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Updates to the Hawaiian grass flora and selected keys to species: Part 3

Kevin Faccenda, Mitsuko Yorkston, and Clifford W. Morden
School of Life Sciences, University of Hawai'i at Mānoa, 3190 Maile Way, St. John 101, Honolulu, Hawai'i 96822, USA; emails: faccenda@hawaii.edu, mitsuko@hawaii.edu, cmorden@hawaii.edu

During the course of a complete taxonomic revision of the Hawaiian introduced grass flora (see Faccenda 2022, 2023), several taxa were found that were difficult to identify morphologically. Genetic methods were therefore sought to identify these plants. Although efforts were principally focused on members of the *Sporobolus indicus* complex, other groups were also added as this work progressed. DNA barcoding was used as the genetic technique to identify these species. All material cited below was identified by the first author.

SEQUENCING

The internal transcribed spacer (ITS) is a non-coding DNA region between the small and large ribosomal subunits in the nucleus and is a widely used barcoding region for plants (Cheng *et al.* 2016). At least one sample of each species in this study had the ITS region sequenced. The rpl32-trnL chloroplast region was also used for some samples, but was of comparatively little value and is only discussed for certain samples. These regions were chosen due to the abundance of reference sequences in databases such as the NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and they are generally variable between closely related species.

Plant material was largely collected in the field and immediately dried in silica gel, but some material was destructively sampled from herbarium specimens at BISH. Herbarium specimens were made for almost all genetic samples collected in the field and are stored at BISH. All other vouchers are also stored at BISH, unless otherwise indicated.

Fresh material, silica-dried leaves, or fragments from pressed specimens were used for DNA extractions using the CTAB method (Doyle & Doyle 1987), with some modifications (Morden *et al.* 1996). The concentration and quality of DNA were determined using a NanoDrop Spectrophotometer (ND-1000, v 3.6.0, Thermo Scientific). Extra DNA materials were deposited into the Hawai'i Plant DNA library (HPDL) (Morden *et al.* 1996; Randell & Morden 1999).

The primers ITS5a and ITS4 were used to sequence the ITS region and trnl(UAG) and rpL32-F were used for rpl32-trnL region based on sequences reported in Peterson *et al.* (2010). PCR amplifications were carried out with GoTaq G2 Colorless Master Mix (Promega, Madison, WI, USA). The successful PCR products were cleaned using Exo-Sap-It (Affymetrix, Santa Clara, CA, USA). Sanger sequencing of the PCR products was performed by Azenta (Chelmsford, MA) or at the University of Hawai'i ASGPB sequencing facility with both forward and reverse amplification primers. The raw sequences were aligned using Geneious Prime 2022.0.1 (https:www.geneious.com) utilizing the Geneious aligner with default parameters. Final adjustments were done by visual inspection. Most sequences have been submitted to NCBI GenBank (Table 1).

Table 1. **Material sampled for this analysis, HPDL#, and GenBank accession number**. Several *Cynodon* specimens could not have their sequences submitted to GenBank, as the sequences showed multiple peaks at most sites. See comments under *C. nlemfuensis*.

Species	Location	Year	Specimen	HPDL#	GenBank Acc.
Andropogon bicornis	Hawai‘i, Volcano	2022	*K. Faccenda 2622*	12957	ITS: OR056243
Bothriochloa pertusa	O‘ahu, Mānoa	2022	none	12986	ITS: OR056271
Chloris pycnothrix	O‘ahu, Wahiawā	2023	*K. Faccenda 3045*	13002	ITS: OR056278 rpl32-trnL: OR129967
Cynodon aethiopicus	Maui, Kula	1982	*R. Hobdy s.n.* (BISH 460060)	13010	ITS: OR056282
Cynodon aethiopicus × C. nlemfuensis	Maui, Nāpili	1999	*H. Oppenheimer H49919*	13009	ITS: not submitted
Cynodon aethiopicus × C. nlemfuensis	Kaho‘olawe, summit	1984	*R. Hobdy 1964*	13011	ITS: not submitted
Cynodon aethiopicus × C. nlemfuensis	O‘ahu, Kualoa	2023	*K. Faccenda 3057*	13018	ITS: not submitted rpl32-trnL: OR129970
Cynodon nlemfuensis	O‘ahu, Pearl Harbor	2005	*L. Crago 2005-068*	13012	ITS: OR056283
Cynodon nlemfuensis	Maui, Waihe‘e	2004	*C. Imada 2004-46*	13013	ITS: not submitted rpl32-trnL: OR129968
Cynodon nlemfuensis	Maui, Wailuku	2001	*H. Oppenheimer H120103*	13014	ITS: OR056284
Cynodon nlemfuensis	O‘ahu, Honolulu	2023	*K. Faccenda 3073*	13015	ITS: OR056285 rpl32-trnL: OR129969
Cynodon nlemfuensis	O‘ahu, One‘ula	2023	*M. Ross 1919*	13020	ITS: not submitted
Cynodon nlemfuensis	O‘ahu, Kaimana	2023	*M. Ross 1916*	13021	ITS: OR056287
Cynodon nlemfuensis	O‘ahu, Airport	2023	*M. Ross 1912*	13022	ITS: OR056288
Digitaria velutina	Maui, Kula	2022	*K. Faccenda 2804*	12985	ITS: OR056270
Echinochloa crus-galli	Hawai‘i, Hilo	2022	*K. Faccenda 2336*	12960	ITS: OR056246
Echinochloa crus-galli	Kaua‘i, Hanalei	2022	*K. Faccenda 2435*	12964	ITS: OR056250
Echinochloa crus-galli	Maui, Keālia	1995	*C. Imada 98-25*	12970	ITS: OR056256
Echinochloa crus-galli	O‘ahu, Wailua	1992	*C. Imada 92-35*	12973	ITS: OR056257
Echinochloa crus-galli	O‘ahu, Bellows AFB	1984	*C. Morden 1171* (HAW)	196	ITS: OR056277
Eriochloa procera	O‘ahu, ‘Āhuimanu	2022	*K. Faccenda s.n.*	12956	ITS: OR056242
Eriochloa procera	Kaua‘i, Kalāheo	2022	*K. Faccenda 2471*	12968	ITS: OR056254
Eriochloa procera	O‘ahu, Queen’s Beach	1985	*C. Morden 1273* (HAW)	336	ITS: OR056280
Festuca rubra	Hawai‘i, Volcano	2022	*K. Faccenda 2621*	12958	ITS: OR056244
Festuca rubra	Hawai‘i, Volcano	2022	*K. Faccenda 2657*	12959	ITS: OR056245
Festuca rubra	Hawai‘i, Volcano	2022	none	12962	ITS: OR056248
Polypogon fugax	O‘ahu, Ka‘ala	2022	*K. Faccenda 2206*	12966	ITS: OR056252
Schizachyrium microstachyum	Hawai‘i, Kahuku	2022	*K. Faccenda 2607*	12961	ITS: OR056247
Sporobolus africanus	Hawai‘i, Pu‘u Huluhulu	2022	*K. Faccenda 2305*	12941	ITS: OR056227 rpl32-trnL: OR129952
Sporobolus africanus	Hawai‘i, Waimea	2022	*K. Faccenda 2308*	12942	ITS: OR056228 rpl32-trnL: OR129953
Sporobolus africanus	O‘ahu, Hawai‘i Loa Ridge	2022	*K. Faccenda 2691*	12943	ITS: OR056229 rpl32-trnL: OR129954
Sporobolus africanus	Hawai‘i, Kahuku	2022	*K. Faccenda 2611*	12947	ITS: OR056233 rpl32-trnL: OR129958

Table 1. (continued)

Species	Location	Year	Specimen	HPDL#	GenBank Acc.
Sporobolus africanus	Hawai‘i, Hualālai	2022	*K. Faccenda 2280*	12952	ITS: OR056238 rpl32-trnL: OR129963
Sporobolus africanus	Hawai‘i, Nā‘ālehu	2022	*K. Faccenda 2269*	12954	ITS: OR056240 rpl32-trnL: OR129965
Sporobolus africanus	Kaho‘olawe, Lua Makika	1990	*S. Warren KAH-90-6*	12974	ITS: OR056258
Sporobolus africanus	Hawai‘i, Pu‘u Wa‘awa‘a	1989	*W. Takeuchi 5755*	12976	ITS: OR056260
Sporobolus africanus	Hawai‘i, Pōhakuloa	1985	*C. Morden 1317* (HAW)	410	ITS: OR056275
Sporobolus diandrus	O‘ahu, Mānoa	2022	*K. Faccenda s.n.*	12990	ITS: OR056274
Sporobolus domingensis	Midway, Sand Island	2022	*F. Starr 22-062701*	12979	ITS: OR056263
Sporobolus elongatus	O‘ahu, Waimānalo	2022	*K. Faccenda 2693*	12944	ITS: OR056230 rpl32-trnL: OR129955
Sporobolus elongatus	O‘ahu, base of Wa‘ahila Ridge	2022	*K. Faccenda 2692*	12945	ITS: OR056231 rpl32-trnL: OR129956
Sporobolus elongatus	O‘ahu, Windward CC	2022	*K. Faccenda 2697*	12946	ITS: OR056232 rpl32-trnL: OR129957
Sporobolus elongatus	O‘ahu, ‘Ōhikilolo	2022	*K. Faccenda 2849*	12955	ITS: OR056241
Sporobolus elongatus	Kaua‘i, Lī‘hu‘e	2022	*K. Faccenda 2420*	12969	ITS: OR056255
Sporobolus elongatus	O‘ahu, Ka‘ō‘io Point	1966	*D. Herbst 100*	12978	ITS: OR056262
Sporobolus elongatus	Maui, Lāhainā	2022	*K. Faccenda 2734*	12991	ITS: OR056265
Sporobolus elongatus	Maui, Pā‘ia	2022	*K. Faccenda 2729*	12983	ITS: OR056268
Sporobolus elongatus	O‘ahu, Mānoa	2022	*K. Faccenda 2850*	12984	ITS: OR056269
Sporobolus elongatus	O‘ahu, Mānoa	2022	*K. Faccenda s.n.*	12989	ITS: OR056273
Sporobolus elongatus	O‘ahu, Bellows AFB	1984	*C. Morden 1167* (HAW)	192	ITS: OR056276
Sporobolus fertilis	O‘ahu, He‘eia	2022	*K. Faccenda 2701*	12951	ITS: OR056237 rpl32-trnL: OR129962
Sporobolus fertilis	Hawai‘i, Hilo	2022	*K. Faccenda 2340*	12953	ITS: OR056239 rpl32-trnL: OR129964
Sporobolus fertilis	O‘ahu, Wailupe Valley	2022	*K. Faccenda 2101*	12963	ITS: OR056249
Sporobolus fertilis	Kaua‘i, Kalāheo	2022	*K. Faccenda 2450*	12965	ITS: OR056251
Sporobolus fertilis	Hawai‘i, Kahuku	2022	*K. Faccenda 2603*	12967	ITS: OR056253
Sporobolus fertilis	Maui, Lāhainā	2002	*H. Oppenheimer H10205*	12975	ITS: OR056259 rpl32-trnL: OR129966
Sporobolus fertilis	Maui, Halepua‘a	2005	*F. Starr 050405-27*	12977	ITS: OR056261
Sporobolus fertilis	Maui, Kīpahulu	2022	*K. Faccenda 2746*	12980	ITS: OR056264
Sporobolus fertilis	Maui, Pā‘ia	2022	*K. Faccenda 2778*	12981	ITS: OR056266
Sporobolus fertilis	Maui, Hāna	2022	*K. Faccenda 2758*	12982	ITS: OR056267
Sporobolus indicus	Kaua‘i, Hanalei	2022	*K. Faccenda 2438.5*	12948	ITS: OR056234 rpl32-trnL: OR129959
Sporobolus indicus	Kaua‘i, Waimea Canyon	2022	*K. Faccenda 2469*	12949	ITS: OR056235 rpl32-trnL: OR129960
Sporobolus indicus	Kaua‘i, Kapa‘a	2022	*K. Faccenda 2403*	12950	ITS: OR056236 rpl32-trnL: OR129961
Urochloa glumaris	O‘ahu, Mānoa	2022	none	12988	ITS: OR056272

IDENTIFICATION OF PLANTS BASED ON GENETIC DATA

For all other genera outside of *Sporobolus*, NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to assign a name to the sequence by finding the most similar sequence in the NCBI reference library, based on the shared percentage of the bases. *Urochloa glumaris* and *Schizachyrium microstachyum* were also sequenced, but lack suitable reference specimens on GenBank to compare to.

For the *Sporobolus indicus* complex, a phylogeny was created based on ITS sequences using the data from Peterson *et al.* (2014) as well-identified references. The sequences were aligned with MUSCLE (Edgar 2004), and RAxML was used to create the phylogeny with default parameters (Stamatakis 2014). Five clades of Hawaiian species belonging to the *Sporobolus indicus* complex were found (Figure 1). This phylogeny ended up being more useful for determining how many species occurred in Hawai‘i than placing a name on them. *Sporobolus elongatus* lacked a reference sequence, and *S. indicus* and *S. africanus* were placed closest to species that they are morphologically dissimilar to. Once the different clades were established, morphological identification was used to place a name on each clade using the keys in Simon & Jacobs (1999) and Clayton (1965). After seed characters were found to reliably separate the genotyped specimens, all *Sporobolus* material at BISH was critically examined, much of which was misidentified. In total, 49 of the 140 (35%) specimens belonging to the *Sporobolus indicus* complex at BISH were misidentified. Most *S. africanus* vouchers were correctly identified, but nearly all material previously identified as *S. indicus* was incorrectly identified and was actually *S. elongatus* or *S. fertilis*.

Bothriochloa pertusa (L.) A. Camus **Note**

Bothriochloa pertusa was published as occurring in Hawai‘i by O’Connor (1990). The ITS region was sequenced and found to be 564/566 base match to GenBank accession DQ005028.1, supporting its current identity.

Chloris pycnothrix Trin. **Note**

Chloris pycnothrix was published as occurring on O‘ahu by Faccenda (2023). It was also sequenced and found to be a 659/660 base match to GenBank accession KP873271.1, supporting its initial identification.

Cynodon aethiopicus Clayton & J.R. Harlan
× ***C. nlemfuensis*** Vanderyst **New state record**

Upon sequencing several *Cynodon* specimens with affinities to both *C. aethiopicus* and *C. nlemfuensis*, the ITS sequence was found to be entirely unusable due to multiple peaks at each base, up until around 630 bases, where the sequence suddenly becomes high quality. It was soon realized that at the location, there is a deletion in the *C. aethiopicus* ITS region, and the pattern of peaks in the messy region is entirely predicted by the ITS sequences of *C. aethiopicus* and *C. nlemfuensis* (data not shown), giving strong evidence that the sample was a hybrid. Hybridization between these species is easily induced under experimental conditions (De Wet & Harlan 1970), and it is most likely that these hybrid populations are of artificial rather than natural origin.

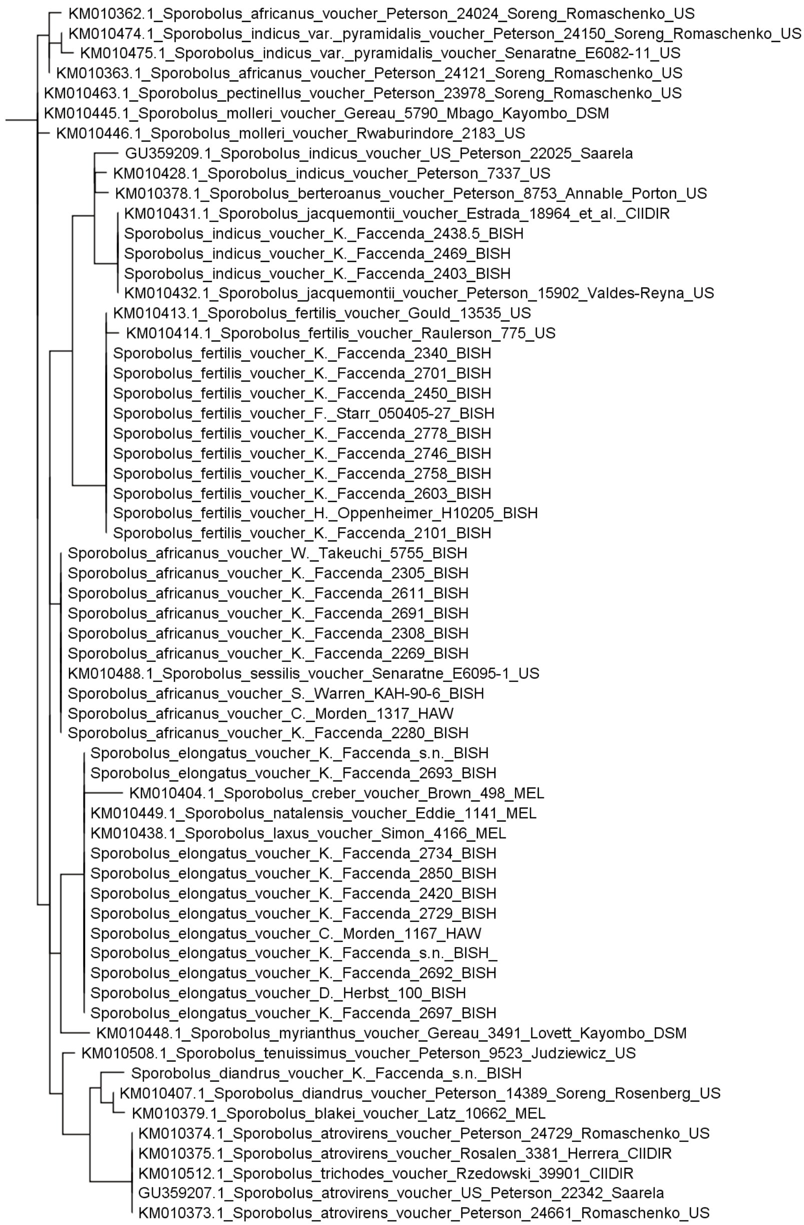

Figure 1. Phylogeny of the *Sporobolus indicus* complex based on only the ITS region. All nodes have low bootstrap support due to low sequence polymorphism among this species complex. Sequences from Peterson *et al*. (2014) are prefixed with their GenBank accession numbers. The scientific names displayed for the species sampled in this study are the final names after morphological identification.

One specimen was cultivated at Kualoa Ranch on Oʻahu, and another was from Maui, which states it was also cultivated and referred to as "Puerto Rican Star Grass." Naturalized specimens have been collected from Maui and Kahoʻolawe; it is unclear if this hybrid can make viable seed or if these populations have spread purely via vegetative means.

Material examined. **OʻAHU:** Kualoa Ranch, pasture immediately behind ranch headquarters, open, rather dry pasture, strongly stoloniferous grass (but not checked for rhizomes) forming a monoculture over at least 100 square meters, quite robust, most inflorescences with one whorl of branches but some rarely with two, very likely planted and given that it has not spread out of the pasture it should not be considered naturalized, 20 m, 21.520237, -157.839328, 07 Mar 2023, *K. Faccenda & J. Lee 3057*. **MAUI:** West Maui, Lāhainā Distr, Moʻomoku, growing on dirt road between pineapple fields at gully bottom, spreading vegetatively by stolons, known to be planted elsewhere on Honolua plantation lands, but not at this locality, 1,200 ft [365 m], 20 Nov 2002, *H. Oppenheimer H110212*; Lāhainā Distr, Nāpili, cultivated to cover areas of bare, exposed soil, mat-forming, 06 Apr 1999, *H. Oppenheimer H49919*. **KAHOʻOLAWE:** Near summit in diversion ditch by LZ-1, long creeping rhizomes, 16 Feb 1984, *R. Hobdy 1964*.

Cynodon nlemfuensis Vanderyst **New island record; Note**

Several populations of *C. nlemfuensis* from across the islands were sequenced (Table 1) and were found to closely match *C. nlemfuensis* sequences in GenBank, e.g. for *K. Faccenda 3073* the rpl32-trnL region is a 683/683(100%) match to KP873541.1 and the ITS region is a 626/628(99%) to KP873323.1.

In the African literature, where these species are native, *C. nlemfuensis* is described as having only stolons and no rhizomes (Clayton & Harlan 1970; Clayton & Renvoize 1982). However, underground rhizomes have been observed in several populations of *C. nlemfuensis* on Oʻahu, including the one documented by *K. Faccenda 3073* that genetically matches *C. nlemfuensis*.

Two populations of plants that morphologically match *C. nlemfuensis* were sequenced and the ITS region shows two peaks at almost all sites, indicating that it has multiple copies of ITS, one of which has an indel relative to the others (data not shown). The rpl32-trnL region of these plants were a close match for *C. nlemfuensis*. It is unclear if these plants are polyploids or hybrids with *C. dactylon*, but it seems likely to be a hybrid with *C. dactylon*, as the species hybridize very easily (De Wet & Harlan 1970). No sequences in GenBank have an indel at the correct location to predict where the other ITS sequence came from and would allow for the determination of the other parent.

Cynodon nlemfuensis is now known to be naturalized on Kauaʻi at Kekaha and Waiakea, based on collections from the 1970s, and Lānaʻi at Kaʻā and Kamoku, where it was collected over 100 years ago as well as by the author in 2023. Based on these specimens, it is also likely that the "Giant bermuda" introduced to other islands at the same time was also *C. nlemfuensis*. As *C. nlemfuensis* was only described in 1922, the correct scientific name could not have been applied at that time.

Material examined. **KAUAʻI:** Waiakea Golf Course, manmade dune, 23 Sep 1977 *C. Corn s.n* (PTBG 068240); Waimea Distr, along road from Kekaha to Kōkeʻe State Park, 25 ft, *D. Herbst & G. Spence 5564* (HAW). **LĀNAʻI:** Kaʻā, introduced to the island in 1914, [no date], *G.C. Munro sn.* (BISH 118482); Kamoku, giant bermuda, Dec 1914, *G.C. Munro 406*; Airport Rd (440) about 3 km makai of Lānaʻi City, roadside, sunny, mowed area, from exposed hardpan soil where no other plants were growing, 414 m, 20.800175, -156.949977, 19 Jun 2023, *K. Faccenda 3125*.

Key to *Cynodon* in Hawai‘i

This key is provisional and is bound to fail with certain plants despite being largely based on the key in Barkworth *et al.* (2003). The genus *Cynodon* is in need of a modern revision and some Hawaiian populations seem to introgress, especially those with affinities to *C. nlemfuensis*. It is very likely that a *C. dactylon* × *C. nlemfuensis* hybrid occurs here, in addition to the *C. aethiopicus* × *C. nlemfuensis* hybrid that certainly occurs here.

1. Plants 5–40 cm tall; inflorescence with 3–6 branches always in a single whorl .... *C. dactylon*

1′. Plants 20–100 cm tall; inflorescence with 5–20 branches in one or more whorls

2. Inflorescences often with >1 whorl; lemma keel glabrous or minutely hairy; plants stiff and woody; inflorescence usually red to purple .... *C. aethiopicus*

2′. Inflorescence typically with 1 whorl, sometimes with 2 in hybrid populations; lemma keel densely hairy; plants softer; inflorescence typically green but sometimes with red coloration

3. Plants with consistently one whorled inflorescence; found in coastal through montane sites .... *C. nlemfuensis*

3′. Plants with at least some inflorescences with multiple whorls (examine large sample, at least 40 flowers); not currently known from coastal sites .... *C. aethiopicus* × *C. nlemfuensis*

Digitaria velutina (Forssk.) P. Beauv. **Note**

Digitaria velutina was published as occurring on Maui by Faccenda (2023). It was also sequenced and found to be a 663/669 base match to GenBank accession HM347010.

Echinochloa crus-pavonis (Kunth) Schult. **Correction**

Echinochloa crus-pavonis was first published as occurring in Hawai‘i by the Smithsonian *Flora of the Hawaiian Islands* online checklist by Wagner *et al.* (2012) and that record was then incorporated into the Imada (2019) checklist. This record was based on many specimens filed as *E. crus-pavonis* in the Smithsonian (US) herbarium; however, this species does not occur in the BISH herbarium, as all specimens formerly annotated as *E. crus-pavonis* were annotated as *E. crus-galli* by W.D. Clayton in the work leading to the publication of Herbst & Clayton (1998). Attempts to contact the botanists who annotated the *E. crus-pavonis* material at US did not reveal why that name was applied to Hawaiian material.

Duplicate specimens identified as *E. crus-pavonis* at US were closely examined at BISH and all were determined to be *E. crus-galli*, based on their morphology. Examination of the photographed specimens of *E. crus-pavonis* also showed them to most closely match *E. crus-galli*. Furthermore, when DNA was extracted from two BISH duplicates of sheets annotated as *E. crus-pavonis* at US (*Imada 98-25, Imada 92-35*), the ITS region was sequenced and searched via BLAST only to find that they were close matches to other specimens of *E. crus-galli* in the GenBank database. Three other recently collected specimens (*Faccenda 2336, Faccenda 2435, Morden 1171,*HAW) were also similarly sequenced and also found to be most similar to *E. crus-galli* via BLAST. We therefore conclude that the US specimens are misidentified and that *Echinochloa crus-pavonis* does not occur on any of the islands of Hawai‘i.

The key to *Echinochloa* in *Flora of North America* (Barkworth *et al.* 2003) does not work on Hawaiian material for distinguishing *Echinochloa crus-galli*. On no specimens of *E. crus-galli* examined was a line of minute hairs noticed at the tip of the fertile lemma. It is also worth noting that while researching this species, many taxonomic treatments of *Echinochloa* were examined, some of which used entirely different characters for their circumscription of *E. crus-pavonis*. Among the most markedly so were Clayton & Renvoize (1982) and Wu *et al.* (2006), which both state that *E. crus-pavonis* has long awns and compound panicle branches, whereas nearly all other treatments define the species by its short or absent awns (Gould *et al.* 1972). The characters that appear to be applied more consistently than others are the panicle nodding and spikelets with short awns (Gould *et al.* 1972; Michael 1983; Weakley 2020), which is how we circumscribed the species.

Eriochloa procera (Retz.) C.E. Hubb. **New island records**

Specimens of *Eriochloa* from across the islands have been critically examined and almost all wild specimens published as *Eriochloa punctata* were found to be misidentified and are a better match for *E. procera*. The two species differ in duration and length of the florets; see the key below. *Eriochloa procera* was previously reported as naturalized on Molokaʻi (Oppenheimer 2008), Midway (Snow & Lau 2010), and Oʻahu (Imada & Kennedy 2020), and is now also known from Niʻihau, Kauaʻi, and Maui.

Several specimens of *Eriochloa* morphologically resemble *Eriochloa barbatus* (syn. *E. fatmensis*), as they both have an aristate spikelet. However, when one of these samples (*Morden 1273*, HAW) was sequenced, it was a close match to *E. procera* (654/660 base match to GenBank# MH768193.1) and quite distant to any *E. barbatus* accession. Two other specimens of *E. procera* were also sequenced (Table 1) that did not have aristate spikelets, and these were also most similar to GenBank# MH768193.1. *Eriochloa procera* and *E. barbatus* are very closely related, differing only in whether the spikelet is aristate or acute at the apex (Shaw & Webster 1987). Launert & Pope (1989) report that these species also intergrade in southern Africa. Specimens from Hawaiʻi also vary continuously between acute, short-acuminate, and long-acuminate florets. This continuous variation and lack of genetic differentiation between the extreme forms does not warrant recognition of *E. barbatus* in Hawaiʻi, and as such, all material has been identified as *E. procera*. Further taxonomic work is needed to determine if these species are conspecific globally.

Material examined. **NIʻIHAU:** Old Makanikahau Reservoir, on dried muddy bed of apana, 400 ft [122 m], 01 Apr 1949, *H. St. John 23634*. **KAUAʻI:** Intersection of Lauoho Rd and Rt 50, shady, roadside, moist, with other weeds, rare, one plant seen, 142 m, 21.922714, -159.515804, 30 May 2022, *K. Faccenda 2427*. **MAUI:** Lāhainā, Kahana, neglected, irrigated area, 20.966688, -156.680489, 13 Nov 2005, *H.L. Oppenheimer H110504*.

Eriochloa punctata (L.) Ham. **Correction; New island record**

Eriochloa punctata has previously been published as occurring on, Niʻihau, Oʻahu, Molokaʻi, and Maui (Imada 2019). However, all these reports were based on specimens that were misidentified and were truly *Eriochloa procera*; see further comments above. One specimen from 1961 on Kauaʻi documents the naturalization of this species. *Eriochloa punctata* was intentionally imported by the Hawaii Agricultural Experiment Station (*Lyman s.n.*, BISH 129219), most likely for trial as a forage grass.

Material examined. **KAUA‘I:** Kekaha, rare in swampy place, May 1961, *B. Kumbe s.n.* (BISH 785875).

KEY TO *ERIOCHLOA* IN HAWAI‘I

1. Perennial (but sometimes flowering first year); florets 4.2–5.7 mm long .... *E. punctata*

1′. Annual or short-lived perennial; florets 2.7–5 mm long

2. Florets 2.7–3.6 mm long (sometimes with an acuminate tip reaching to 4.6 mm); axis of inflorescence usually glabrous; leaves usually <4 mm wide .... *E. procera*

2′. Florets 3.8–5.0 mm long; axis of inflorescence usually pubescent; leaves usually >5 mm wide .... *E. acuminata* var. *acuminata*

Figure 2. Spikelets of *Eriochloa* from Hawai‘i. **A,** *E. procera*, acute floret form (*H. Oppenheimer H71019*). **B,** *E. procera*, acuminate form (*P. O’Connor s.n.,* BISH 510049). **C,** *E. acuminata* var. *acuminata* (*H. Oppenheimer H59002*). **D,** *E. punctata* (*R. Lyman s.n.,* BISH 447489). All photographs taken at BISH under 20× magnification.

Festuca rubra L. **Note**

Festuca rubra was published as occurring in Hawai‘i by O’Connor (1990). A specimen from Volcano, Hawai‘i, was also sequenced and found to be 98.2% match to GenBank accession OQ874980.1.

Polypogon fugax Nees ex Steud. **New island records**

Polypogon fugax was published as occurring in Hawaiʻi by Herbst & Clayton (1998) based on one specimen from the Nuʻuanu Pali on Oʻahu. This record is, however, quite problematic as many other vouchers from the same population were also annotated as *P. interruptus* by W.D. Clayton (one of them being a duplicate sheet of the specimen annotated as *P. fugax*!). Morphological identification of these species proved challenging, as they differ only by their duration and lobing of the glumes (Barkworth *et al.* 1993). The Hawaiian specimens had a glume lobe of ~1 mm, making that character equivocal (Barkworth *et al.* 1993). After examining herbarium material of both species in their native range, it was also found that duration cannot be reliably implied from herbarium specimens.

Therefore, genetic analysis was undertaken. DNA was extracted from a herbarium specimen from the Nuʻuanu Pali and one from Makaʻeha, Maui. Unfortunately, neither of these successfully amplified, as the DNA was likely too fragmented. The Nuʻuanu Pali was also visited several times and plants were also unable to be located in the field (but were later located after DNA sequencing was completed). ITS was only successfully amplified from a plant collected at the summit of Kaʻala and found to be a 642/643 base match to *P. fugax* (GenBank accession MH808886.1), compared to a 669/677 match to GenBank accession KX873141.1 (*P. interruptus*). It is therefore concluded that all plants in Hawaiʻi are actually *P. fugax*, as there is no considerable difference between the lobing of the glume from the Kaʻala plant and any of the other Hawaiian specimens formerly labeled as *P. interruptus*. Field observations of the *Polypogon* plants at the Kaʻala summit also suggest that they are annual, further supporting the *P. fugax* identification (Barkworth *et al.* 1993). *Polypogon fugax* is now known from Kure, Midway, Kauaʻi, Oʻahu, Maui, and Hawaiʻi; only the first collection from each island is reported below.

Material examined. **KURE:** Near tennis court, 08 May 1986, *R. Saito s.n.* (BISH 511549). **MIDWAY:** Sand Island, in saturated soil below dripping air conditioner, 29 Jun 1980, *D. Herbst & W. Takeuchi 6383* (US). **KAUAʻI:** Nā Pali Coast, about 25 min past Hanakāpīʻai Beach, on rocky cliff face, locally common, 07 May 1976, *J. Arakawa s.n.* (PTBG 1000036808). **OʻAHU:** Nuʻuanu Pali, 17 Jun 1916, *A.S. Hitchcock 13789*. **MAUI:** Kula, 01 Oct 1902, *J.G. Smith s.n.* (BISH 786511). **HAWAIʻI:** Kanehoha, Kona, 25 Jun 1911, *C.N. Forbes 261.H.*

Polypogon interruptus Kunth **Correction**

Polypogon interruptus is no longer known to occur in Hawaiʻi, as all material has been reidentified as *P. fugax*. See note above.

Sporobolus domingensis (Trin.) Kunth **Note**

Sporobolus domingensis was published by Faccenda (2023). It was also sequenced and found to be a match to 620/622 base match to KM010410.1.

Sporobolus elongatus R. Br. **New island records; Note**

Sporobolus elongatus is now known to be naturalized on Kauaʻi, Molokaʻi, and Maui, in addition to being previously reported on Midway, Oʻahu, Lānaʻi, and Hawaiʻi (Imada 2019; Faccenda 2022). *Sporobolus elongatus* was first collected on Oʻahu, where it was naturalized as a weed at an experiment station in 1920 (*Westgate 32*). It was intentionally imported between 1901 and 1915 as "*Sporobolus indicus*" but was stated to be from Australia, and must be *S. elongatus*, as *S. indicus* does not occur in Australia (McClelland

1915; Simon & Jacobs 1999). It quickly spread to Lāna‘i, where it was found in 1925 and was also found on Hawai‘i Island as a weed at the Kohala Ditch Trail in 1924 (*Lee 111*). It was found to be widespread on Kaua‘i, Moloka‘i, and Maui during roadside surveys and has surely been present but overlooked on these islands for many years.

Sporobolus elongatus and *S. indicus* are very similar species, and finding adequate characters to identify them was a difficult task. The inflorescence structure is very similar, although *S. elongatus* tends to have a looser structure and more flexible branches. The florets are basically identical, although they differ slightly based on seed position and seed shape (Clayton 1965). Examining the grain free of the pericarp at high magnification is the best way to reliably identify these species. The grain on *S. indicus* is 1 mm long × 0.6 mm wide and oblong with a rounded tip, and the embryo also tends to be dark on *S. indicus* (Figure 4C). The grain on *S. elongatus* is 0.7 mm × 0.5 mm with an acute base and concave to truncate tip. The embryo is the same color as the rest of the grain on *S. elongatus* (Figure 4D).

Material examined. **KAUA‘I:** Ninini Point Road running along the edge of Lī‘hu‘e Airport, roadside weed, sunny, dry area, common, 45 m, 21.971244, -159.352368, 29 May 2022, *K. Faccenda 2420*; Kalāheo, National Tropical Botanical Garden, native plant garden area, weed in infrequently mowed grass, sunny, moist area, common, 57 m, 21.904929, -159.508933, 31 May 2022, *K. Faccenda 2440* (PTBG). **MOLOKA‘I:** Maunaloa town, intersection of Maunaloa Rd and N Wai‘eli St, in mowed lawns around town, rather dry, sunny areas, uncommon in mowed lawns, <10 plants seen, 314 m, 21.131932, -157.212399, 26 Dec 2022, *K. Faccenda 2905*; Rt 450, ca. 6 km E of ‘Ualapu‘e, roadside weed in sunny, moist area, uncommon, 11 m, 21.081986, -156.784592, 29 Dec 2022, *K. Faccenda 2949* (PTBG); ‘Ualapu‘e, Wavecrest Resort, weed in irrigated, mowed grass at resort in sunny area, common here and also along roadsides, 7 m, 21.054636, -156.840225, 30 Dec 2022, *K. Faccenda 2971* (US). **MAUI:** Pā‘ia, Holomua Rd, Old Maui High School, MISC Baseyard, weed in mowed lawn, common, clump-forming, 93 m, 20.915407, -156.348011, 22 Oct 2022, *K. Faccenda 2729*; Lāhainā, Lāhainā Recreation Center off of Shaw St, mowed and irrigated grass field, weed, uncommon, 9 m, 20.867549, -156.668351, 22 Oct 2022, *K. Faccenda 2734*.

Sporobolus fertilis (Steud.) Clayton **New state record**

Sporobolus fertilis has been present in Hawai‘i since at least 1936, when it was first collected on Kaua‘i and Hawai‘i, then subsequently found on O‘ahu in 1937. It has now spread to Kaua‘i, O‘ahu, Moloka‘i, Maui, and Hawai‘i and is common and widespread on each of these islands.

Herbarium specimens of this grass were found misidentified as *S. africanus*, *S. indicus*, and *S. elongatus*. *Sporobolus fertilis* is very similar to these species, and small plants are often indistinguishable unless the grains are examined under a microscope. However, *S. fertilis* can reach much larger sizes than those species, approaching 1.5 m tall and with panicles up to 50 cm, whereas the other species rarely have panicles >30 cm long. *Sporobolus fertilis* also has straw-colored inflorescences (Figure 3), whereas the other members of the *S. indicus* complex have inflorescences that are various shades of green or gray.

Sporobolus fertilis is native from South to Southeast Asia, and is naturalized in Australia and throughout the Pacific (POWO 2023). In Australia, where it is a weed of pastures (Yobo *et al.* 2009), it is reported to be invasive (Grice *et al.* 2013). Even in its native range, it is referred to as a common weed, where it is found on roadsides, field margins, grassy places, and mountain valleys (Wu *et al.* 2006).

Figure 3. *Sporobolus fertilis* showing its distinctive straw-colored inflorescence and rather loose branches that separate from the main axis when the inflorescence is bent. Photo taken in Volcano, Hawai‘i.

The following description is taken from Wu *et al.* (2006:484):

> "Perennial. Culms densely tufted, erect, rigid, 25–100(–120) cm tall. Leaf sheaths glabrous but margin ciliolate, basal sheaths papery, lightly keeled; leaf blades linear, flat or involute, 15–50(–65) × 0.2–0.5 cm, glabrous or adaxial surface thinly pilose, tapering to a long filiform apex; ligule ca. 0.5 mm. Panicle linear, contracted to spikelike, often interrupted especially at base, 7–45 × 0.5–1.5 cm; branches 1–2.5(–5) cm, erect and appressed to main axis, or looser and narrowly ascending, densely spiculate throughout. Spikelets grayish or yellowish green, 1.7–2 mm; lower glume oblong, ca. 0.5 mm, veinless, apex truncate-erose; upper glume oblong-elliptic, 1/2–2/3 spikelet length, 1-veined, ± acute; lemma ovate, as long as spikelet, indistinctly 1(–3)-veined, acute. Anthers 3, 0.8–1 mm. Grain red-brown, obovate-elliptic, 0.9–1.2 mm, distinctly shorter than its lemma and palea, these gaping widely beyond its top, apex truncate. Fl. and fr. Mar–Dec. 2n = 36, 48, 54."

Material examined. **KAUA‘I:** Kālaheo, Pu‘u Rd about 1 km S of Pu‘u Lani Pl, in valley, partly sunny area in forest along road, 179 m, 21.911338, -159.534860, 02 Jun 2022, *K. Faccenda 2450*; Wailua Game Reserve, above Wailua, plots stripmined for bauxite in 1958–1960, 20 Oct 1985, *R.A. Howard 20191*; roadside near end of road at Hā‘ena Beach, 16 Jun 1978 *C. Corn s.n.* (BISH 667185); Kapa‘a, common on roadsides, 400 ft, 28 Oct 1936, *E.Y. Hosaka 1632*; Kalalau Trail, along first mile or so, 21 Dec 1983, *W.L. Wagner et al. 6173*. **O‘AHU:** Wailupe middle ridge, partly sunny under mixed native and invasive forest, collected from habitat 23 May, cultivated in pot for about 5 months before flowering and pressing, 21.313427, -157.753800, 28 Aug 2021, *K. Faccenda 2101*; Kamehameha Hwy outside of He‘eia State Park, weedy, partly sunny, moist area, to 80 cm tall, inflorescence loose, 2 m, 21.440399, -157.809360, 20 Sep 2022, *K. Faccenda 2701*; Wai‘alae Nui Ridge, “dry forest zone” along trail, occasional, 27 May 1937, *F.E. Egler 37-53*. **MOLOKA‘I:** Maunaloa, intersection of Rt 460 and Kalua Koi Rd, roadside weed in dry, sunny area, uncommon, forming dense clumps, 309 m, 21.147184, -157.198381, 26 Dec 2022, *K. Faccenda 2906*; Ho‘olehua, end of pavement on Rt 482 on its western end, disturbed, occasionally mowed roadside, dry, sunny, uncommon, scattered patches seen around the island, 163 m, 21.181892, -157.091685, 27 Dec 2022, *K. Faccenda & C. Daehler 2920* (US); Kalaupapa National Park, restoration site at switchback 1, 07 Oct 2004, *M.L. Wysong 385*. **MAUI:** Hāna Hwy ca. 6 km W of Wai‘ānapanapa State Park, wet, sunny roadside dominated by weeds, common along road, 197 m, 20.798314, -156.061320, 23 Oct 2022, *K. Faccenda 2758*; Pā‘ia, Lower Pā‘ia Park, dry, sunny area on edge of parking lot, compressed soil, 1 m, 20.915075, -156.384976, 24 Oct 2022, *K. Faccenda & B. Hobdy 2778*; West Maui, Lāhainā Distr, Nāpili, growing along unimproved road through gulch between pineapple fields, 700 ft, 20° 58’ 47” -156° 38; 38”, 08 Jan 2002, *H. Oppenheimer H10205* (PTBG); Palikea Stream, Haleakalā National Park, Kīpahulu Valley, 29 Apr 1977, *P.K. Higashino 5779*; Hāna Distr, Ka Iwi o Pele, secondary forest, steep NE slope, common with *Panicum* in *Casuarina* grove, 40–440 ft., 13 Nov 1987, *T. Flynn 2624*. **HAWAI‘I:** Hilo, Lili‘uokalani Gardens, weed under shade trees, moist area, common, 1 m, 19.727753, -155.067817, 06 Mar 2022, *K. Faccenda 2340*; Kahuku Unit of Hawai‘i Volcanoes National Park, near main parking lot and toilet, one plant seen, killed, 654 m, 19.064500, -155.678610, 09 Aug 2022, *K. Faccenda with HAVO I&M Vegetation Crew 2603*; Hawai‘i Volcanoes National Park, outside of Visitor Center, near trailhead for Sulfur Banks Trail, shady area on edge of forest, one plant seen, killed, 1214 m, 19.430718, -155.259337, 12 Aug 2022, *K. Faccenda & J. Gross 2613*; Hilina Pali, above cliff, common in trampled parking lot area, 25 Mar 1984, *F.R. Fosberg 64301*; Hāwī, Kohala, weed along roadside in moist places, 500 ft [152 m], 19 Sep 1936, *E.Y. Hosaka 1631*.

Sporobolus indicus (L.) R. Br. **Corrections; New island record**

Sporobolus indicus was previously published as occurring on Lāna‘i, Maui, and Hawai‘i by O’Connor (1990); however, no specimens could be found from Lāna‘i to support this record, nor could any be found in the field. Also, all Maui specimens previously filed as *S. indicus* have been redetermined as *S. fertilis* and all Hawai‘i Island specimens have been reidentified as *S. elongatus*. During fieldwork on Moloka‘i, the first record of *S. indicus* was found for that island. *Sporobolus indicus* is now only known from Midway, Kaua‘i, O‘ahu, and Moloka‘i (Imada 2019).

Sporobolus indicus was likely accidentally introduced as a seed contaminant as the first specimen (*Anon s.n.* BISH 591291) was made from the Pensacola Experiment Station and was described as a “Volunteer with *Brachiaria ciliatissima*”. This specimen was not dated, but examining the HAES accession inventory (HAES n.d.), *B. ciliatissima* was only imported in 1938 making the accidental introduction of *S. indicus* most likely 1938. Three live plants of *S. indicus* were also imported in 1912 from Louisiana (HAES n.d), but given that no specimens were made until later, it is likely they did not naturalize.

Material examined. **MOLOKA‘I:** Ho‘olehua, 100 m SE of intersection of Rt 482 and Ala ‘Ēlua St, partly shaded, moist roadside, uncommon on the island, small colony of <10 plants at this spot, 239 m, 21.169220, -157.052205, 27 Dec 2022, *K. Faccenda & C. Daehler 2928*.

Key to *Sporobolus* in Hawai‘i

Morphological identification of plants in the *S. indicus* complex (*S. africanus*, *S. diandrus*, *S. elongatus*, *S. fertilis*, and *S. indicus*) is notoriously difficult and, in the author's opinion, mostly impossible in the field. If species level ID is required, a microscope is usually required to examine the seeds. The seed characters listed in this key are for the seed after it has been freed from the pericarp (the wrinkly, greenish or yellowish coating around the seed). If no seeds naturally freed from their pericarp are available on the specimen, a fruit can be dissected from its lemma, soaked in water for 5 minutes, then gently separated from the pericarp with a needle. Note that fresh material must be dried before the soaking technique works to remove the pericarp. Identification characteristics for these species have been largely adapted from the excellent *Sporobolus* treatment by Simon & Jacobs (1999), with minor influence from the treatments by Clayton (1965), Baaijens & Veldkamp (1991), and Barkworth *et al.* (2003).

1. Plant annual, rarely surpassing 30 cm tall
   2. Lower inflorescence branches not whorled; often associated with horticulture or moist disturbed areas .... *S. tenuissimus*
   2′. Lower inflorescence branches whorled; often associated with dry saline or calcareous conditions
      3. Leaves often with papillose-based hairs and conspicuously spiny margins; lemmas minutely scabrous; panicle usually open even when immature; panicle with secondary branches usually divergent from the main axis .... *S. coromandelianus*
      3′. Leaves without papillose-based hairs, margins scabrous; lemmas smooth; panicle spikelike when immature; panicle with secondary branches appressed .... *S. pyramidatus* (in part)

1′. Plant perennial, 20–150 cm tall
   4. Upper glume >⅔ as long as floret; only found in coastal areas
      5. Rhizomatous; blades distichous [native] .... *S. virginicus*
      5′. Not rhizomatous; blades not conspicuously distichous
         6. Lower inflorescence node whorled .... *S. pyramidatus* (in part)
         6′. Lower inflorescence node not whorled .... *S. domingensis*
   4′. Upper glume <⅔ as long as floret; uncommonly found in coastal areas, if in coastal area, in areas with low soil salinity (*S. indicus* species complex)
      7. Panicle racemelike, secondary branches strongly divergent from main axis at >45° angle
         8. Secondary inflorescence branches lacking florets on lower 1/6–1/4, these branches 1–4 (rarely up to 10) cm long; spikelets 1.2–1.6 mm long ... *S. diandrus*
         8′. Secondary inflorescence branches with florets to the base, these branches <2 cm long; spikelets 1.6–2.0 mm long .... *S. fertilis* (in part)
      7′. Panicle spikelike, secondary branches ascending and loosely to tightly contracted to main axis, if divergent from main axis only up to <30° angle
         9. Spikelets 2.0–2.5 mm long; spike densely contracted; spike 6–20 cm long; inflorescence usually gray .... *S. africanus*
         9′. Spikelets <2 mm long; spike densely or loosely contracted; spike 13–50 cm long; inflorescence various shades of green, gray, or straw-colored

10. Seed blunt, bearing no sharp edges, generally 0.4–0.5 mm thick, minutely rugose in texture (Figure 4A); mature inflorescence straw-colored; inflorescence 15–50 cm long; plants 50–150 cm tall .... *S. fertilis* (in part)
10′. Seed with or without sharp edges, generally ≤0.3 mm thick, smooth or rugose in texture; mature inflorescence generally green or greenish gray (the only straw-colored ones observed were old sun-bleached ones); inflorescence 13–30 cm long (may be up to 35 cm in robust *S. elongatus*); plants <100 cm tall

11. Seed with a blunt and convexly rounded apex (Figure 4C); grain of mature florets held such that it appears to be 80–90% as long as the lemmas; stamens always 3 .... *S. indicus*
11′. Seed with a truncate apex with sharp edges, often concave distally (Figure 4D); grain of mature florets held lower, appearing closer to 60% as long as the lemmas; stamens usually 2 but can infrequently be 3 .... *S. elongatus*

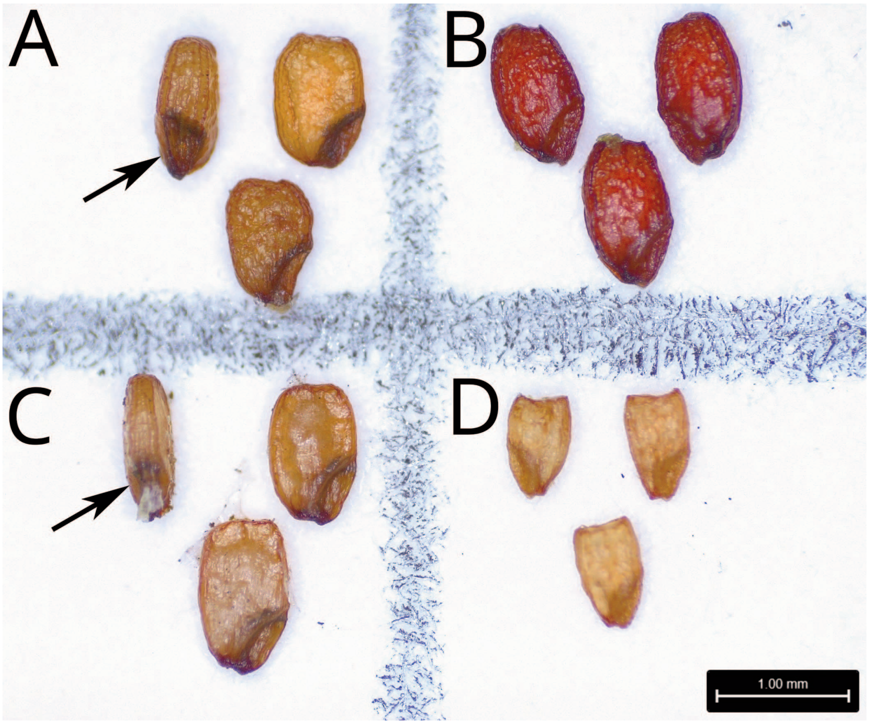

Figure 4. Grains of selected members of the *Sporobolus indicus* complex; all three grains of each species came from the same plant. **A,** *S. fertilis*, note that the grain indicated with the arrow is sitting on its dorsal face. **B,** *S. africanus*, note that the color of the grain is often lighter than these photographed. **C,** *S. indicus*, note that the grain indicated with the arrow is sitting on its dorsal face. **D,** *S. elongatus*.

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