Invasion Alert

Taxonomic Confusion Permits the Unchecked Invasion of Vernal Pools in California by Low Mannagrass (*Glyceria declinata*)

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Chloroplast DNA sequences and recently established morphological characters were used to confirm the widespread invasion of California's vernal pools by European low mannagrass. Morphological similarities between low mannagrass and western mannagrass have led to different taxonomic treatments, depending on the geographical extent of a particular flora. When California's flora was last revised, the two species were combined as western mannagrass, which was then considered to be a native species. Unfortunately, the revised flora was published just as low mannagrass began to rapidly expand its range within the state and, because it was considered to be a native species in the new flora, no actions were initiated to limit the invasion. Our data show that low mannagrass was present at all localities in the Central Valley of California that were investigated, indicating a widespread and undetected invasion. The invasion has led to a degradation of the vernal pool ecosystems, which are the habitat of many federal and state protected endangered and threatened species.

Nomenclature: Low mannagrass, *Glyceria declinata* Bréb.; western mannagrass *Glyceria occidentalis* (Piper) J. C. Nelson.

Key words: *Glyceria*, chloroplast DNA, cryptic invader, invasiveness risk assessment, control and containment actions, vernal pools.

Taxonomic confusion can be a serious barrier to the early detection, risk assessment, rapid response, and implementation of control methods. While there may be many technical causes for taxonomic confusion, here we use the term to describe a situation in which changing taxonomic treatments lead to confusion or uncertainty in the native status of plant species. This can prevent timely management action. Ultimately, a delay during the early establishment stage can permit a nascent invasive species to expand its range to the point where eradication is impossible and the only reasonable management action is local control.

Mannagrass (*Glyceria*), a genus of approximately 40 species, is represented in North America by approximately 15 species. During their evaluation of the genus for the Poaceae section of the Flora of North America, Barkworth et al. (2007) determined that it was impossible to distinguish some *Glyceria* species, including low mannagrass and western mannagrass from each other using published morphological characteristics. These morphological similarities have led to the species being either separated or combined depending on the geographical extent of a particular flora. Western mannagrass [*Glyceria occidentalis* (Piper) J. C. Nelson] is distributed in the western United States and Canada, south to northern California, and low mannagrass (*Glyceria declinata* Bréb.) is a Eurasian species. The first herbarium specimen of low mannagrass in California was collected in 1946 (Calflora 2000). When California's flora was revised in 1993 (Hickman 1993) the two species, which were previously

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Interpretive Summary

Changing taxonomic treatments can lead to confusion or uncertainty in the native status of plant species. This can prevent timely management action. A delay during the early establishment stage can permit a nascent invasive species to expand its range to the point where eradication is impossible and the only reasonable management action is local control.

Morphological similarities between low mannagrass (*Glyceria declinata*) and western mannagrass (*Glyceria occidentalis*) have led to different taxonomic treatments, depending on the geographical extent of a particular flora. When California's flora last was revised, the two species were combined as western mannagrass, which was then considered to be a native species. Unfortunately, the revised flora was published just as low mannagrass began rapidly to expand its range within the state and, because it was considered to be a native species in the new flora, no actions were initiated to limit the invasion.

Vernal pools along the east side of the Central Valley are especially prone to being invaded by low mannagrass, which has been found in very high densities in some surveyed pools. Vernal pools are considered a major conservation priority because they contain many endemic plant and animal species, of which several have been given formal protection as threatened or endangered species.

This incident shows that it is important to consider the possibility of the introduction of foreign populations or of unrecognized species when rapid range expansion of a putative native species occurs. This is especially true if, as was the case with low mannagrass, revised taxonomic treatments lead to the inclusion of a foreign species into a native one.

treated as separate species (Munz and Keck 1968), were combined as western mannagrass, which was then considered to be a native species.

After the 1993 revision of the flora, the ''western'' mannagrass began a period of rapid range expansion in California's Central Valley (CH2MHill 1998; Pacific Mutual Consultants 2005). Vernal pools along the east side of the Central Valley are especially prone to being invaded by mannagrass, which has been found in very high densities in some surveyed pools. Vernal pools are considered a major conservation priority because they contain many endemic plant and animal species, of which several have been given formal protection as threatened or endangered species.

Whipple et al. (2007) sequenced chloroplast DNA to separate the species and to identify unique morphological features for taxonomic keys. During their molecular analysis they tested two samples of low mannagrass from Denmark; one sample from Oregon; and seven California herbarium samples from Pacific coastal areas, the Sierra Nevada foothills, and a degraded vernal pool in the Central Valley. All samples were determined to be low mannagrass and the clearest morphological characteristic identifying low mannagrass was found to be the presence of two lobes on either side of the lemma tip (see Fact Sheet images at http://utc.usu.edu/). Whipple et al. (2007) found that low mannagrass contained chloroplast sequence haplotypes that differ from those found in western mannagrass; this indicates that the 1993 taxonomical treatment of the species was misleading. In addition, their determination that low mannagrass was present at one site in the Central Valley of California revealed that it is likely that the observed invasion of vernal pools in California is caused by low mannagrass, an exotic species. Because of the importance of the California's Central Valley vernal pool ecosystems for threatened and endangered species, we undertook a study to confirm the taxonomic status of *Glyceria* occurring in the vernal pool system in California by sampling a larger number of vernal pool sites, including those associated with herbarium specimens.

Materials and Methods

Herbarium specimen data were consulted to identify all Central Valley locations of *Glyceria* species. Distribution information for either low mannagrass or western mannagrass in vernal pools in California's Central Valley and in two other nearby vernal pool regions that are closer to the Pacific coast was taken into account. Most vernal pools are located on private ranches or in vernal pool preserves. We used the collection data of herbarium specimens as well as publicly available information from conservation agencies to determine accessible localities.

Central Valley specimens of *Glyceria* deposited in the University of California Davis herbarium were examined. Low mannagrass has a clearly wavy to dentate lemna tip whereas western mannagrass does not, and low mannagrass has the more evidently bifurcate palea (Munz and Keck 1968; Whipple et al. 2007; M. Barkworth personal communication; see figures at http://herbarium.usu.edu/webmanual/). Lemna tip morphology is a character that easily is accessible in the field and effectively differentiates low mannagrass from every other congeneric species. Using these characters, the occurrence of mannagrass in 24 accessible localities have been confirmed during botanical surveys (Tables 1 and 2). One individual from each of six locations was collected for DNA sequence analysis to verify the result of the field surveys. This collection took place across a variety of vernal pool types in the central portion of the distribution. Fresh leaf samples were frozen in liquid nitrogen, freeze dried, and ground. DNA extraction was performed using the ChargeSwitch kit[1] following the manufacturer's protocols. Voucher specimens of each occurrence evaluated with our molecular markers were submitted to the University of California Davis Herbarium.

A 600-bp sequence of the trnK(AAA)-rps16 spacer was sequenced comparatively. Amplification and sequencing were performed using the trnK-rps16 primer set and methods described in Whipple et al. (2007). Sequences were aligned manually and compared with their results

Table 1. List of herbarium records that were used to infer the distribution of low mannagrass in Central Valley vernal pools in California. Determination was performed in the field using the characters described in the text. The status of six accessions as low mannagrass was verified using DNA sequence analysis of chloroplast loci according to Whipple et al. (2007).

Herbarium Records					
Central Valley					
Site	County	Herbarium[a]	Year	Collector	Determined by
Cherokee Mine	Butte	CHSC	2000	L. Ahart 8377	M. E. Barkworth
Rock Creek	Nevada	CHSC	2002	L. Ahart 9673	M. E. Barkworth
Mill Creek	Tehama	CHSC	2000	L. P. Janeway & B. Castro	M. E. Barkworth
Mill Creek Trail	Tehama	CHSC	2004	L. Ahart & S. M. Hillaire	M. E. Barkworth
Red Bluff	Tehama	CHSC	1998	V. Oswald & L. Ahart 8978	M. E. Barkworth
Shintaffer Farm	Yuba	CHSC	1999	L. Ahart 8248	M. E. Barkworth
Michigan Bar Road	Amador	CDF	2004	G. F. Hrusa 16267	M. E. Barkworth
Red Hill Ranch	Butte	CDF	2006	M. Stewart PDR 1290445	G. F. Hrusa
Wildcat Canyon Park	Contra Costa	CDF	2002	D. G. Kelch	M. E. Barkworth
Tomales Bay	Marin	CDF	1979	G. H. True	M. E. Barkworth
CDFA Facility	Sacramento	CDF	1999	G. F. Hrusa 14932	M. E. Barkworth
Mather Field[b]	Sacramento	CDF	2002	G. F. Hrusa 16012	M. E. Barkworth
Meiss Road	Sacramento	CDF	1994	G. F. Hrusa 11764	M. E. Barkworth
Rancho Cordova	Sacramento	CDF	2003	J. Davidek	M. E. Barkworth
Strawberry Creek	Sacramento	CDF	1993	G. F. Hrusa 10935	M. E. Barkworth
Valensin Ranch[b]	Sacramento	CDF	1996	G. F. Hrusa 12796	M. E. Barkworth
Linden	San Joaquin	CDF	1974	J. B. Gianelli	M. E. Barkworth
Santa Rosa	Sonoma	CDF	1968	H. F. McCracken	M. E. Barkworth
Sebastopol	Sonoma	CDF	1969	Farm Advisor Office	M. E. Barkworth
Calpine Rice Field	Sutter	CDF	1997	G. F. Hrusa 13681	M. E. Barkworth
Rice Field	Sutter	CDF	2004	M. Stelmok PDR 1411715	M. E. Barkworth
Fort Bragg	Mendocino	CDF	1928	C. S. Myaska	M. E. Barkworth
Copperopolis	Calaveras	DFA	2000	G. F. Hrusa 15478	M. E. Barkworth
Sutter Ione Road	Sacramento	DFA	2001	G. F. Hrusa 15868	M. E. Barkworth
Keifer Landfill[b]	Sacramento	DFA	2005	G. F. Hrusa 16459	G. F. Hrusa
Jesus Maria Creek	Calaveras	AHUC/DAV	1959	B. Crampton 40922	J. D. Gerlach
Copperopolis	Calaveras	AHUC/DAV	2000	G. F. Hrusa 15478	J. D. Gerlach
Roadside ditches	Modesto	AHUC/DAV	1993	M. Flesher	J. D. Gerlach
Cosumnes River Preserve	Sacramento	AHUC/DAV	1994	H. Brink	J. D. Gerlach
Valensin Ranch[b]	Sacramento	AHUC/DAV	1996	G. F. Hrusa 12796	J. D. Gerlach
Vintage Park[b]	Sacramento	AHUC/DAV	1995	B. Crampton 10303	J. D. Gerlach
Sloughouse vernal pools	Sacramento	AHUC/DAV	1995	R. E. Preston 838	J. D. Gerlach
Oakdale	Stanislas	AHUC/DAV	1953	B. Crampton 1247	J. D. Gerlach
Modesto Irrigation Canal	Stanislas	AHUC/DAV	1969	P. S. Allen 469	J. D. Gerlach
Rice Field	Yuba	AHUC/DAV	1984	J. F. Williams	M. E. Barkworth
Rice Field	Yuba	AHUC/DAV	1984	J. F. Williams	J. D. Gerlach
Other regions					
Arcata	Humboldt	CDF	1968	E. C. Whitney	M. E. Barkworth
Dome Creek	Siskiyou	AHUC/DAV	1956	B. Crampton 3792	J. D. Gerlach
Bolder Creek	Siskiyou	AHUC/DAV	—	A. Beetle	J. D. Gerlach

[a] Abbreviations used for herbaria: AHUC/DAV: University of California, Davis; CDF: CDFA Botany Laboratory; CHSC: California State University, Chico.

[b] Site also visited during the field surveys.

Table 2. List of surveys in the field (Visited localities) that were used to infer the distribution of low mannagrass in Central Valley vernal pools in California. For localities visited in the course of this study, UTM coordinates are listed. Determinations were performed in the field using the characters described in the text. The status of six accessions as low mannagrass was verified using DNA sequence analysis of chloroplast loci according to Whipple et al. (2007).

Visited localities/Central Valley			
Site	County	Projection	UTM Coordinates
Field surveys			
Broyles Road	Butte	UTM 10 NAD 83	10 S 0588771 4413358
Ridgewood	Mendocino	UTM 10 NAD 83	10 S 0474017 4353766
Mann Ranch	Mendocino	UTM 10 NAD 83	10 S 0472076 4339862
Kelsey Ranch	Merced	UTM 10 NAD 83	10 S 0734423 4157563
Richards Ranch	Merced	UTM 10 NAD 83	10 S 0727277 4159293
Roduner Ranch	Merced	UTM 10 NAD 83	10 S 0731087 4144370
Flying M Ranch	Merced	UTM 10 NAD 83	10 S 0738135 4143015
Ichord Ranch	Merced	UTM 10 NAD 83	10 S 0734763 4138918
Butler Ranch	Merced	UTM 10 NAD 83	10 S 0743519 4136287
Chance Ranch	Merced	UTM 10 NAD 83	10 S 0731144 4150439
Nelson Ranch	Merced	UTM 10 NAD 83	10 S 0746970 4132656
Knapp Ranch	Merced	UTM 10 NAD 83	10 S 0752950 4117634
Phoenix Field	Sacramento	UTM 10 NAD 83	10 S 0655266 4280154
Howard Ranch[a]	Sacramento	UTM 10 NAD 83	10 S 0668476 4245206
Valensin Ranch	Sacramento	UTM 10 NAD 83	10 S 0649407 4245744
Rancho Seco	Sacramento	UTM 10 NAD 83	10 S 0667355 4244397
Bouveire Preserve[b]	Sonoma	UTM 10 NAD 83	10 S 0542860 4246597
Dye Creek	Tehama	UTM 10 NAD 83	10 S 0580362 4439803
Field surveys verified by sequence analysis			
Twelve Bridges	Placer	UTM 10 NAD 83	10 S 0648055 4300897
Mather Field	Sacramento	UTM 10 NAD 83	10 S 0651254 4268019
Kiefer Landfill	Sacramento	UTM 10 NAD 83	10 S 0657636 4266375
Sloughhouse Mitigation	Sacramento	UTM 10 NAD 83	10 S 0655602 4261448
Vintage Park	Sacramento	UTM 10 NAD 83	10 S 0645036 4258483
Lane Ranch	San Joaquin	UTM 10 NAD 83	10 S 0669450 4232538
Reported occurrences not verified by field survey			
Ahart Ranch	Butte	UTM 10 NAD 83	10 S 0626935 4355907
Kennedy Table	Fresno	UTM 11 NAD 83	11 S 0270618 4108352
Lone Tree	Butte	UTM 10 NAD 83	10 S 0626002 4362894
Oakdale	Butte	UTM 10 NAD 83	10 S 0691429 4177348
Oroville Airport	Butte	UTM 10 NAD 83	10 S 0618255 4372557

[a] Determined by J. Buck.

[b] Determined by D. Guelsenkamp.

(Genebank accession numbers DQ665551 to DQ665637). Phylogenetic and distance analysis was performed using the program PAUP (Swofford, 2002) with the indels included and coded as presence/absence data.

Results and Discussion

All specimens of the genus *Glyceria* of the University of California Davis Herbarium, that had been collected from Central Valley vernal pools, showed lemna morphology that is characteristic of low mannagrass. In addition, specimens at all localities that we surveyed were determined to be low mannagrass. We were not able to visit reported field survey locations at the northernmost site, the southern group of sites, and the two northwestern sites, but the presence of low mannagrass seems likely because local botanical surveys, California State University Chico Herbarium collection location data, and/or other nongeoreferenced surveys in those areas found low mannagrass to be present (Figure 1). None of the

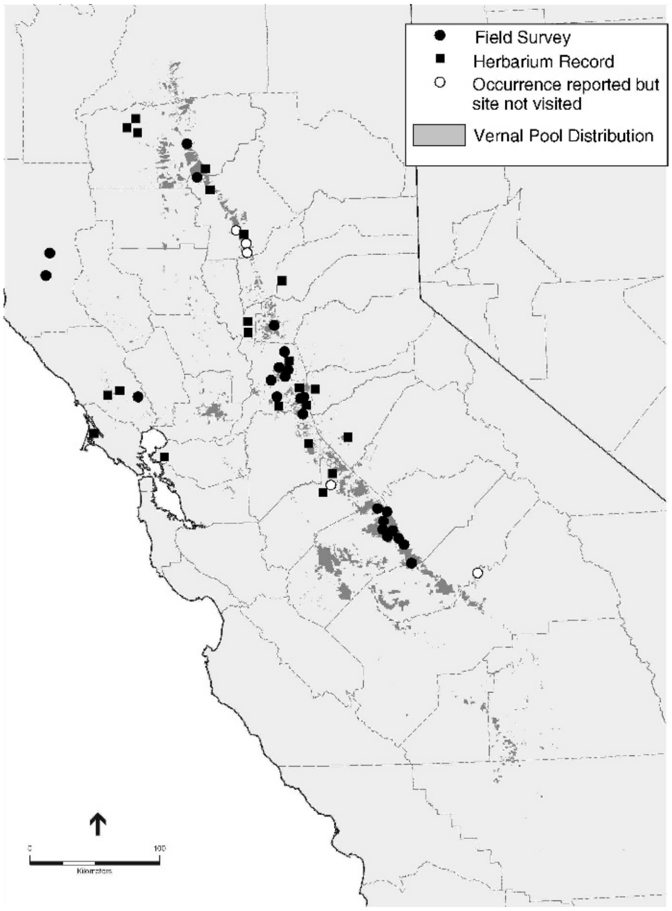

Figure 1. Map of the Central Valley vernal pool system in California, indicating the localities investigated. Identification of *Glyceria declinata* was performed by sequencing of chloroplast DNA, morphological examination of collected specimen, and examination of herbarium specimen.

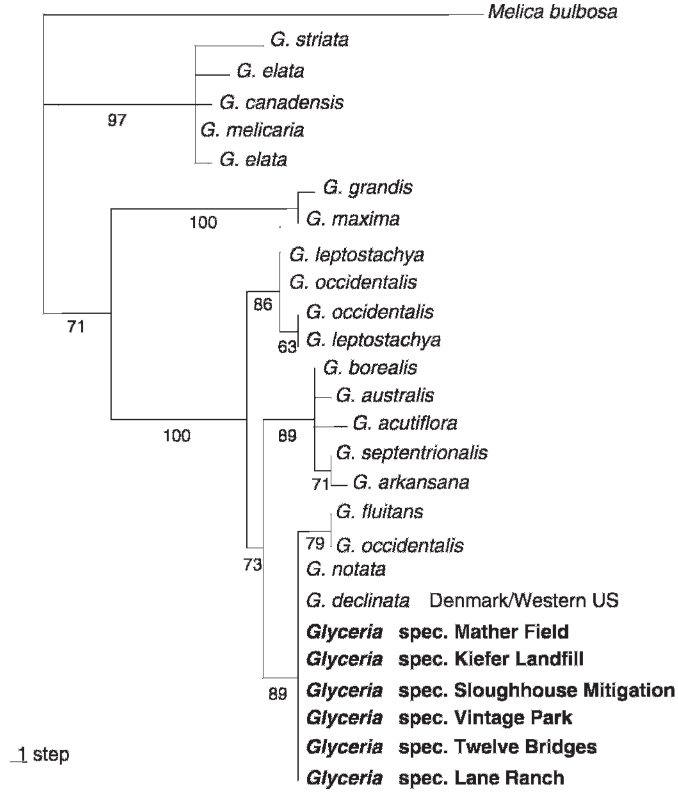

Figure 2. Maximum parsimony analysis of trnK-rps16 spacer sequences of samples investigated in this study (bold) and *Glyceria* species published by Whipple et al. (2007; DQ665551 to DQ665637). Only one sequence per species was included and each differed in at least in one position from the remaining samples. Shown is a phylogram of one randomly chosen tree from the 150 shortest trees of 83 steps. Bootstrap values from 1,000 replicates are indicated below the branches. All investigated *Glyceria* samples were equivalent to published low mannagrass (*G. declinata*) sequences and differed in at least two positions from western mannagrass (*G. occidentalis*) and from water mannagrass (*G. fluitans*).

occurrences in vernal pools that we surveyed or present in collections in the University of California Davis Herbarium were found to be western mannagrass. Including recently determined herbarium specimen from other herbaria (Tables 1 and 2), a total of 52 locations in the Central Valley for low mannagrass were documented (Figure 1).

All trnK-rps16 spacer sequences determined were identical to sequences obtained from low mannagrass by Whipple et al. (2007); gi: DQ665567 to 665576. Maximum Parsimony analysis of the spacer region of *Glyceria* samples investigated in this study in comparison to samples investigated by Whipple et al. (2007) indicated that, with the exception of marked mannagrass (*Glyceria notata* Chevall), all species differ at least in two positions from low mannagrass (Figure 2). Western mannagrass shares chloroplast haplotypes with two species: narrow mannagrass, (*G. leptostachya* Buckley, western United States) and water mannagrass (*G. fluitans* L., Eurasia), indicating a hybrid origin of western mannagrass from these two species (Whipple et al. 2007). However, all samples of western mannagrass included in the study of Whipple et al. (2007) are clearly differentiated from samples from low mannagrass using the trnK-rps16 spacer.

The presence of chloroplast haplotypes from low mannagrass supports our analyses of lemna morphology. This suggests that only low mannagrass is present in the Central Valley (Figure 1). Low mannagrass is a known dominant of vernal pool ecosystems in Portugal and Spain (Gallego-Fernández et al. 1999; Molina 1996; Rivas-Martínez 2002). It is easily dispersed by seeds that are able to float and that become attached to waterfowl and grazing animals (Hubbard 1942). Our results indicate that this species has been introduced in California's Central Valley where it now is probably the only *Glyceria* species invading vernal pools.

The recent invasion of California's Central Valley vernal pools by low mannagrass provides an example of how taxonomic confusion can result in the widespread invasion of a rare ecosystem, despite close observation by the federal and state endangered and threatened species programs (Rogers 1998; Volmar 2002) and invasive species risk assessment programs (CalEPPC 1999). Many human activities in the invaded vernal pool ecosystems are regulated by the U.S. Army Corps of Engineers (Corps) under Section 404 of the U.S. Clean Water Act, and the U.S. Fish and Wildlife Service (USFWS) under Section 7 of the U.S. Endangered Species Act, because vernal pool ecosystems have been designated as critical habitat for federally listed threatened and endangered species. Because of these regulatory programs, the recent rapid range expansion of low mannagrass has been extensively documented under the Corps' wetland permitting process and reported in both federal (see CH2MHill 1998) and nonfederal environmental review processes (Pacific Mutual Consultants 2005).

Field collection labels associated with herbarium specimens (University of California Davis and California State University Chico) suggest that that the 1993 flora, which treated both species as conspecific, was published just as low mannagrass began rapidly to expand its range within California. The consolidation into a single native species effectively stopped all management pending further research. This incident shows that it is important to consider the possibility of the introduction of foreign populations or of unrecognized species when rapid range expansion of a putative native species occurs. This is especially true if, as was the case with low mannagrass, revised taxonomic treatments lead to the inclusion of a foreign species into a native one.

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Source of Material

[1] ChargeSwitch® Plant Kit, Invitrogen, Carlsbad, CA 92008.

Literature Cited

Barkworth, M. E., K. M. Capels, S. Long, L. K. Anderton, and M. B. Piep, eds. 2007. *Magnoliophyta* Commelinidae (in part: Poaceae, part 1). Flora of North America North of Mexico. Vol. 24. New York: Oxford University Press. 911 p.

CalEPPC. 1999. The CalEPPC List: Exotic Pest Plants of Greatest Ecological Concern in California. October, 1999. Berkely, CA: California Invasive Plant Council, 8 p.

Calflora. 2000. Information on California Plants for Education, Research and Conservation. Berkeley, California: The Calflora Database. http://www.calflora.org/. Accessed March 1, 2008.

CH2MHill. 1998. Final Training Manual to Evaluate Habitat Quality of Vernal Pool Ecosystem Sites in Santa Rosa Plain. Prepared for U.S. Army Corps of Engineers, San Francisco District, USA. December, 1998. San Francisco, CA: CH2MHill, 75 p.

Gallego-Fernández, J. B., M. R. García-Mora, and F. García-Novo. 1999. Small wetlands lost: a biological conservation hazard in Mediterranean landscapes. Environ. Conserv. 26:190–199.

Hickman, J. C., ed. 1993. The Jepson Manual: Higher Plants of California. Berkeley, CA: University of California Press. 1424 p.

Hubbard, C. E. 1942. Annual meeting in the University Department of Botany, Oxford. J. Ecol. 30:227–233.

Molina, J. A. 1996. Sobre la vegetación del los humedales de la Peninsula Ibérica (1. Phragmiti-Magnocaricetea). Lazaroa 16:27–88.

Munz, P. A. and D. D. Keck. 1968. A California Flora and Supplement. Berkeley, CA: University of California Press. 1905 p.

Pacific Mutual Consultants. 2005. Yuba Highlands Specific Plan Draft Environmental Impact Report Sch No. 2001032070. Prepared for Yuba County, California, USA. January, 2005. Chico, CA: Pacific Mutual Consultants, 616 p.

Rivas-Martínez, S., T. E. Díaz, F. Fernández-González, J. Izco, J. Loidi, M. Lousã, and A. Penas. 2002. Vascular plant communities of Spain and Portugal (12.2.7). Itinera Geobotanica 15:5–922.

Rogers, D. C. 1998. Aquatic macroinvertebrate occurrences and population trends in constructed and natural vernal pools in Folsom, California. Pages 224–235 *in* C. Witham, E. T. Bauder, D. Belk, W. R. Ferren Jr., and R. Ornduff, eds. Conference Proceedings: Ecology, Conservation, and Management of Vernal Pool Ecosystems. June 19–21, 1996, Sacramento, CA. Sacramento, CA: CNPS.

Swofford, D. L. 2002. PAUP*: Phylogenetic Analysis using Parsimony (* and Other Methods). Version 4.0b10. Sunderland, MA: Sinauer Associates.

Vollmar, J. E. 2002. Wildlife and Rare Plant Ecology of Eastern Merced County's Vernal Pool Grasslands. Berkeley, CA: Vollmar Consulting. 444 p.

Whipple, I. A., M. E. Barkworth, and B. S. Bushman. 2007. Molecular insights into the taxonomy of *Glyceria* (Poaceae: Meliceae) in North America. Am. J. Bot. 94:551–557.

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