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A NOTE ON NON-BINARY STROBILI IN *PINUS JEFFREYI*, A RARE PHENOMENON IN WILDLANDS

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ABSTRACT

The occurrence of non-binary strobili (N-BS) in gymnosperms is of interest to understanding the origins of flowering in angiosperms. One case of non-binary strobili with distal ovulate bracts was discovered during ~50,000 field observations of *P. ponderosa* Douglas ex Loudon. and *P. jeffreyi* A.Murray on 1100 trees over a 1500-km transect and a period of 20 years in western North America. The N-BS was observed once in mature *P. jeffreyi* during a year of above-average precipitation, with low tree-to-tree competition, moderate nitrogen deposition, and moderately high ozone exposure in Sequoia National Park, California. An updated, verified compendium of non-binary strobili is reported for the subfamily Pinoideae of Pinaceae.

Key Words: androgynous, bisexual, bisporangiate, cone, hermaphroditic, *Pinus*, teratological.

The occurrence of single strobili with both pollen and ovule-bearing structures (non-binary strobili, N-BS) in the subfamily Pinoideae of Pinaceae are rare, with 50 uniquely reported instances since 1838 (Cramer 1838 plate published in Masters 1869; Table 1). Nonetheless, Masters (1869) and Chamberlain (1935) have stated that most field-going botanists will have 'certainly' observed them, suggesting that the occurrence of N-BS is more common than reported. Early evolutionary botanists were interested in teratological features of strobili (Masters 1869; Penzig 1890; Masters 1889 as seen in Doak 1935) because developmental morphology and their variations suggested shared evolutionary origins with angiosperms. The capacity for N-BS is thought to have arisen prior to the angiosperm split from gymnosperms (ca. 300 million years ago), and its occurrence is now believed to be a retained, occasional feature (Frohlich 2006; Flores-Rentería et al. 2011). Interestingly, genetic (chloroplast DNA linked to microsporangia, mitochondrial DNA linked to megasporangia, and nuclear DNA contributed by both; X. Q. Wang et al. 2000), and transcriptomic analyses (Ran et al. 2018) support the classic morphological divisions of Pinaceae in which *Pinus*, *Cathaya*, *Picea*, *Pseudotsuga* and *Larix* are grouped into the pinoid clade. Of these genera, *Pinus* and *Picea* have the greatest number of reported N-BS occurrences, and *Abies, Pseudotsuga, Tsuga,* and *Larix* the least. Hybridization appears to increase N-BS occurrence (Mergen 1963) but is not discussed further here.

Bisporangiate cones in gymnosperms were first mentioned in Dickson (1860); morphological and developmental investigations of these structures over the next 75 years led to a proposed evolutionary ranking of gymnosperms (summarized in Chamberlain 1935). This approach (structure and development) in subsequent investigations led to the understanding that megasporangia develop from modified, terminal (subterminal) long shoots, and microsporangia develop from lateral needle primordia (short shoots) borne in the axils of needles (Tosh and Powell 1986; D. Y. Wang et al. 2000; Rudall et al. 2011). The occurrence, variability, plasticity, and viability (Flores-Rentería et al. 2011) of N-BS structures and their development in gymnosperms still holds interest.

Bisporangiate reproductive structures in fossils predating angiosperms are believed to support the hypothesis of angiosperm flower evolution in a common ancestor shared by both gymnosperms and angiosperms. Doyle (2008) combined morphological, molecular, and fossil evidence, to conclude that the relationships among gymnosperms (glossopterids, *Pentoxylo*n, Bennettitales, and *Caytonia*) and angiosperms are supported. However, the combination of stamens and carpels in a single structure may have arisen independently in Bennettitales. Accepting the molecular evidence, the morphological similarities of *Pentoxylon* and *Caytonia* may have arisen independently.

With some exceptions, there is temporal and spatial separation of female and male strobili. Male strobili dominate in young trees, while female strobili predominate in upper branches and male in midcrown (summarized in Tosh and Powell 1986; Caron and Powell 1990; Theissen and Becker 2004). Within a branch, female strobili are distal, and male are proximal (as in *Agathis*; Lanner 1966a). Teratological (or plastic) features suggest a historical and potential capacity for strobili to evolve. Hormonal

Table 1. Occurrences of Non-manipulated, Non-binary Strobili in Pinoideae of Pinaceae. Instances of non-binary strobili occurring in hybrids or in response to hormonal manipulation have not been included. The species names have been reported based on the modern authority according to the EPPO Global Database, and footnotes indicate species as reported in the article. When information was not clearly reported, a "n/i" is indicated. Species' name as cited in reference, cross-referenced with EPPO Global Database:[1] *Larix europaea*; [2] cannot substantiate occurrence; [3] *Larix leptolepis*; [4] *Larix macrocarpa*; [5] *Abies excelsa*; *Picea excelsa*; [6] *Picea excelsa*; [7] *Abies excelsa*; [8] *Pinus montigena*; [9] *Pinus morinda*; [10] *Pinus tonyosho*; [11] *Pinus cubensis* (Mohr); *Pinus heterophylla* (Jack), both authors reported Mellichamps' field observation; [12] *Pinus montana*; [13] *Pinus laricio*; [14] *Pinus maritima*; [15] *Pseudotsuga douglasii*; [16] *Pseudotsuga taxifolia*. Additional notes: [a] observations of *Tsuga carolinana* and *T. dumosa* reported as seen in Rudall et al. 2011, but the data for years of observation and the number of trees was supplied for both species from Ruddall, Royal Botanic Gardens, personal communication.

Species	Distal position of sporangia	Native location	Observed location	Year(s) observed	Number of trees	Reference
Larix decidua Mill.[1]	mega-	Central EU	England	1912	n/i	Bartlett 1913
Larix decidua Mill.[1,2]	mega-	Central EU	Texas (assumed), US	n/i	n/i	Doak 1935
Larix kaempferi Fortune ex Gordon	mega- or micro-	Japan	Nagano, Japan	1959	n/i	Momose 1961
Larix kaempferi Fortune ex Gordon[3]	mega- or micro-	Japan	New York, US	1958	1	Chandler 1959
Larix laricina (Du Roi) K.Koch	micro-	N America	New Brunswick	1983, 1984	(many)	Tosh and Powell 1986
Larix laricina (Du Roi) K.Koch[2,4]	micro-	N America	n/i	n/i	n/i	Masters 1869
Larix occidentalis Nutt.	mega-	W N America	Montana, US	1915	1	Kirkwood 1916
Picea abies (L.) H.Karst[2,5]	micro-	N EU	n/i	n/i	n/i	Dickson 1860
Picea abies (L.) H.Karst.[5]	mega-	N EU	Scotland	n/i	(some)	Dickson 1860
Picea abies (L.) H.Karst.[7]	mega-; midpt micro-	C EU	Sudetic Mountains	n/i	n/i	Stenzel 1876
Picea abies (L.) H.Karst.[6]	mega-	N EU	n/i	n/i	n/i	Chamberlain 1935
Picea abies (L.) H.Karst.[6]		N EU	Pennsylvania, US	1974–1982	7	Tabor 1990
Picea asperata Masters	mega-	W China	China	1958	2	Santamour 1959
Picea balfouriana Rehder & E.H.Wilson	mega-	China	Pennsylvania, US	1958	1	Santamour 1959
Picea glauca (Moench) Voss	micro-	boreal, N America	Michigan, US	1941	1	Pauley 1942
Picea glauca (Moench) Voss	1° mega-; also micro-	boreal, N America	Ontario, Canada	1980	(few)	Ho 1984
Picea glauca (Mönch) Voss	mega- or micro-	boreal, N America	Alaska, US	1970	(many)	Zasada et al. 1978
Picea glauca (Moench) Voss	mega-	boreal, N America	Pennsylvania, US	1958	5	Santamour 1959
Picea likiangensis subsp. *montigena* (Mast.) Silba[8]	mega-	Himalayan China	Pennsylvania, US	1958	2	Santamour 1959
Picea mariana Britton, Sterns & Poggenb.	mega-	boreal, N America	NW Territory, Canada	1977	(several)	Elliott 1979
Picea mariana Britton, Sterns & Poggenb.	mega-	boreal, N America	New Brunswick, Canada	1980–1983	16	Caron & Powell 1990
Picea mariana Britton, Sterns & Poggenb.	mega-; vertical and lateral separation	boreal, N America	New Hampshire, US	1967	n/i	Weidlich and Teeri 1976
Picea retroflexa Masters	mega-	Himalayan China	Pennsylvania, US	1958	1	Santamour 1959
Picea smithiana Boiss.	micro- or mega-	Himalayan China	Pennsylvania, US	1958	1	Santamour 1959
Picea smithiana Boiss.[9]	mega-	Himalayan China	Himalayas	1928	1	Rao 1931
Picea wilsonii Shaw	mega-	Central Asia	Pennsylvania, US	1958	1	Santamour 1959
Pinus contorta Bol.	mega-	W N America	Scotland	1960	>3	Black 1961
Pinus densiflora Siebold & Zucc.	n/i	E Asia	Japan	1892–1894	2	Fujii 1895, p274
Pinus densiflora Siebold & Zucc.	mega-	E Asia	California, US	1931	1	Righter 1932
Pinus densiflora Siebold & Zucc.[10]	mega-	E Asia	Illinois (assumed), US	n/i	1	Doak 1935
Pinus densiflora Siebold & Zucc.	mega-	E Asia	S Korea	2019	4	Kwon et al. 2021
Pinus echinata hort. ex Carrière	mega-	E U.S.	Georgia, US inferred	n/i	n/i	Ruark & Jones 1966

TABLE 1. CONTINUED

Species	Distal position of sporangia	Native location	Observed location	Year(s) observed	Number of trees	Reference
Pinus elliottii Engelm.[11]	mega-; vertical or lateral separation	SE U.S.	South Carolina, US	1893–1895	2	Jack1895
Pinus jeffreyi A.Murray	mega-	W U.S.	California, US	2007	1	<this paper>
Pinus johannis M.-F.Robert	mega-	SW U.S.	San Luis Potosi, MX	2005–2009	6	Flores-Rentería et al. 2011
Pinus longifolia Roxb.	mega-	Himalayas	Himalayas	1929, 1930	1	Rao 1931
Pinus massoniana Zucc.	mega-	China	California, US	1931	1	Richter 1932
Pinus mugo Turra[12]	mega-	Central EU	Wisconsin, US	1915	1	Steil 1918
Pinus nigra Marshall	mega-	Central & S EU	Greece	1990	1 (clone)	Matziris 2002
Pinus nigra Marshall[13]	mega-	Central & S EU	Ohio (assumed), US	1905	1	Fisher 1905
Pinus palustris Mill.	mega-	SE U.S.	Texas	n/i	n/i	Zobel 1952
Pinus pinaster Loudon[14]	mega-	SW EU	n/i	n/i	1	Goebel 1905
Pinus taeda L.	mega-	SE U.S.	Texas	n/i	n/i	Dormán 1976
Pinus thunbergii Parl.	mega-	E Asia	Maui, Hawaii	1965	n/i	Lanner, 1966b
Pinus thunbergii Parl.	mega-	E Asia	n/i	n/i	n/i	Matzuda 1892
Pseudotsuga menziesii (Mirb.) Franco[15]	mega-	W N America	n/i	n/i	n/i	Coulter & Chamberlain 1910
Pseudotsuga menziesii (Mirb.) Franco[16]	mega-	W N America	New York, US	1926	1	Littlefield 1931
Tsuga canadensis L.Carrière	mega-, midpt bisexual	E N America	Illinois	n/i	1	Holmes 1932
Tsuga caroliniana Engelm.	mega-	SE U.S.	England	2009–2011	2,+	Rudall et al. 2011[a]
Tsuga dumosa (D.Don.) Eichler	mega-	W China	England	2009–2011	2,+	Rudall et al. 2011[a]

or nutritional manipulation to obtain N-BS is of interest to silviculturists for the potential to increase and or promote earlier seed production in trees (Dormán 1976; Ross and Pharis 1986).

In general, megasporangia are distal and microsporangia are proximal in N-BS, similar to the arrangement in angiosperm flowers (Flores-Rentería et al. 2011). However, the reverse configuration has been reported in *Larix* (Chamberlain 1935; Pauley 1942; Table 1). In bisporangiate strobili, a neutral zone may be present between the two genders, such as that found in *Larix kaempferi* (Lamb.) Carrière (Momose 1961), *Picea abies* (L.) H.Karst (Stenzel 1876), *Pinus densiflora* Siebold & Zucc. (Doak 1935), and *Tsuga canadensis* L.Carrière (Holmes 1932). Transitional structures may also occur in the neutral zone such as in *Picea abies* (referred to as *Abies excelsea*; Dickson 1860), *L. europaea* DC. (Bartlett 1913), *Larix occidentalis* Nutt. (Kirkwood 1916), *Pseudotsuga menziesii* (Mirb.) Franco (referred to as *Pinus taxifolia*; Littlefield 1931), and *L. laricina* (DuRoi) K.Koch (Caron and Powell 1990).

N-BS occurrence may be associated with environmental stresses such as drought (Doak 1935; Zobel 1952; Black 1961), excess nitrogen (Meehan 1869; Fujii 1892; Doak 1935; Eis 1970), geographically marginal distributions (Fisher 1905; Doak 1935; Masters 1869; Elliot 1979), and in arboretum plantings outside of their natural range (Masters 1869; *Pinus montana Mill.*, Steil 1918; Zobel 1952). Megasporangia are promoted in trees of high vigor and preferentially on higher-vigor branches (Remphrey and Powell 1984), although teratological features were also observed on a low-vigor specimen of *Pinus contorta* (Douglas ex Louden) (Black 1961). Hormonal applications (cytokinins, Wakushima et al. 1997; gibberellins, Owens et al. 1990) also influence the occurrence of N-BS in Pinaceae species were not considered further here.

Here we report the first N-BS observation in *Pinus jeffreyi* A.Murray, in the *Pinus* subsect. Sabinianae (Willyard et al., 2021). The species is largely centered in the eastern Sierra Nevada, but extends to the western slope of the Sierra Nevada at mid to higher elevations (5500–7500 m; Fites-Kaufman et al. 2007), north into central Lake County in Oregon east of the Cascades (Grulke unpublished data), and south in the Peninsular Range to San Pedro Martir National Park, Baja Norte, Mexico. The intent of the retrospective bibliographic survey was in part to verify and identify unique reports of N-BS in the subfamily Pinoideae, to compare occurrences of the phenomenon, in wildlands vs. altered environments, and to explore the roles of extreme drought and nutritional amendments in promoting its expression.

METHODS

Location

The branchlet, with attached bisexual strobili, was collected in a mature, open-grown *P. jeffreyi*, in a dry pine-dominated woodland in Sequoia National Park, CA, near Crescent Meadow (36°33′16″N, 118°45′21″W, 2061 m) in 2006 by K. D. Marrett. Measurements of the strobili were made with a digital micrometer (to the nearest 0.01 mm). The voucher specimen was deposited and is retained in the Sequoia National Park herbarium, in Three Rivers, CA.

Sampling

The collection was made as part of a survey where the 3rd, 4th, or 5th secondary branchlet (2nd order) proximal to a primary branch terminus was sampled (no further back than one quarter of the distal end of the branch) in mid crown (11–13 m) of mature *P. jeffreyi* and *Pinus ponderosa*. These branchlets were sampled for another purpose: to assess tree crown vigor and drought stress level (e.g., needle retention, branchlet diameter, branch and needle elongation relative to the longest observed growth on the branchlet, needle color and condition, and presence of insects and pathogens; Grulke et al. 2020a). The observation of N-BS was within the context of 20 years of field observations of *Pinus jeffreyi* and *P. ponderosa P.* Lawson & C. Lawson var. *ponderosa* across a 750-km latitudinal gradient in four mountain ranges - southern Cascades Mountains, eastern and western slope of the Sierra Nevada, Transverse Range of southern California, and the Peninsular Range in southern California and its extension into Baja Norte, Mexico (San Pedro Martir Mountains) (Fig. 1). In all, approximately 51,000 observations of 1100 trees of the two species were made (Table 2).

Assembly of Observations

To construct Table 1, all unique, verifiable observations of N-BS were searched for until no additional references were discovered. The following sources were searched: Google Scholar, TreeSearch, USDA Forest Service Library, Boyce Thompson Institute Library, Harvard Library, University of Idaho Library, National Agriculture Library, and HathiTrust digital library. An extensive search was made in Google Scholar. Key words included: androgynous, bisexual, bisporangiate, hermaphroditic, teratological, abnormal, and ‘monstrous’ cone. The latter term was common in publications prior to 1900; but often this term denoted proliferated cones, unusual growth patterns (e.g., tissue adherence of multiple unisporangiate strobili), or an unusual abundance or locations of unisporangiate strobili. Species names and authorities were listed according to the International Plant Names Index. The EPPO Global Database was used to cross reference older to more modern nomenclature.

In several publications older than 1875, a reference listed nothing to do with N-BS. For example, Masters (1869) and subsequent authors cite N-BS reported by von Mohl, Schleiden, and A. Braun (1853) in *Flora* Volume 5. However, the article was found and on that page is an argument presented against a colleague by a Dr. Walpers with no reference to N-BS. It is possible

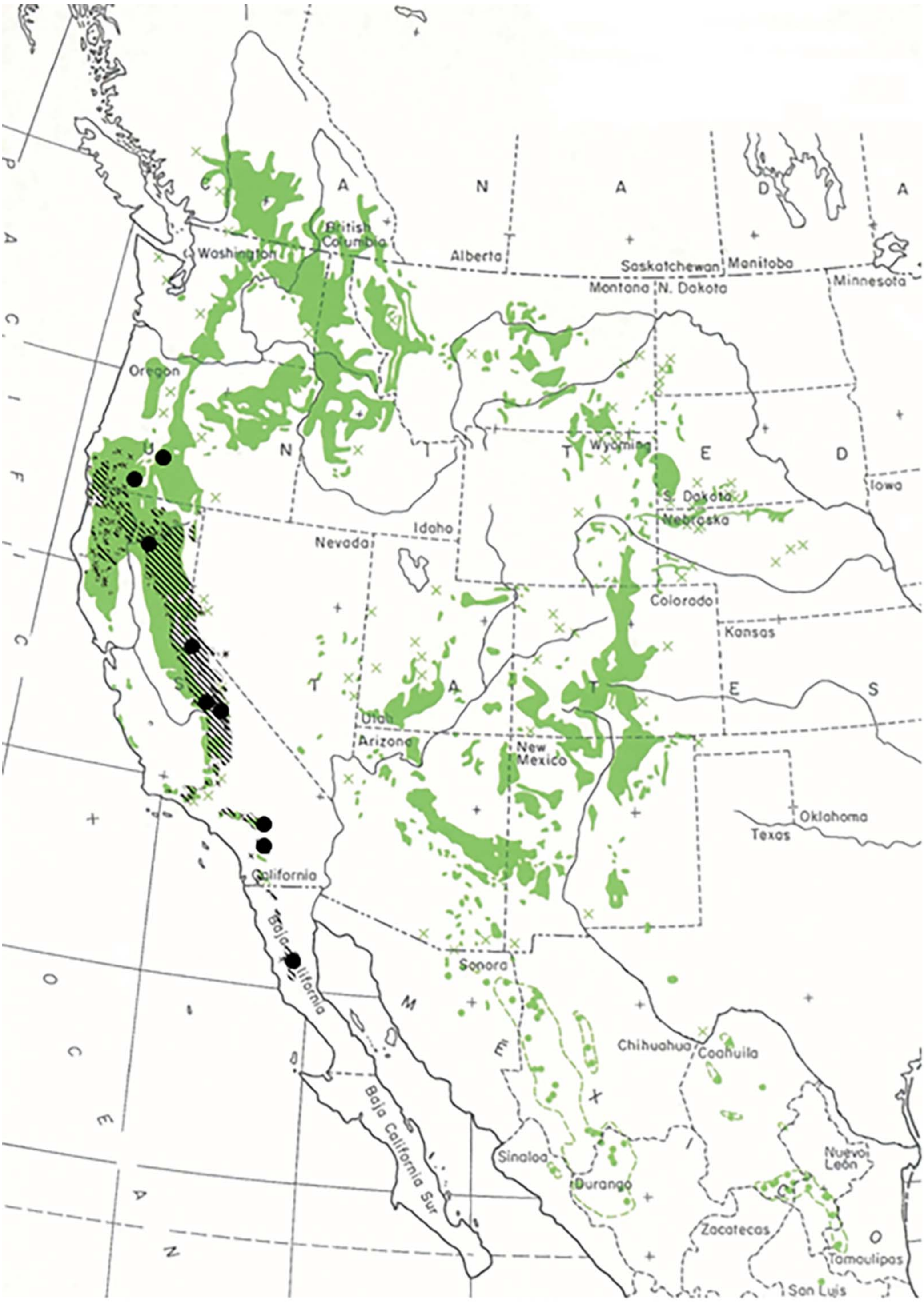

FIG. 1. Location of observations in western North America, listed in Table 2. Base map modified from Jenkinson's (1990) distribution map of *P. jeffreyi*. The coastal length of Oregon is 474 km.

Table 2. Locations of *Pinus jeffreyi* (PJ) and *P. ponderosa* (PP) observed in this study. 'NF' refers to USDA National Forest; 'NP' refers to USDI National Park.

Jurisdictional area	Site	Geophysical location	Aspect/ location	GPS	Species	Years	# Branches	# Trees	# Observations
Sycan Preserve	Sycan Marsh	Basin & Range	eastern slope	42°52′15″N, 120°54′31″W, 1905 m	PP	2014–2019	36	390	14040
Crater Lake NP	Munson Valley	Cascade Mountains	eastern slope	42°56′46″N, 122°01′14″W, 2128 m	PJ, PP	1991	3	16	48
Lassen NF	Mineral, CA	Cascade Mountains	western slope	40°20′29″N, 121°25′19″W, 1725 m	PP	1991	3	32	96
Lassen NF	Fredonyer Butte	Cascade Mountains	eastern slope	40°21′50″N, 120°47′31″W, 1572 m	PJ	2007–2009, 2012, 2014, 2017	36	68	2448
Tahoe NF	Boca Reservoir	Sierra Nevada	eastern slope	39°24′23″N, 120°0319″W, 1984 m	PJ	2007–2009, 2012, 2014, 2017	36	48	1728
Inyo NF	Mammoth Lakes	Sierra Nevada	eastern slope	37°42′09″N, 118°54′16″W, 2444 m	PJ	2007–2009, 2012, 2014, 2017	36	48	1728
Sequoia NF	Stoney Creek	Sierra Nevada	western slope	36°40′00″N, 118°49′52″W, 1979 m	PJ	1998–2012, 2014, 2017	96	64	6144
Sequoia NP	Lodgepole	Sierra Nevada	western slope	36°36′04″N, 118°44′17″W, 2117 m	PJ	1998–2012, 2014, 2017	96	132	12672
Sequoia NP	Crescent Meadow	Sierra Nevada	western slope	36°33′13″N, 118°45′07″W, 2045 m	PJ	1998–2012, 2014, 2017	96	32	3072
Sequoia NP	Crystal Cave Rd.	Sierra Nevada	western slope	36°34′31″N, 118°47′07″W, 1593 m	PJ, PP	1991	3	16	48
Sequoia NF	Kennedy Meadows	Sierra Nevada	eastern slope	36°03′37″N, 118°11′04″W, 2409 m	PJ	2007–2009, 2012, 2014, 2017	36	48	1728
San Bernardino NF	Crestline	Transverse Range	west-side	34°14′15″N, 117°19′15″W, 1619 m	PP	1993–1996	21	30	630
San Bernardino NF	Strawberry Peak	Transverse Range	west-side	34°14′20″N, 117°14′32″W, 1616 m	PP	1993–1995	18	30	540
San Bernardino NF	Fawnskin, CA	Transverse Range	east-side	34°17′06″N, 116°57′08″W, 2266 m	PJ	2007–2009, 2012, 2014, 2017	96	40	3840
San Bernardino NF	Holcomb Valley	Transverse Range	east-side	34°17′38″N, 116°52′23″W, 2239 m	PJ	2007–2009, 2012, 2014, 2017	96	20	1920
San Bernardino NF	Barton Flats	Transverse Range	east-side	34°09′53″N, 116°50′50″W, 2068 m	PP	1993–1995	18	30	540
San Jacinto State Park	Long Valley	Transverse Range	east-side	33°48′45″N, 116°38′03″W, 2417 m	PJ, PP	1991	3	16	48
San Jacinto State Park	Idyllwild	Transverse Range	east-side	33°47′23″N, 116°44′19″W, 1879 m	PJ, PP	1991	3	16	48
San Pedro Matir NP	Vallejito	Peninsular Range	western slope	30°53′19″N, 115°34′07″W, 1856 m	PJ	1991	3	32	96

that the year, volume number, or page number was incorrect; however, a search of a 2 years before and after publication in this journal still did not yield the reported observation of N-BS. In some cases, the same observation was repeated as a new observation by different authors; we reported the first publication that could be verified. For example, N-BS in *Pinus elliotii* Engelm. observed by Melchamps was reported in a letter to Jack (1895), who quoted the letter in his publication. A second publication of N-BS in the same species (Christ 1895) was identified as the same observation by Jack (1895). Dormán (1976) published a photo of an occurrence of N-BS in *Pinus taeda* L. attributed to Zobel and Goddard (1954), but as the latter authors did not identify it as such, so it was attributed to Dormán (1976); the photo was the same. Bencat (1960, in Czech) reported a N-BS occurrence in *Pinus mugo* Turra, cited in Giertych 1960 (in Polish), but there were no photos of the occurrence and the nuances of the Czech observation could not be verified by a Russian language expert. An observation of N-BS in *Picea abies* (L.) H. Karst by Flandung et al. in 1999 (as reported in Theissen and Becker 2004) was not included because the book containing the original could not be obtained to verify. Fujii (1892) and Matzuda (1892) are both cited as observing N-BS in *Pinus thunbergii* Parl. in the literature (e.g., Atkinson 1897), but are believed to be the same observation (same year, same location). Only Matzuda's publication (in Japanese) is cited in the table because a plate labeled with the species name in Latin is included in his publication. A report of N-BS in *Pinus palustris* Mill. described by Dormán 1976 (Pg. 33, Fig. 23) showed terminal strobili (subtending next year's bud) with distal staminate flowers and a swelling above, but no differentiated ovules. The location of two observations is marked as 'assumed' (*Larix decidua* Mill.; *Pinus densiflora* (Siebold & Zucc.; Doak 1935) because the author was employed by Texas A&M before, after, and at the time of observation, and other observations reported in the same publication cite 'Stations in Texas' and 'College Station, Texas.'

RESULTS

One non-binary strobilus was observed on one branch, in one year, on the southern exposure in the upper mid-crown of mature *P. jeffreyi*. Similar branch samples had been taken on this tree for the previous 8 years, and subsequently for the following 11 years, without an additional N-BS observation. The N-BS differentiated in 2006, which was not a drought year in Sequoia National Park (Kim et al. 2022).

The total length of the strobilus was 38 mm, with the distal female portion 12 mm long and the proximal male portion 14 mm long (Fig. 2). The diameter at the base of the male portion was 14.5 mm and was thicker (7.3 mm) relative to a wholly male strobilus, similar to that reported in Dickson (1860) and others. The stem was 12 mm in length and 6.2 mm diameter at its base. The female portion was of the same developmental stage as fully megasporangiate cones. At the time of this report, no transitional forms between microsporophylls and leaves were found. No N-BS were observed in mature *P. ponderosa*. Proliferated seed cones were not observed in either species.

FIG. 2. Branchlet with non-binary strobili in Jeffrey pine collected in Sequoia National Park. The total length of the strobili was 38 mm.

DISCUSSION

Flores-Renterìa et al. (2011) and others have suggested that N-BS are seen and noted more frequently in plantations, parks, domestic plantings, or on the margins of the tree distributions, perhaps because they are more frequently observed in those locations. They posit that trees in these locations may have access to greater or lesser nutrition or experience other types of stress than in their native habitat, and thus N-BS may occur more frequently. In plantations, N-BS are rare (e.g., ~0.5% in *Picea mariana* Du Roi, Caron, and Powell 1990); in our study, N-BS of *P. jeffreyi* in wildlands is rarer (ca. 0.00002%). However, outplanting of plantation-produced seeds may have undesirable outcomes, such as fewer seeds produced on N-BS (Doak 1935; Dormán 1976).

The N-BS in mature *P. jeffreyi* was collected at a site that experienced moderately high nitrogen deposition (Fenn et al. 2003) in a National Park. However, other sites sampled and reported here have much higher nitrogen deposition, such as Crestline and Strawberry Peak in the San Bernardino Mountains (Grulke et al. 1998; Fenn et al. 2003), but with no observations of N-BS. Drought has been suggested as an elicitor of N-BS; however, it was not a particularly dry or hot year during bud differentiation (2005) in Sequoia National Park (Kim et al. 2022), and many trees experienced greater physiological drought stress at other sites in this Park (Lodgepole, Table 2; Grulke et al. 2003; Grulke et al. 2020b), and outside the Park (Barton Flats, San Bernardino Mountains [Grulke 1999]; Sycan Preserve in south central Oregon [Grulke et al., 2020b]; and San

Pedro Matir National Park, MX [Grulke 2010]). No other occurrence of N-BS was observed in *P. jeffreyi*, and no occurrence was observed in *P. ponderosa*. An unexplored environmental stress is the effect of toxins aerially transported into Sequoia National Park from upwind agricultural fields in the California Central Valley (Jovan and McCune 2006).

Caron and Powell (1990) posit that environmental stress was not likely to have a role in the occurrence of N-BS in *Picea mariana*. They suggest that "the mechanisms controlling gender differentiation on some trees may be weakly separated when strobilus bearing starts." In young plantation-grown *Larix laricina*, N-BS occurred repeatedly (Tosh and Powell 1986) but did not appear to differ among the three years of observation. Our observation suggests that N-BS may develop at any tree age, albeit rarely. Flores-Rentería et al. (2011) argue that perhaps the occurrence of N-BS is not teratological, but instead should be considered an ancient, albeit occasional, retained trait.

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