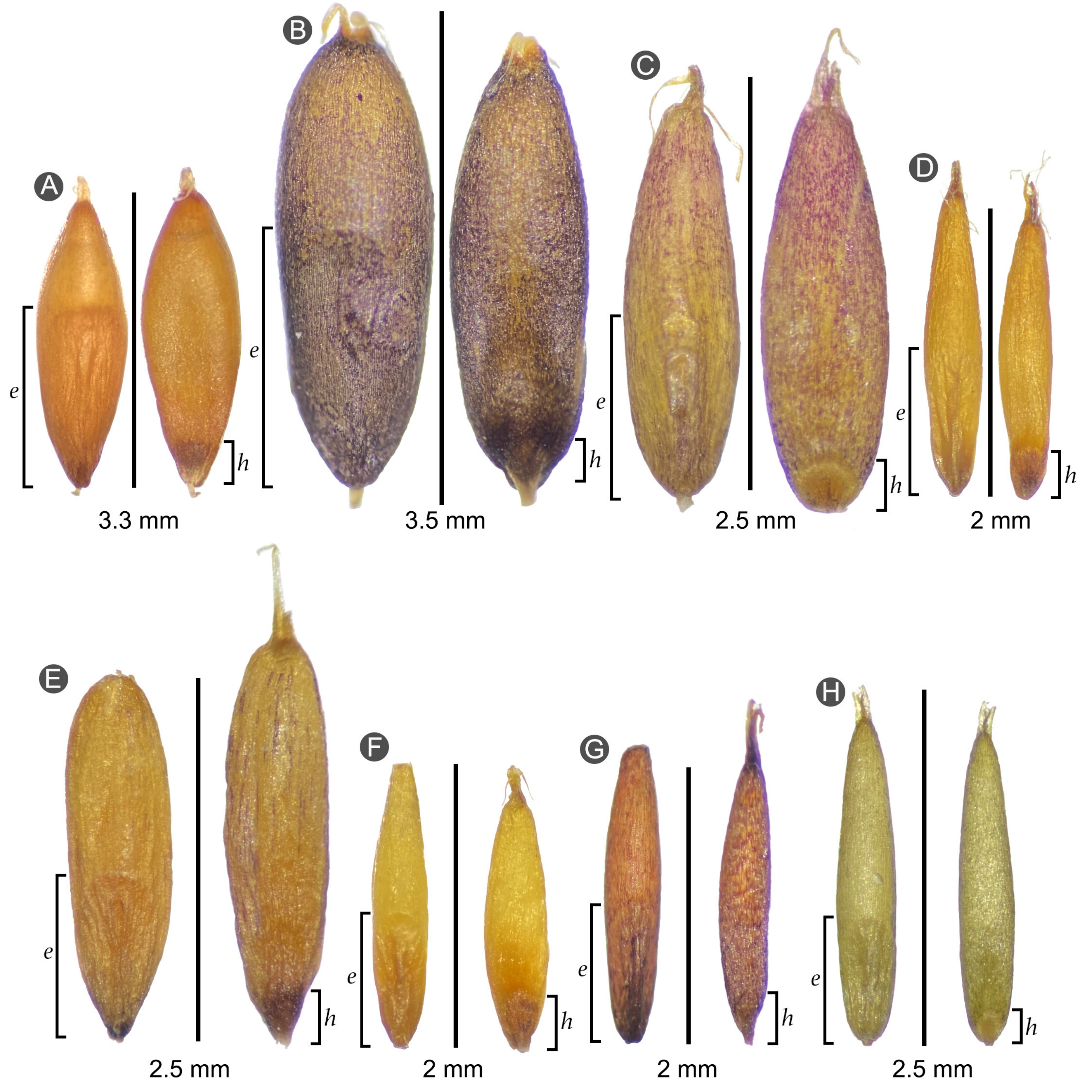

Figure 1: Ventral and dorsal views of *Andropogon* L. Caryopses: A. *Andropogon fastigiatus* Sw.; B. *Andropogon gayanus* Kunth; C. *Andropogon gerardi* Vitman; D. *Andropogon liebmannii* Hack. var. *liebmannii*; E. *Andropogon pringlei* Scribn. & Merr.; F. *Andropogon selloanus* (Hack.) Hack.; G. *Andropogon tenuispatheus* (Nash) Nash.; H. *Andropogon virginicus* L. var. *virginicus. e*=embryo; *h*=hilum.

Andropogon gerardi (*Andropogon*-type): caryopses ovate, 2.4-2.5 × 0.6-0.7 mm wide, embryo 1.1-1.3 mm long, hilum punctiform, circular, 0.3-0.4 mm long (Fig. 1C); simple starch grains widely elliptic, irregularly elliptic, irregularly squarish to polygonal, some with flat facets (Fig. 2C), 5-13(-14) µm diameter, size distribution uneven, loosely packed; compound starch grains 10-13 µm diameter, with 2-5 granules, moderate abundance; granules 4-6 µm, cir-

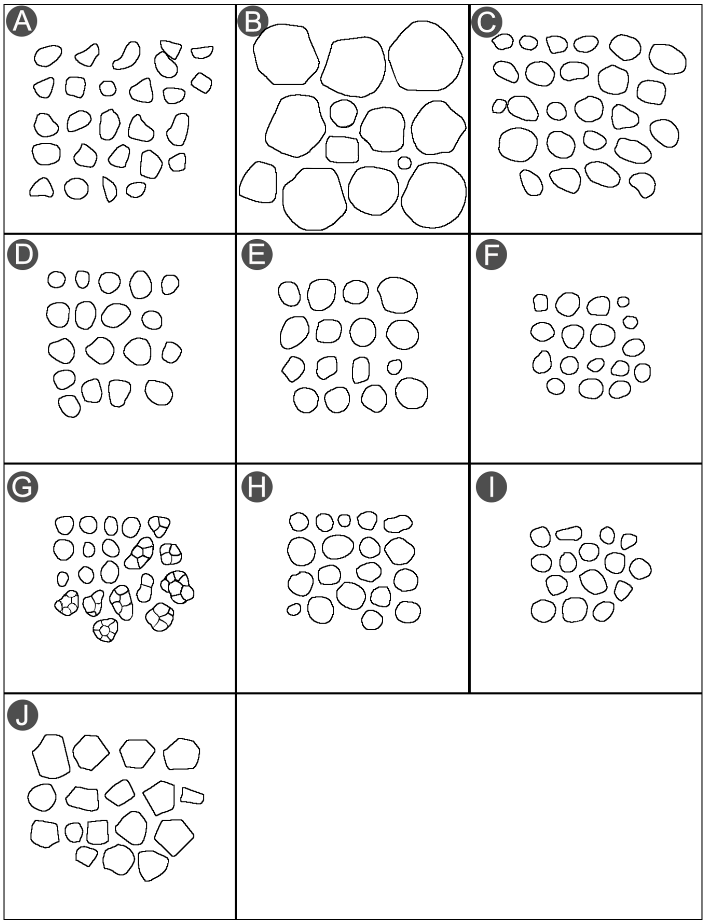

Figure 2: Outlines of starch grains observed. A. *Andropogon fastigiatus* Sw.; B. *Andropogon gayanus* Kunth; C. *Andropogon gerardi* Vitman; D. *Andropogon liebmannii* Hack. var. *liebmannii*; E. *Andropogon pringlei* Scribn. & Merr.; F. *Andropogon selloanus* (Hack.) Hack.; G. *Andropogon tenuispatheus* (Nash) Nash.; H. *Andropogon virginicus* L. var. *virginicus*; I. *Arthraxon hispidus* (Thunb.) Makino; J. *Hyparrhenia rufa* (Nees) Stapf.

cular to squarish, flat facets towards the center, rounded externally (Fig. 3C).

Andropogon liebmannii var. *liebmannii* (*Andropogon*-type): caryopses ovate-elliptic, 1.9-2.1 × 0.4-0.5 mm wide, embryo 1-1.1 mm long, hilum punctiform, circular, 0.3-0.4 mm long (Fig. 1D); simple starch grains irregularly circular, widely elliptic, few triangular to squarish, with flat facets (Fig. 2D), (2-)4-10(-12) µm diameter, size distribution uneven, but larger towards the center, loosely packed; compound starch grains 8-13 µm diameter, with 2-4 granules, low abundance; granules 5-7 µm diameter, circular to polygonal, flat facets towards the center, rounded externally (Fig. 4A).

Andropogon pringlei (*Andropogon*-type): caryopses obovate, 2.5-2.6 × 0-4-0.5 mm wide, embryo 1.1-1.2 mm long, hilum punctiform, circular, 0.4-0.5 mm long (Fig. 1E); simple starch grains circular, irregularly circular, squarish to polygonal, some with flat facets (Fig. 2E), 5-13(-15) µm diameter, size distribution uneven, closely packed; compound starch grains 13-14 µm diameter, with 2-4 granules, moderate abundance; granules 4-6 µm diameter, circular to squarish, flat facets towards the center, rounded externally (Fig. 4B).

Andropogon selloanus (*Andropogon*-type): caryopses elliptic, 2-2.1 × 0.3-0.4 mm wide, embryo 0.9-1 mm long, hilum punctiform, triangular, 0.4-0.5 mm long (Fig 1F); simple starch grains circular, irregularly circular, squarish, some with somewhat flat facets (Fig. 2F), (3-)5-11 µm diameter, size distribution uneven, loosely packed; compound starch grains 7-12 µm, with 2-3 granules, low abundance; granules 5-6 µm diameter, circular to squarish, flat facets towards the center, rounded externally (Fig. 4C).

Andropogon tenuispatheus (previously *A. glomeratus* Britton, Sterns & Poggenb. var. *pumilus* (Vasey) L.H. Dewey (Wipff and Shaw, 2018, "*Andropogon*-type"): caryopses elliptic, 2-2.1 × 0.4-0.5 mm wide, embryo 1-1.1 mm long, hilum punctiform, triangular, 0.3-0.4 mm long (Fig. 1G); simple starch grains circular to elliptic (Fig. 2G), 3-10 µm diameter, low abundance, sparsely distributed, closely packed; compound starch grains (6-)7-15 µm diameter, irregular, with (2-)3-9 granules, circular, squarish, triangular, larger compound grains towards the center, size distribution uneven, very high abundance; granules 4-6 µm diameter, circular to squarish, flat facets towards the center, rounded externally (Fig. 5A).

Andropogon virginicus var. *virginicus* (*Andropogon*-type): caryopses ovate-elliptic, 2.4-2.5 × 0.6-0.7 mm wide, embryo 0.9-1 mm long, hilum punctiform, circular to slightly oblong, 0.2-0.3 mm long (Fig. 1H); simple starch grains circular to irregularly circular, irregularly elliptic (Fig. 2H), 4-12(-14) µm diameter, size distribution uneven, larger towards the center, loosely packed; compound starch grains 10-13 µm diameter, with 2-3 granules, low abundance;

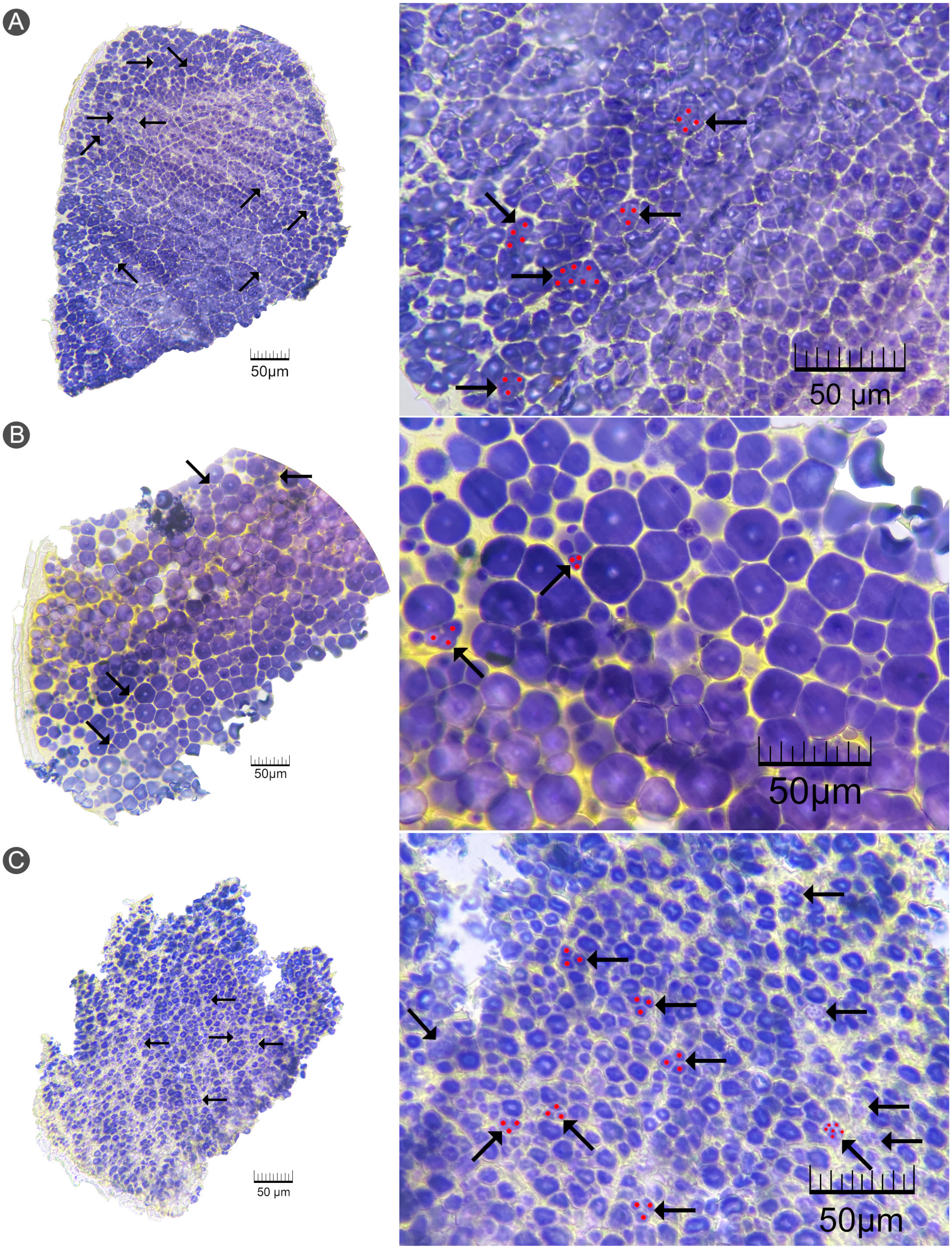

Figure 3: Starch grains. A. *Andropogon fastigiatus* Sw.; B. *Andropogon gayanus* Kunth; C. *Andropgon gerardi* Vitman. Left column=lower magnification; right column=higher magnification. Arrows point to compound starch grains; red dots are granules.

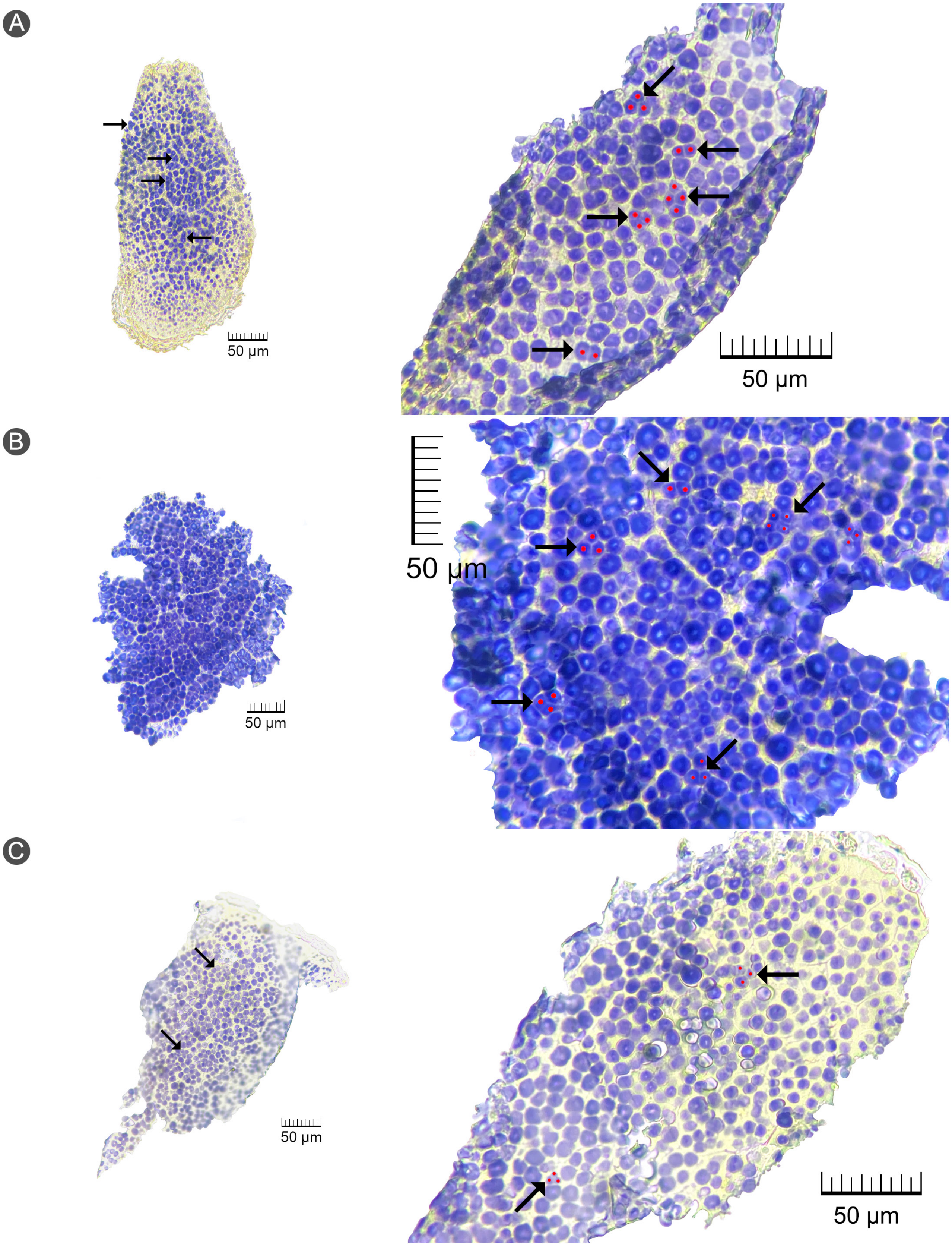

Figure 4: Starch grains. A. *Andropogon liebmannii* Hack. var. *liebmannii*; B. *Andropogon pringlei* Scribn. & Merr.; C. *Andropgon selloanus* (Hack.) Hack. Left column=lower magnification; right column=higher magnification. Arrows point to compound starch grains; red dots are granules.

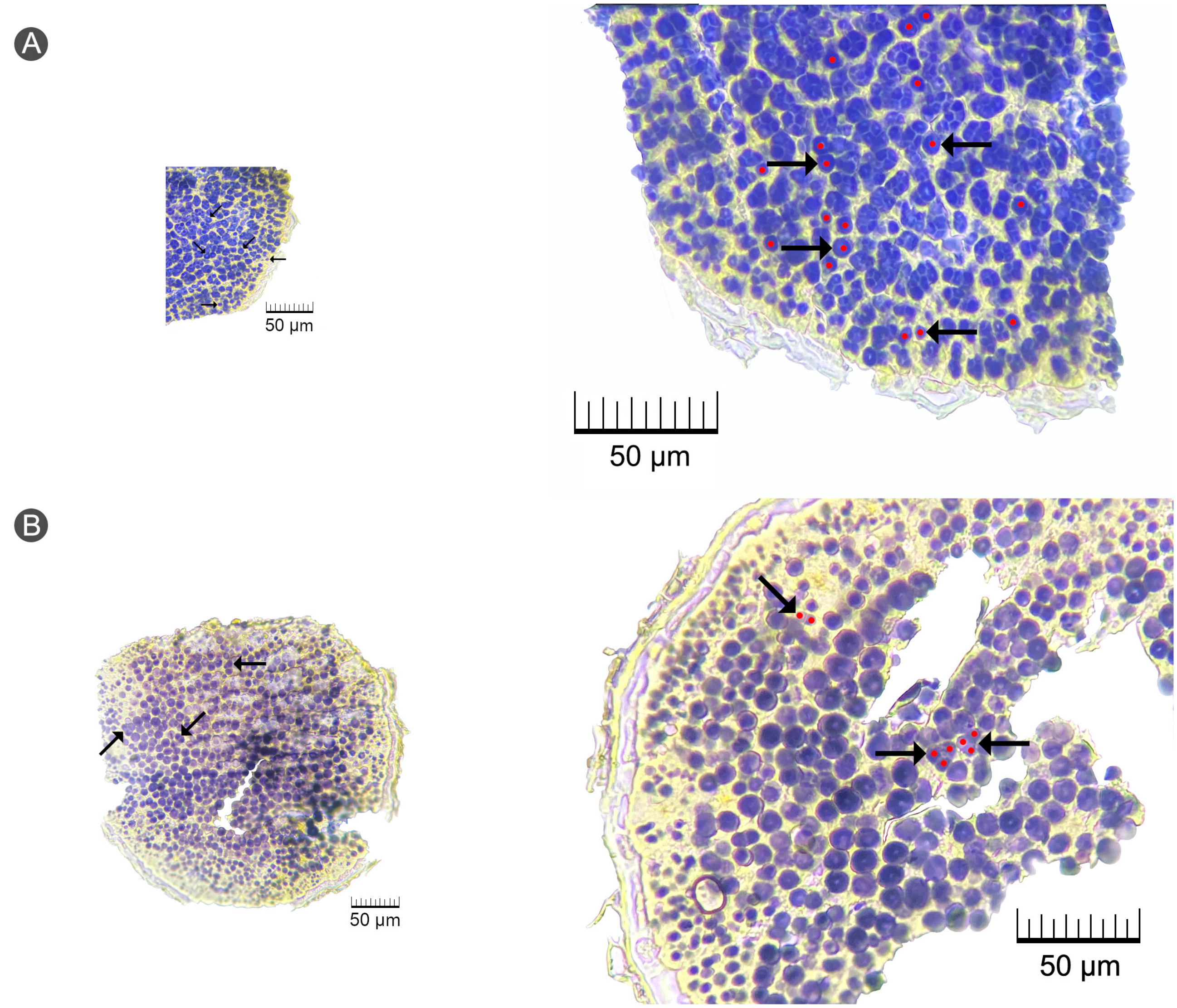

Figure 5: Starch grains. A. *Andropogon tenuispatheus* (Nash) Nash (arrows point to simple grains, red dots are simple grains); B. *Andropogon virginicus* L. var. *virginicus* (arrows point to compound starch grains, red dots are granules). Left column=lower magnification; right column=higher magnification.

granules 3-7 µm diameter, circular to squarish, flat facets towards the center, rounded externally (Fig. 5B).

Arthraxon hispidus (*Andropogon*-type): caryopses elliptic, 1.9-2 × 0.2-0.3 mm wide, embryo 0.6-0.7 mm long, hilum punctiform, slightly oblong, 0.2-0.3 mm long (Fig. 6A); simple and compound starch grains; simple starch grains circular, irregularly elliptic to polygonal, some with flat facets due to pressure (Fig. 2I), (3-)5-10(-12) µm diameter, size distribution uneven, larger towards the center, closely packed; compound starch grains 9-13 µm diameter, with 3 granules, low abundance; granules 4-9 µm diameter, circular to squarish, flat facets towards the center, rounded externally (Figs. 6A-C).

Hyparrhenia rufa (*Panicum*-type): caryopses obovate, 2.5-2.6 × 0.7-0.8 mm, embryo 1.1-1.2 mm long, hilum punctiform, triangular, 0.2-0.3 mm long (Fig. 6D); simple starch grains; simple grains triangular to polygonal with flat facets due to pressure, those towards the aleurone layer irregularly circular (Fig. 2J), 9-18(-20) µm diameter, larger grains towards the center, tightly packed (Figs. 6D-F).

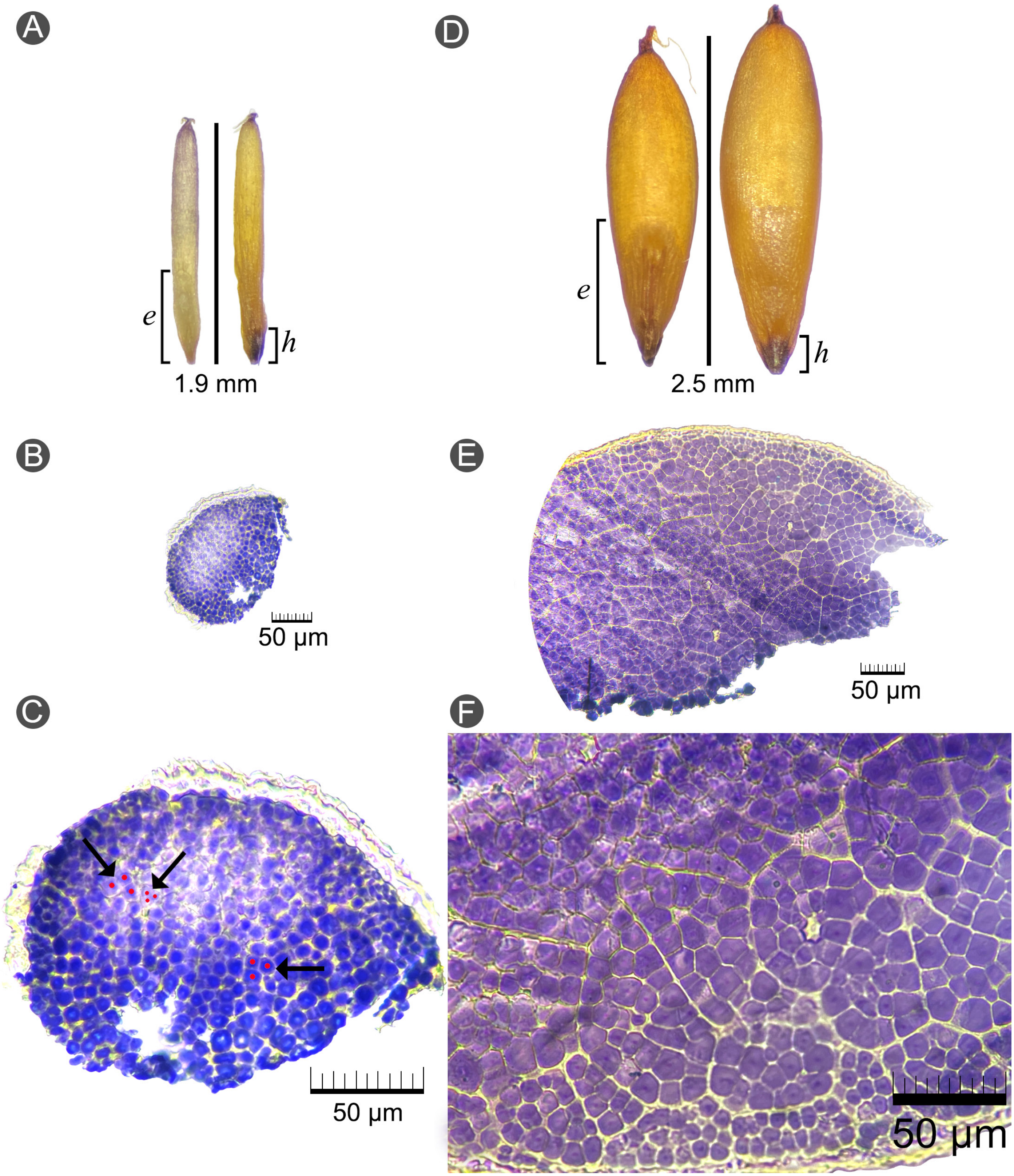

Figure 6: Caryopses and endosperm starch grains. A-C. *Arthraxon hispidus* (Thunb.) Makino: A. caryopses; B. endosperm in transversal view, lower magnification; C. endosperm in transversal view, higher magnification. D-F. *Hyparrhenia rufa* (Nees) Stapf: D. caryopses; E. endosperm in transversal view, lower magnification; F. endosperm intransversal view, higher magnification. Caryopses: Left=ventral view; right=dorsal view; *e*=embryo; *h*=hilum.

Discussion

It has been assumed that the morphology of the starch grains within a genus is unique, except for some genera of the subfamilies Panicoideae and Bambusoideae (Tateoka, 1962 and references therein). Tateoka (1962) reviewed 766 species from 244 genera, from all these genera representing the whole family, most have only one type of starch grains. Few genera of some subtribes of Andropogoneae (*Andropogon* L., *Arthraxon*, *Chrysopogon* Trin., *Ischaemum* L., and *Mischanthus* Andersson), one genus from Paspaleae (*Arthropogon* Nees), and two genera of Paniceae (*Panicum* L. and *Digitaria* Haller), according to the current classification (Soreng et al., 2015), apparently have more than one type of starch grains. Several genera have been segregated from *Andropogon* and *Panicum* since then and because Tateoka (1962) only reported the number of the species but not their names, those species with a different type of starch grains remain unknown.

All the species of *Andropogon* and *Arthraxon* are characterized by *Andropogon*-type starch grains. Seven out of eight species have a major percentage of simple starch grains with low (2-5) to moderate abundance (6-30) of compound starch grains. These compound starch grains have two to five starch granules, except those in *Andropogon tenuispatheus* with up to nine granules. Among all these species, *A. tenuispatheus* has a unique morphology, an inverted *Andropogon*-type, where the compound starch grains are the most abundant compared to the simple grains, which may account for less than 5% of those observed. So far, this inverted *Andropogon*-type has been observed in *Tridens chapmannii* (Small) Chase, Chloridoideae), *Thuarea sarmentosa* Pers., Paniceae), and the Andropogoneae taxa *Microstegium vimineum* (Trin.) A. Camus, *Chrysopogon aciculatus* (Retz.) Trin., and *Themeda triandra* Forssk. (Tateoka, 1962).

Regarding the simple starch grains size, all *Andropogon* species range from (2-)3-13(-15) µm diameter (numbers in parenthesis indicate rare or unique grains with those sizes), whereas in *A. gayanus* the grains can reach up to 28 µm diameter, more than twice the size of the grains of the other species. The arrangement of the grains may be tightly packed like in *A. fastigiatus* where the facets of the grains appear to be flattened; the same goes for those species with closely but not tightly packed in species such as *A. gayanus* and *A. pringlei* judging by the roundness of the grains. In the remaining species (*A. gerardi*, *A. liebmannii* var. *liebmannii*, *A. selloanus*, and *A. virginicus* var. *virginicus*), the simple grains are loose, so the facets are rounded. In *A. tenuispatheus* the compound starch grains are closely packed but the facets of the granules are flattened because they are part of a compound grain.

Whether *Andropogon fastigiatus* belongs or not in the genus *Andropogon*, remains unclear (McAllister et al., 2018; Welker et al., 2020) but for the time being, it is still considered within the genus. The morphology of the caryopses and starch grains, at least in the reviewed species, is a little different, which may suggest that the species should be considered in its own genus, *Diectomis* Kunth. Tateoka (1962) reported *Panicum*-type starch grains for a nameless species, which does not agree with the *Andropogon*-type found here. Even though *A. gerardi* (sect. *Andropogon* L.) and *A. pringlei* (probably sect. *Leptopogon* Stapf) belong to different sections, both have similar caryopses and starch grains. Only the packing of the starch grains is different, in the former they are loosely packed and, in the latter, they are moderately packed. Regarding the caryopsis shape, all the remaining species (*A. liebmannii* var. *liebmannii*, *A. selloanus*, *A. tenuispatheus*, and *A. virginicus* var. *virginicus*) belong to the section *Leptopogon* (Nagahama and Norrmann, 2012), sharing similar caryopses and morphology of the starch grains. From these four species, Reichert (1913) reported *A. virginicus* var. *virginicus* to have *Andropogon*-type starch grains, like those found here. Of these four species, three belong to the *A. virginicus* complex (*A. liebmannii*, *A. tenuispatheus*, and *A. virginicus* var. *virginicus*) according to Campbell (1983); *A. liebmannii* var. *liebmannii* and *A. selloanus*, a species putatively related to that complex appeared in a well-supported group of the section *Leptopogon* in the phylogenetic analysis by Welker et al. (2020). This assumption is also supported by the size and shape of the caryopses, as well as embryo and hilum length; however, they differ in the color of the pericarp and the shape of the hila. Starch grains are similar in *A. liebmanni* var. *liebmanni*, *A. selloanus*, and *A. virginicus* var. *virginicus*, in which the simple starch grains are more common than the compound ones. The opposite occurs in

A. tenuispatheus, where the compound starch grains are the most common compared to the simple ones, a condition also found in *Gymnopogon spicatus* (Spreng.) Kuntze, *Digitaria ciliaris* (Retz.) Koeler (previously *D. adscendens* (Kunth) Henrard) and other taxa mentioned by Tateoka (1962).

Tateoka (1962) only concluded that *Arthraxon* had *Panicum*-type and *Andropogon*-type without naming the species. *Arthraxon hispidus* is very similar to the species of *Andropogon* reviewed here having the *Andropogon*-type.

Finally, *Hyparrhenia* is in the same situation of *Andropogon*; its monophyly is not clear yet. However, the *Panicum*-type description given by Reichert (1913) is like in this study: the simple grains of *H. rufa* are large, circular with flattened facets due to pressure, in the range size of 9-18(-20) µm diameter, a range a little smaller than the 15-25 µm diameter reported by Reichert (1913) for several species of *Hyparrhenia.*

Conclusions

It is confirmed so far that all the reviewed *Andropogon* species have only one type of endosperm starch grain morphology, the *Andropogon*-type. However, because the species names studied earlier remain nameless, the *Panicum*-type and *Festuca*-type are still considered for the genus. *Andropogon fastigiatus* or *Diectomis fastigiata* (Sw.) P. Beauv. and *Arthraxon hispidus* have *Andropogon*-type and *Hyparrhenia rufa* has *Panicum*-type starch grains.

Author contributions

JGSK conceived and designed the study, reviewed the collections, realized the anatomical study, edited the photographs, wrote, and edited the manuscript after the revision.

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