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## Spontaneous hybridization among invasive Poaceae in Hawai‘i: *Chloris* × *pseudosagrana nothosp. nov.* and *Cenchrus* × *peregrinus nothosp. nov.*

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### Abstract

During grass surveys across the islands of Hawai‘i in 2022, two novel hybrids among non-native Poaceae were found growing on roadsides. Spontaneous hybridization was observed among *Chloris barbata* and *C. divaricata* on O‘ahu and among *Cenchrus clandestinus* and *C. setaceus* on Hawai‘i island. Morphological evidence initially suggested these plants may be hybrids and molecular analysis using the ITS and *rpl32-trnL* regions confirmed that the plants are hybrids and identified their parentage. These hybrids are named *Chloris* × *pseudosagrana nothosp. nov.* and *Cenchrus* × *peregrinus nothosp. nov.* No developed seeds could be found on either of these hybrids and they are suspected to be sterile F1 hybrids. The occurrence of hybridization events among other non-native species are also discussed.

**Key words:** hybrid, Hawai‘i, ITS, Poaceae, invasive species

### Introduction

Non-native grasses have dramatically transformed the landscape of Hawai‘i over the past 250 years with over 270 species being reported as naturalized or questionably naturalized (Faccenda 2023). These grasses are now found in almost every habitat across Hawai‘i and many are aggressive invasive species (Edwards & Still 2008; Gillespie *et al.* 2008). Despite this diversity of non-native grass species in Hawai‘i, there are no prior reports of any hybridization events involving non-native grasses on the islands, although hybrids among non-native species outside of the Poaceae have been reported (Wagner *et al.* 1990).

As part of a revision and update to the Hawaiian grass flora, roadside surveys focusing on invasive grasses were conducted during 2022 across the islands of Kaua‘i, O‘ahu, Moloka‘i, Maui, and Hawai‘i (Faccenda 2023). The goal of these surveys was mapping and early detection of incipient invasive species. During these surveys, two hybrids were incidentally found due to their unusual morphology but were only recognized while examining the specimens after fieldwork had been largely completed.

Natural hybridization is relatively common within Poaceae, especially within the Triticeae tribe, but natural hybrids exist in many tribes (Kellogg 2015; Bernhardt *et al.* 2017). Ancient hybridization events within Poaceae are increasingly being uncovered with molecular investigations into differences between nuclear and plastid inheritance (e.g. Bernhardt *et al.* 2017; Tkach, *et al.* 2020). The high rates of hybridization and polyploidy in plants is a key driver in plant evolution and speciation, as the formation of hybrid polyploids leads to immediate genetic isolation and frequently the formation of a new species, among other benefits that hybridization can lend to diploid populations (Alix *et al.* 2017).

Hybridization in the genus *Chloris* (Chloridoideae: Cynodonteae: Eleusininae) is also well documented but relatively rare, with its evolutionary impacts being largely unstudied (Peterson *et al.* 2015). Spontaneous hybridization among *C. cucullata* Bisch., *C. andropogonoides* E.Fourn., and *C. verticillata* Nutt. in Texas is found (Anderson 1974), but this is the only prior report of spontaneous hybridization within the genus that could be found. An intergeneric hybrid among *Chloris divaricata* R.Br. and *Cynodon dactylon* (L.) Pers., (×*Cynochloris macivorii* Clifford & Everist) has also been reported once from Australia, but only from a single colony (Clifford & Everist 1964).

At least 14 hybrids have previously been reported in *Cenchrus* (Panicoideae, Paniceae, Cenchrinae) and are largely of artificial origin with the exception of two spontaneous hybrids and are thus of little known importance. The following hybrids have been reported that do not have *C. americanus* (L.) Morrone as a parent: *Cenchrus* × *longistylus* (Hochst. ex A.Rich.) Thulin & S.M.Phillips is a spontaneous hybrid among *C. clandestinus* (Hochst. ex Chiov.) Morrone and *C. longisetus* M.C. Johnst. that is only known from the type specimen (Thulin & Phillips 2015) and whose hybrid status is only based on morphological data. *Cenchrus* × *cupreus* (Thorpe) Govaerts (syn. *C. × advena* (Wipff & Veldkamp) Morrone) is a hybrid that is widespread in cultivation. The parentage of *C. × cupreus* is unclear, although one of its parents is suspected to be *C. setaceus* (Forsk.) Morrone (Simpson & Bashaw 1969; van Valkenburg *et al.* 2021). Several artificial hybrids have been created among *C. ciliaris* L. and other *Cenchrus* species including *C. orientalis* (Rich.) Morrone (Ramu *et al.* 1996), *C. pedicellatus* (Trin.) Morrone (Jauhar 1981), *C. setaceus* (Jauhar 1981), and a fertile hybrid with *C. setigerus* Vahl. (Goel *et al.* 2011).

Extensive investigations into breeding *C. americanus* have created many artificial hybrids including hybrids with *C. ciliaris* (Veldkamp 2014), *C. orientalis* (Jauhar 1981), *C. purpureus* (Schumach.) Morrone (Gupta & O Mhere 1997) although this hybrid can also form spontaneously (Jauhar 1981), *C. ramosus* (Hochst.) Morrone, *C. setaceus* (Dujardin & Hanna 1989), *C. schweinfurthii* (Pilg.) Zon, and *C. squamulatus* (Fresen.) Morrone, (Marchais & Tostain 1997). One of, if not the most, phylogenetically unusual hybrids in the Poaceae has also been created artificially among *Oryza sativa* L. and *C. orientalis* (Su-hsuen & Chi-kuei 1963; Jauhar 1981 and Fig. 11.10 within).

## Materials and methods

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 (Morden *et al.* 1996; Randell & Morden 1999).

Eleven plants of the *Cenchrus* hybrid were found at the intersection of Saddle Road and Rt. 190 on Hawai'i Island, all growing within 3 meters of each other. This area was principally dominated by a *Cenchrus setaceus* monoculture. *Cenchrus ciliaris* was also common, and *Cenchrus clandestinus* was the least common *Cenchrus* on the roadside. In the field, this plant was not recognized as a hybrid, so neither herbarium specimens nor DNA from putative parents were collected. Instead, DNA was obtained from a nearby collection of *C. setaceus* from the Hawai'i Plant DNA Library (HPDL# 120; Randell & Morden 1999) and destructive sampling of a *C. clandestinus* herbarium specimen collected nearby (W. Takeuchi 5746 BISH). DNA was extracted from the Saddle Rd. specimen one year after it was collected, and was of good quality.

Seven plants of the *Chloris* hybrid were found, all on O'ahu. One plant was found at the Kualoa Ranch on the windward side of O'ahu. Six plants were found along the stretch of Kaukonahua Rd. about 5 km S. of Waiialua. Four of these six individuals were found within 5 meters of each other at the University of Hawai'i Poamoho Experimental Farm, one individual was found about 500 SE of the farm, and one further plant was found about 1.6 km SE of the farm. At Kualoa Ranch, *C. barbata* Sw. and *C. divaricata* were also seen in close proximity to the hybrid. At the Kaukonahua Rd. sites *C. barbata*, *C. divaricata*, *C. gayana* Kunth, *C. pycnothrix* Trin, and *C. virgata* Sw. were present. DNA was extracted and sequenced from three individuals of the *Chloris* hybrid (K. Faccenda 3002, 3038, & 3064). The ITS region was amplified from all samples and *rpl32-trnL* was amplified from two (K. Faccenda 3002 & 3038).

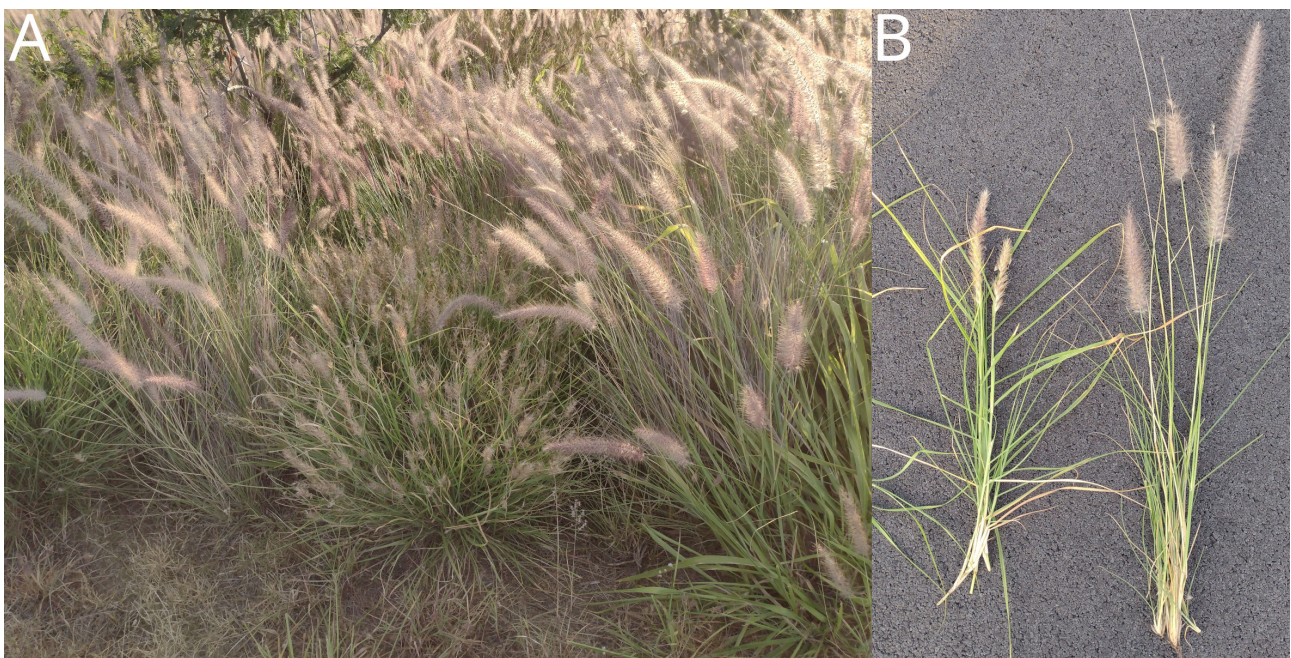
## Results

### *Cenchrus* hybrid

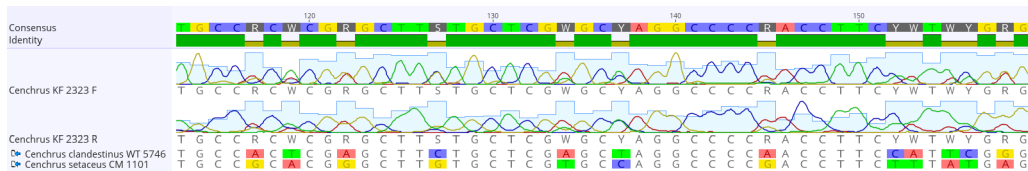
The ITS sequence of the *Cenchrus* hybrid contained 27 heterozygous sites within the first 470 base pairs, at which point an indel among the parents dramatically increases the number of heterozygous sites in the hybrid. Every one of these 27 pre-indel heterozygous sites were predicted if *C. clandestinus* and *C. setaceus* are the parents (Fig. 2). The *rpl32-trnL* sequence of the hybrid was identical to *C. clandestinus*, indicating it as the maternal parent, whereas there were 13 and 55 bases which differed from *C. setaceus*, and *C. ciliaris* respectively.

Examination of herbarium specimens at PTBG found one specimen of *Cenchrus* similar to the plants seen off of Saddle Rd. This plant (*D.H. Lorence 7632 PTBG*) was found at mile 30 on the Hawai‘i Belt Rd. in 1995, about 32 km distant from the Saddle Rd. plants. These plants were growing in a lawn and being mowed as if they were a turf grass. The Saddle Rd. plants and the Hawai‘i Belt Rd. plants were nearly identical in their morphology. Two further specimens were found at BISH: *W. Takeuchi 8521* from 1992 at an unspecified location in the North Kona District and *N. Matayoshi s.n.* from Hualālai Ranch collected in 1990.

Morphologically, this hybrid was intermediate among the two putative parents (Fig. 1). The inflorescence was only partially exerted from the sheath, whereas *C. clandestinus* is entirely inserted and *C. setaceus* is entirely exerted. The leaves were soft and flat, much more similar to the leaves of *C. ciliaris* or *C. clandestinus* compared to the stiff, narrow, v-shaped leaves of *C. setaceus*. The spikelets, 8–11 mm long, were intermediate between the 10–22 mm spikelets of *C. clandestinus* and the 4.5–7 mm spikelets of *C. setaceus*. The spikelets were borne on a pubescent pedicel like *C. setaceus*. Over 1000 mature florets from one population were examined for seed, and none was found, indicating that this hybrid is sterile or typically sterile. It must be noted, that this hybrid form has formed spontaneously many times, and there is no evidence that the hybrid is uniform genetically indicating that, while it is unlikely, other hybrid individuals could be fertile. Given that this hybrid forms regularly where the two parents overlap, and has clear evidence of hybrid origin, it is hereby published as a hybrid species.



**FIGURE 1.** *Cenchrus* × *peregrinus* comparison with *C. setaceus* as seen at the base of Saddle road on Hawai‘i island (plant collected as holotype). (A) *Cenchrus* × *peregrinus* in the center surrounded by the taller *C. setaceus*. (B) *Cenchrus* × *peregrinus* on left, *C. setaceus* on right.



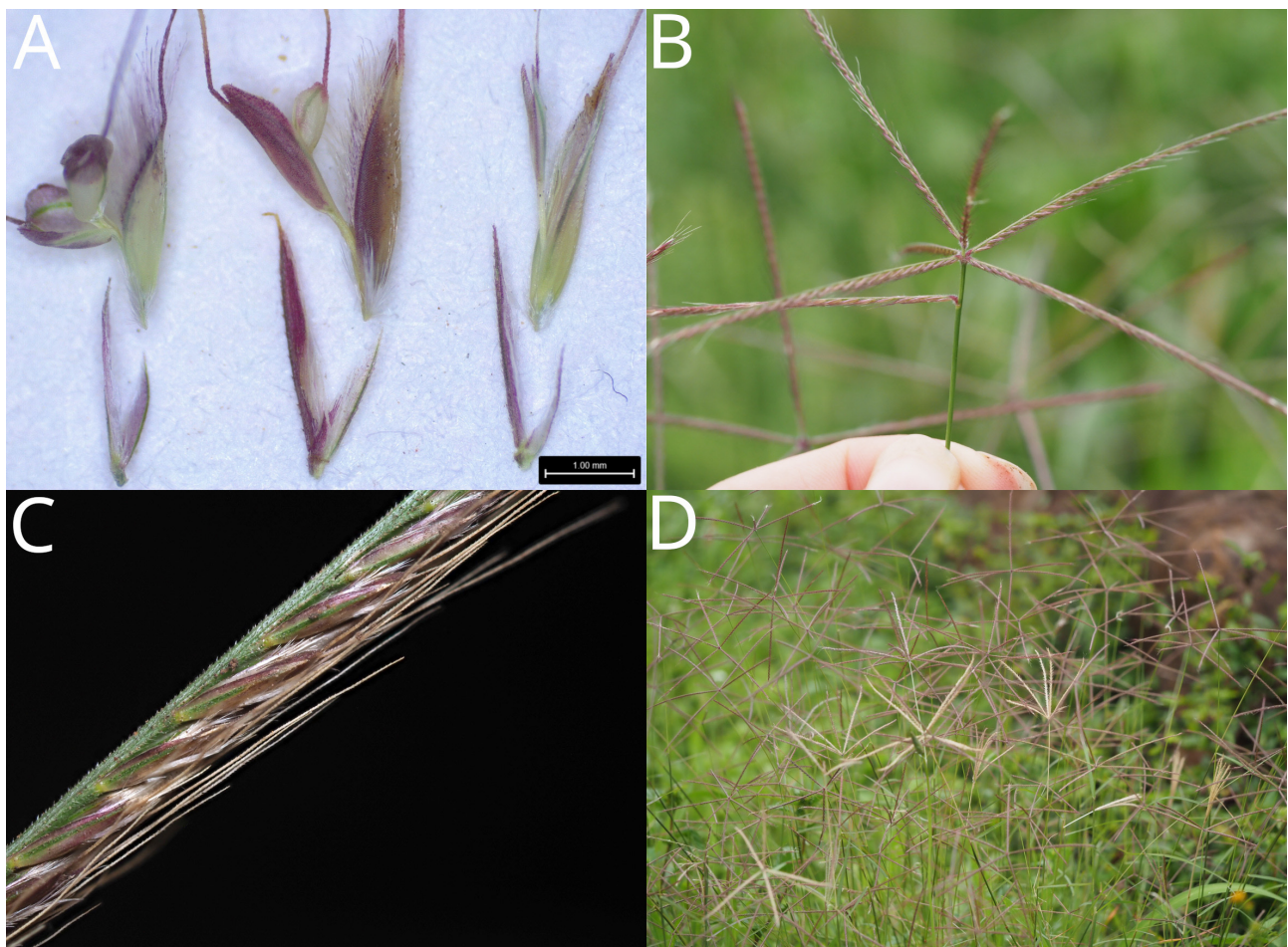
**FIGURE 2.** Sanger sequencing chromatogram for forward and reverse reads of the ITS region for the *Cenchrus* hybrid (*K. Faccenda 2323*) and its parents. The chromatogram shows an additive pattern with 27 “double peaks” for the hybrid where the bases differ among the two parents. Only one “double peak” was observed for each of the parents.

### *Chloris* hybrid

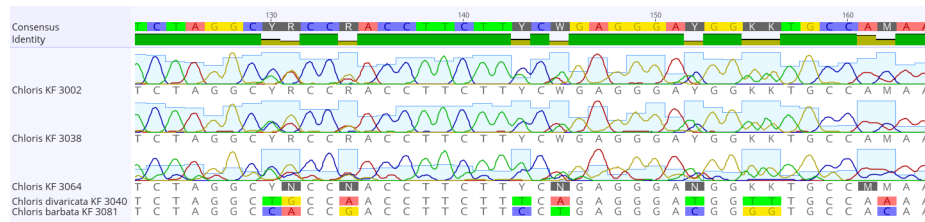
Examination of the chromatogram of the ITS sequences of the *Chloris* hybrid showed 63 heterozygous sites out of 669 total bases. Alignments of the hybrid sequences and the other *Chloris* species also found in Hawai‘i showed that the pattern of heterozygosity was most accurately predicted by hybridization between *C. barbata* and *C. divaricata*, correctly predicting 61 of the 63 heterozygous sites (Fig. 4). The second most accurate was a putative parentage of

*C. barbata* and *C. pycnothrix*, but that only correctly predicted 56 of the heterozygous sites and did not match the morphology observed on the hybrid. Other parentage combinations predicted < 36 of the heterozygous sites correctly. Comparing the *rpl32-trnL* sequences between the hybrid and the putative parents revealed zero differences between it and *C. divaricata* whereas there were 8, 19, 3, and 8 differences for *C. barbata*, *C. gayana*, *C. pycnothrix*, and *C. virgata* respectively, indicating *C. divaricata* is the material parent.

Morphologically, the hybrid *Chloris* bears a stronger resemblance to *C. divaricata* than any other *Chloris* in its vegetative features (Fig. 3). It is a stoloniferous perennial, unlike *C. barbata* which is a caespitose annual or short lived perennial. The hybrid is intermediate in maximum height between the parents at 15–75 cm tall, compared to 15–95 cm for *C. barbata* and 20–50 cm for *C. divaricata* (Barkworth *et al.* 2003). The floret anatomy is also intermediate between both parents. For example, the lowest lemma has a conspicuous pilose edge unlike the short or absent hairs on *C. divaricata*, but similar to the long hairs on *C. barbata* (Fig. 3). The second lemma is either sterile or bisexual; this was variable on the hybrid as sterile florets would often be found adjacent to bisexual ones. Overall, the ratio of the two types was approximately equal. This was extreme compared to the parents as the second lemma is always sterile in *C. divaricata* and *C. barbata*. The third floret was present and sterile (Fig. 3); it was rarely reduced to a rudiment 0.1 mm long and awn 0.4 mm long. A third floret is never present on *C. divaricata*, but it is always present on *C. barbata*. No fruits were found after examining many hundreds of mature florets, suggesting that this hybrid is sterile. No herbarium specimens of this hybrid were found at BISH or HAW. As there is clear evidence that the plants found represent a novel hybrid which has recurrently formed in four localities, it is named herein.



**FIGURE 3.** *Chloris* × *pseudosagrana*. (A) Comparison of hybrid and parents: from left to right, *Chloris barbata*, *Chloris* × *pseudosagrana*, *Chloris divaricata*. The sterile florets were manipulated to make them more visible. (B) Inflorescence. Note that a lower branch is infrequently encountered and most inflorescences are digitate. (C) Close-up of florets showing long hairs on the lemma keels. (D) Habit (Holotype: K. Faccenda & M. Ross 3038).



**FIGURE 4.** Sanger sequencing chromatogram for forward reads of the ITS sequences for three individuals of the *Chloris* hybrid along with the sequence of the putative parents. Reverse reads are identical to the forward reads and are not shown. An additive pattern is shown on the chromatogram of the hybrid at sites where the parents differ in sequence. No additive pattern is seen within the ITS region of the parents. The KF # refers to the collection number of the specimen stored at BISH.

## Taxonomy

### *Cenchrus* × *peregrinus* K. Faccenda, *nothosp. nov.* (Fig. 1)

Type:—UNITED STATES. Hawai‘i: Hawai‘i island, intersection of Saddle Road and Rt. 190, sunny, open meadow, 795 m, 19.934094, -155.687076, 05 Mar 2022, *K. Faccenda 2323* (holotype: BISH!, isotypes US!, PTBG!, Fig. 1)

Plant caespitose, perennial 50–100 cm tall. **Leaf** sheaths pilose; ligules 1.5 mm long; blades 2–4 mm wide, thin, folded along midvein, sparsely pilose on adaxial surface. **Inflorescences** both terminal and axillary, 4–15 cm long, partially exerted from their sheaths; inflorescence axis ridged, scabrous and with cilia in lines on ridges; fascicles pediceled, pedicel long hairy, about 1 mm; bristles 10–20 mm long, inner long ciliate; outer antrorsely scabrous, with one primary bristle longer and thicker than others; 1–4 spikelets per fascicle; pedicel of spikelets 0.2 mm long. **Spikelets** 8–11 mm long; lower glume absent–1.5 mm long, without veins; upper glume 2.5–3 mm long, without veins; sterile lemma 9 mm long, 5 veined; palea of sterile lemma present, 8 mm long; sterile palea enclosing 3 stamens; fertile lemma 9–10 mm long, 7 veined; fertile palea 8 mm long, enclosing 3 stamens; style long exerted, up to 7–10 mm long beyond the floret; anthers 3.8–5 mm long. **Caryopses** not observed.

Origin:—spontaneous hybrid among *Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone ♀ and *Cenchrus setaceus* (Forssk.) Morrone ♂.

Identification:—*Cenchrus* × *peregrinus* is easily distinguished from all other *Cenchrus* by its partially exerted inflorescences and the length of its lemmas. Some resemblance is shared with *Cenchrus* × *longistylus* but it has shorter inflorescences and is decumbent stoloniferous, whereas *C* × *peregrinus* is caespitose.

Paratypes:—North Kona District, mile 30 on Hawai‘i Belt Road (Mamalaha Hwy. 190) between Kailua-Kona and Waimea, 70–3403 Mamalaha Hwy, cultivated as lawn grass, originally collected in vicinity, 2000 ft [609 m], 19.7586 N–155.96362 W [coordinates added by KF based on mile marker] 13 Feb 1995, *D.H. Lorence 7632* (PTBG!). North Kona District, alleged hybrid between fountain grass and kikuyu grass, 07 Oct 1992, *W. Takeuchi 8521* (BISH!). Kona, Hualālai Ranch, *N. Matoyoshi s.n.* (BISH 778149!). Intersection of Saddle Road and Rt. 190, 795 m, 19.933765, -155.686809 *K. Faccenda & E. Judziewicz 3259*. (BISH!, PTBG!, US!, NY!, K!, MO!, MVSC!)

Other material examined:—*Cenchrus setaceus*: Hawaii, S. Kona, lower end of MacNut Farms, Mar 1993, *C. Morden et al. 1101* (HAW!). *Cenchrus clandestinus*: Hawai‘i, Pu‘u Wa‘awa‘a Ranch, near ranch headquarters, 914m, May 1989, *W. Takeuchi 5746* (BISH!).

### *Chloris* × *pseudosagrana* K. Faccenda, *nothosp. nov.* (Fig 3)

Type:—UNITED STATES. Hawai‘i, O‘ahu, central valley, Rt 803 c.a. 1 km N of its intersection with Rt. 801, roadside, *Megathyrsus maximus* dominated area, 279 m, 21.527420 N–158.078503 W, 20 Feb 2023, *K. Faccenda & M. Ross 3038* (holotype: BISH!; isotypes: HAW!, K!, MO!, PTBG!, US!, Fig. 3).

Plant perennial, stoloniferous. **Culms** 15–70 cm tall, nodes and internodes glabrous; sheaths glabrous; ligule a ciliate membrane~0.4 mm long. **Leaves** 2–3 mm wide by 3–16 cm long, collar with a few long hairs or none. **Inflorescence** digitate or subdigitate, often with one branch above or below the main whorl of branches, 3–8 branches (average 6), 2.5–8 cm long (average 6); rachis pilose at basal 5–10 mm, scabrous along remainder of length. **Spikelet** 2.9–3.3 mm long excluding awns, green with red or pink coloration, bearing three florets; callus hairs up to 1 mm long; glumes lanceolate, one veined, keeled, scabrous on keel, lower glume 1.3–1.6 mm long × 0.4 mm wide upper glume 2.3–2.6

mm long by 0.5–0.8 mm wide; lowest lemma bisexual, 2.9–3.3 mm long, minutely rugose, bilobed 0.3 mm deep at apex, awn 4–7 mm long, appressed hairy on margins, hairs up 0.6 mm long, usually scabrous near apex; second lemma sterile or bisexual, elliptic, 1.5–2.1 mm long, glabrous or with few hairs on margin at apex, bilobed 0.2–0.3 mm deep at apex, awn 4–6 mm long, palea ranges from a rudiment 0.1 mm long to a well developed at 1.7 mm long; third lemma sterile, rarely reduced to a rudiment, but typically 0.5–1.2 mm long, truncate, awn 0.8–3.5 mm long; anthers 3, 0.5 mm long. **Caryopses** not observed.

Origin:—spontaneous hybrid among *Chloris barbata* Sw. ♂ and *C. divaricata* R.Br. ♀.

Identification:—*Chloris* × *pseudosagrana* can be distinguished from *Chloris divaricata* by its hairy margin of the lowest lemma, presence of a third lemma, and second lemma being bilobed up to only 1/5 of its length compared to 2/5 to 1/2 its length in *C. divaricata*. The hybrid is also morphologically quite similar to *Chloris sagrana* A. Rich, a Caribbean species unrelated to either *C. divaricata* nor *C. barbata* (Peterson *et al.* 2015).

Paratypes:—Hawai‘i, O‘ahu, Kaukonahua Rd (801) about 2 km N of where it intersects with Wilikina Dr. (803), 279 m, 21.527070, -158.078080, 18 Jan 2023, *K. Faccenda 3002* (BISH!). Hawai‘i, O‘ahu, Kualoa Ranch, Ka‘a‘awa valley, 3 m, 21.540372, -157.846635, 07 Mar 2023, *K. Faccenda & J. Lee 3064* (BISH!, US!). Hawai‘i, O‘ahu, University of Hawai‘i Poamoho Experimental Farm, Kaukonahua Rd., about 5 km S of Waialua, weedy area along edge of dirt road, full sun, rather moist area dominated by weeds, all stems collected from a single clump, 218 m, 21.538179, -158.089588, 29 Mar 2023, *K. Faccenda & M. Ross 3088* (BISH!, K!, MO!, MVSC!, NY!, PTBG!, US!).

Other material examined:—***Chloris barbata***: Central Valley, Kaukonahua Rd., about 5 km S of Waialua, 226 m, 21.536369, -158.088543, 29 Mar 2023, *K. Faccenda & M. Ross 3081* (BISH!). ***Chloris divaricata***: Central Valley, Kaukonahua Rd., about 5 km S of Waialua, 233 m, 21.535140, -158.086949, 29 Mar 2023, *K. Faccenda & M. Ross 3087* (BISH!); Central valley, Rt 803 c.a. 2 km N of its intersection with Rt. 801, 229 m, 21.535572, -158.087540, 20 Feb 2023, *K. Faccenda & M. Ross 3040* (BISH!).

## Discussion

As the ITS region is biparentally inherited with many copies throughout the genome, an additive pattern is expected to be seen in the Sanger chromatogram for recently derived hybrids as both parental regions are sequenced simultaneously (Baumel *et al.* 2001; Álvarez & Wendel 2003; Kaplan & Fehrer 2004). This additive pattern is consistent in F1 hybrids but may be quickly lost in subsequent generations as ITS will tend back towards a parent (Fuentes Aguilar *et al.* 1999; Clarkson *et al.* 2011). As a consistent, additive pattern was seen in the ITS region of all hybrids sequenced, it is likely that these are very recently derived hybrids, likely F1 hybrids.

No cytological work was undertaken as part of this research. As the chromosome count of *Chloris divaricata* is unknown, little can be inferred regarding the *Chloris* hybrid. However, the cytology of *Cenchrus* is well documented. Within *Cenchrus setaceus*, only  $3n = 27$  has been reported, although some references cite  $2n = 54$ , this actually refers to *C. × advena* (Simpson & Bashaw 1969; van Valkenburg *et al.* 2021). *Cenchrus setaceus* is allotriploid and obligately apomictic, leading to low genetic diversity within the species (Jauhar 1981; Poulin *et al.* 2005). Cytological evidence also supports *C. setaceus* being the male parent of *C. × peregrinus* as *C. setaceus* has a pollen fertility of 48–58% (Rangasamy 1972) but should not be able to receive pollen given its apomictic nature. Reported chromosome counts for *Cenchrus clandestinus* include  $2n = 36$  and  $2n = 54$ , both of which are facultative apomictic (Jauhar 1981). It is possible the *C. × peregrinus* is either  $2n = 27$  as *C. setaceus* has well developed molecular machinery for maintaining a triploid state (Ozias-Akins *et al.* 2003), or that it is  $2n = 36$  if the pollen grain from *C. setaceus* was  $n = 18$ . Future work should examine the cytology of these hybrids, especially the *Cenchrus*, to determine if it could make viable seed or pollen.

*Chloris divaricata* and *C. barbata* overlap widely in their habitat preference in Hawai‘i as both are common in moist-to-wet, sunny, lowland areas, giving much opportunity for hybrids to form. It is likely that this hybrid also exists on other islands of Hawai‘i besides O‘ahu. It is hypothesized that *Chloris divaricata* and *C. barbata* may also hybridize in Australia, Fiji, Japan, Marquesas, New Caledonia, Taiwan, United States (South Carolina, Texas) or Vanuatu as the two parents also grow in these areas (POWO 2023) and are expected to overlap in their habitats there as well.

There is relatively little habitat overlap between the distribution of *Cenchrus setaceus* and *C. clandestinus* across the islands of Hawai‘i as *C. setaceus* grows best in dry, lowland environments whereas *C. clandestinus* prefers higher elevation and moister conditions. There is only a limited band of land where these species’ climatic niches and current

range overlap, only on the western side of Hawai'i island. A climatic overlap is also expected at similar elevations on the leeward side of Haleakalā on Maui, but *C. setaceus* is very rare on Maui due to an eradication campaign. Both *C. clandestinus* and *C. setaceus* have been widely introduced across the world, but it is unlikely they grow side-by-side in most areas, decreasing the chance of hybridization.

Hybridization among introduced or invasive species has been reported across the world, although they are outnumbered by hybrids among two natives or a native and a naturalized species (Vilà *et al.* 2000). Most of these hybrids reported among non-native species tend to be fertile, and many are invasive (Abbott 1992; Ellstrand & Schierenbeck 2000). Few sterile hybrids among non-native plants have been reported, including *Ammophila arenaria* (L.) Link × *A. breviligulata* Fernald (Mostow *et al.* 2021), *Mimulus* × *robertsii* Silverside (Vallejo-Marin & Lye 2013) from outside of Hawai'i, and *Pluchea* × *fosbergii* Cooperr. & Galang from Hawai'i (Cooperrider & Galang 1965). There has been no recent analysis or review of hybridization among introduced species, but during our literature review, the paucity of reports of sterile hybrids between introduced species was notable; given how many thousands of plants have become naturalized outside of their native ranges, it is hypothesized that such hybrids are currently underreported. Dedicated investigations into non-native floras are likely to reveal many new such hybrids with the ability to inform our knowledge of evolution or with potential economic utility.

Three other so-called anthropogenic hybrids (*sensu* Viard *et al.* 2020) between two naturalized plants have been previously reported as spontaneously forming in Hawai'i: *Pluchea* × *fosbergii* (Asteraceae; Wagner *et al.* 1990), *Leucaena* × *spontanea* C.E.Hughes & S.A.Harris (Fabaceae; Oppenheimer 2004), *Prosopis juliflora* (Sw.) DC. × *P. pallida* (Humb. & Bonpl. ex Willd.) Kunth (Fabaceae; Gallaher & Merlin 2010). Of these, only the *Pluchea* hybrid is known to be sterile. Two other fertile species of potential recent hybrid origin have also been reported from Hawai'i, but it is unknown if they formed spontaneously between their parents which occur in Hawai'i, or arrived via seed: *Emilia praetermissa* Milne-Redh. (Asteraceae; Ross & Faccenda 2023) and *Malvastrum corchorifolium* (Desr.) Britton ex Small (Malvaceae; Faccenda in review).

The ecological implications of *Cenchrus* × *peregrinus* and *Chloris* × *pseudosagrana* existing in Hawaii are currently negligible, but it is possible that if *Cenchrus* × *peregrinus* were to become fertile, it may take after its parents and be an aggressive invasive species. This has previously occurred when the sterile hybrid *Spartina* × *townsendii* underwent chromosome doubling to become *Spartina angelica*, which is an aggressive invasive species in Europe (Strong & Ayres 2013). However, if both species remain sterile, they are likely to have negligible ecological impacts.

## Conclusion

The first report of spontaneous hybridization between invasive grasses is reported from the islands of Hawai'i: *Cenchrus* × *peregrinus* and *Chloris* × *pseudosagrana*. Molecular and morphological analyses support the hybrid nature of these plants and identified the parent species. No developed seeds have been found on either hybrid and they are both presumed to be sterile.

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## Author contributions

K. Faccenda conceptualized this work, assisted with laboratory and herbarium work, and manuscript preparation.  
M. Yorkston assisted with laboratory work and experimental design.  
M. Ross assisted with field work  
C. Morden assisted with manuscript preparation

## Data Availability Statement

All sequence data has been uploaded to the NCBI Genbank database <https://www.ncbi.nlm.nih.gov/genbank/>. All specimens have been deposited in public herbaria.

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