Botrychium ascendens W.H. Wagner (trianglelobe moonwort), *Botrychium crenulatum* W.H. Wagner (scalloped moonwort), and *Botrychium lineare* W.H. Wagner (narrowleaf grapefern): A Technical Conservation Assessment

B. ascendens

B. lineare

B. crenulatum

Prepared for the USDA Forest Service,
Rocky Mountain Region,
Species Conservation Project

November 12, 2003

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Peer Review Administered by
Center for Plant Conservation

Beatty, B.L., W.F. Jennings, and R.C. Rawlinson (2003, November 12). *Botrychium ascendens* W.H. Wagner (trianglelobe moonwort), *B. crenulatum* W.H. Wagner (scalloped moonwort), and *B. lineare* W.H. Wagner (narrowleaf grapefern): a technical conservation assessment. [Online]. USDA Forest Service, Rocky Mountain Region. Available: http://www.fs.fed.us/r2/projects/scp/assessments/botrychiums.pdf [date of access].

ACKNOWLEDGEMENTS

We acknowledge several botanists and land management specialists for providing helpful input, including Kathleen Ahlenslager, George Cameron, Alan Carpenter, Walter Fertig, Craig Freeman, Scott Gall, Kent Houston, Cindy Johnson-Groh, Andrew Kratz, David Ode, Peter Root, Richard Schneider, Susan Spackman, Steve Tapia, Mary Clare Weatherwax, and Tara Williams. Natural Heritage Programs and herbaria within USFS Region 2 supplied current occurrence records of this species from their databases and collections. We thank Mike Hays/Idaho Conservation Data Center (photo), Walter Fertig/Wyoming Natural Diversity Database (illustration), Linda Swartz/Idaho Conservation Data Center (photo), David Wagner (illustration), J. Sellers/Colorado Natural Heritage Program (photo), and Janet Wingate/Colorado Natural Heritage Program (illustration) for permission to use their *Botrychium* images. Funding for this document was provided by U.S. Forest Service, Rocky Mountain Region (Region 2) contract 53-82X9-1-123.

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COVER PHOTO CREDIT

Botrychium ascendens photo was taken by Mike Hays, reprinted with permission from the Idaho Conservation Data Center, *B. crenulatum* photo was taken by Linda Swartz, reprinted with permission from the Idaho Conservation Data Center, and *B. lineare* photo was taken by J. Sellers, reprinted with permission from the Colorado Natural Heritage Program.

NEW INFORMATION

New information on distribution and abundance of *Botrychium lineare* is available at http://www.fs.fed.us/r2/projects/scp/assessments/newinfo.shtml.

Summary of Key Components for Conservation of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare*

Status

The Global Heritage Status Ranks for *Botrychium ascendens* (trianglelobe moonwort), *B. crenulatum* (scalloped moonwort), and *B. lineare* (narrowleaf grapefern) are G2G3 (between imperiled and vulnerable), G3 (vulnerable), and G1 (critically imperiled), respectively (NatureServe 2003). Within U.S. Forest Service (USFS) Region 2, *B. ascendens* is known from four occurrences in Wyoming, with three occurrences in Shoshone National Forest and one occurrence in Bighorn National Forest (Wyoming Natural Diversity Database 2003). *Botrychium crenulatum* has been reported, but not confirmed, from one location in Wyoming, in Bighorn National Forest (Wyoming Natural Diversity Database 2003). New information on the distribution and abundance of *Botrychium lineare* is available at http://www.fs.fed.us/r2/projects/scp/assessments/newinfo.shtml. There is no documented occurrence of *B. lineare* on the White River National Forest. *Botrychium lineare* was confirmed on the Black Hills National Forest (Wyoming) in December 2003. *Botrychium lineare* is known from six occurrences in Colorado, with two occurrences in Pike-San Isabel National Forest, one occurrence in White River National Forest, two occurrences in Arapaho-Roosevelt National Forest, and one occurrence in Rocky Mountain National Park (University of Colorado Herbarium 2002, Colorado Natural Heritage Program 2003). *Botrychium ascendens* is ranked in Wyoming as S1 (critically imperiled), *B. crenulatum* is ranked in Wyoming as SR (state-reported), and *B. lineare* is ranked in Colorado as S1 (critically imperiled) (NatureServe 2003). *Botrychium lineare* is currently on the USFS Rocky Mountain Region sensitive species list (U.S. Forest Service 2003). *Botrychium lineare* is a candidate for listing as threatened under the federal Endangered Species Act of 1973 (16 U.S.C. 1531-1536, 1538-1540).

Primary Threats

Botrychium ascendens, *B. crenulatum*, and *B. lineare* are considered to be very rare, with few documented occurrences, small population abundances, and widely-disjunct occurrences within large ranges. *Botrychium* species throughout western North America may be threatened by a variety of factors: road construction and maintenance, herbicide application, recreational activities, grazing and trampling by wildlife and/or livestock, structure construction, timber harvest, competition from non-native species, and changes to natural disturbance regimes. Disturbances and land management activities may create and maintain suitable habitat for this species or may negatively impact existing populations, depending on the disturbance intensity and frequency. The specific threats to *B. ascendens*, *B. crenulatum*, and *B. lineare* within Region 2 are largely unknown or unassessed. Although no immediate concerns have been identified, existing populations of *B. ascendens* and *B. lineare* have few individuals and cover a small area. Thus, a random, catastrophic disturbance could destroy these populations completely. The only population of *B. crenulatum* in Region 2 has not been confirmed or relocated in recent years, and the status of this occurrence is unknown. The primary threats to existing populations of *B. ascendens*, *B. crenulatum*, and *B. lineare* in Region 2, given the current understanding, are: road, trail, or structure construction and maintenance; trampling by wildlife, livestock, or off-trail recreational activities; competition from non-native plant species; natural habitat succession or fire suppression; and changes in hydrology affecting soil moisture or mycorrhizal existence. Specific populations could be at a greater risk than other populations, depending on the landscape context, characteristics of the natural and human disturbance regimes, and biological characteristics of each species. For example, *B. crenulatum* tends to be found in wetter habitats, and some populations of this species could potentially be less threatened by damage from recreational activities than *B. ascendens* or *B. lineare*.

Primary Conservation Elements, Management Implications and Considerations

Not enough data are available to assess the long-term viability of these *Botrychium* species within Region 2. For each of these species, the current distribution, abundance, microhabitat needs, and disturbance regime (intensity, frequency, size, and type of disturbance) optimal for persistence of the species are unknown. The lack of information regarding the dispersal and colonizing ability, mycorrhizal relationships, adaptability to changing environmental conditions, reproductive potential, or genetic variability of these species makes it difficult to predict their long-term vulnerability. Possible key conservation elements for *B. ascendens*, *B. crenulatum*, and *B. lineare* include surveying high probability habitat for new populations; protecting and buffering existing populations from direct damage;

documenting and monitoring the effects of current management activities; preserving landscape diversity to facilitate establishment of additional populations; and studying mycorrhizal relationships, the effects of trophophore and sporophore removal, spore longevity and dispersal, microsite requirements, and effects of environmental fluctuations. These species are all highly dependent on soil mycorrhizal health for successful growth and establishment. They may not thrive where mycorrhizae are absent, dry soils inhibit growth or reproductive processes, or weedy plants dominate and compete for resources. *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* individuals may survive removal of aboveground tissues, as long as primordial tissues are intact. Continuous removal of sporangia before dispersal, however, may affect long-term population or species viability. Landscape diversity may be important in providing a mosaic of patches for *Botrychium* dispersal and establishment through time and space.

Management implications indicate the need for additional, detailed, long-term monitoring programs and research studies. Critical components of these research efforts could include the following:

- direction of additional surveys to locate existing populations within Region 2
- determination of population distribution, abundance, density
- investigation of factors affecting spatial distribution (microhabitat characteristics, disturbance dynamics, dispersal)
- determination of relationships with soil mycorrhizae
- investigation of threats affecting population establishment, growth, and reproduction
- investigation of effects of habitat fragmentation or isolation
- characterization of temporal patterns of abundance
- investigation of effects of climate fluctuations on abundance
- determination of demographics (age structure of population, recruitment, age of breeding, fertility, annual rate of survival)
- investigation into viability and minimum number of plants necessary to perpetuate the species
- investigation on patterns of population size, and spore fertility, dormancy, and viability

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INTRODUCTION

This assessment is one of many being produced to support the Species Conservation Project for the Rocky Mountain Region (Region 2), U.S. Forest Service (USFS). Three *Botrychium* species, *B. ascendens* (trianglelobe moonwort), *B. crenulatum* (scalloped moonwort), and *B. lineare* (narrowleaf grapefern) , are the focus of an assessment, because they are or have the potential to be listed as sensitive species in Region 2 (**Table 1**). *Botrychium lineare* is currently listed as a sensitive species within Region 2, but *B. ascendens* and *B. crenulatum* are not currently listed (U.S. Forest Service 2003). Within the National Forest System, a sensitive species is a species whose population or habitat viability is identified as a concern by a regional forester because of significant current or predicted downward trends in population numbers or habitat capability that would reduce its distribution (U.S. Forest Service 1995). Sensitive species may require special management, so knowledge of their biology and ecology is critical.

Table 1. Conservation and management status of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare*, as ranked by the U.S. Forest Service, U.S. Fish and Wildlife Service, NatureServe, and Natural Heritage Programs in Region 2 states.

Listing	*Botrychium ascendens*	*Botrychium crenulatum*	*Botrychium lineare*
U.S. Forest Service Sensitive Species List[1]	Not listed	Not listed	Sensitive
U.S. Fish and Wildlife Service Endangered Species Act[2]	Not listed	Not listed	Candidate
NatureServe Global Ranking[3]	Imperiled to Vulnerable (G2G3)	Vulnerable (G3)	Critically imperiled (G1)
Wyoming Natural Diversity Database	Critically imperiled (S1)	State reported (SR)	Not listed
Colorado Natural Heritage Program	Not listed	Not listed	Critically imperiled (S1)
Kansas, Nebraska, South Dakota Natural Heritage Programs	Not listed	Not listed	Not listed

[1]As designated by a USFS Regional Forester; population viability is a concern due to downward trends in population numbers, density, or habitat capability.

[2]Candidate is any species being considered for listing as an endangered or a threatened species but not yet the subject of a proposed rule.

[3]Key to rankings: G = Global rank based on rangewide status, S= State rank based on status of a species in an individual state.

G1 Critically imperiled globally because of extreme rarity (five or fewer occurrences or very few remaining individuals) or because of some factor making it especially vulnerable to extinction.

G2 Imperiled globally because of rarity (six to 20 occurrences) or because of factors demonstrably making a species vulnerable to extinction.

G3 Vulnerable throughout its range or found locally in a restricted range (21 to 100 occurrences) or because of other factors making it vulnerable to extinction.

G4 Apparently secure, though it may be quite rare in parts of its range, especially at the periphery.

G5 Demonstrably secure, though it may be quite rare in parts of its range, especially at the periphery.

S1 Critically imperiled in the state because of extreme rarity (five or fewer occurrences or very few remaining individuals) or because of some factor making it especially vulnerable to extinction.

S2 Imperiled in the state because of rarity (six to 20 occurrences) or because of factors demonstrably making a species vulnerable to extinction.

S3 Vulnerable throughout its statewide range or found locally in a restricted statewide range (21 to 100 occurrences) or because of other factors making it vulnerable to extinction.

S4 Apparently secure, though it may be quite rare in parts of its statewide range, especially at the periphery.

S5 Demonstrably secure, though it may be quite rare in parts of its range, especially at the periphery.

This assessment addresses the biology of the three *Botrychium* species throughout their ranges in Region 2. The broad nature of the assessment leads to some constraints on the specificity of information for particular locales. Furthermore, completing the assessment promptly requires establishment of some limits concerning the geographic scope of particular aspects of the assessment and further analysis of existing, but unanalyzed field data. This introduction defines the goal of the assessment, outlines its scope, and describes the process used in its production.

Goal

Species conservation assessments produced as part of the Species Conservation Project are designed to provide forest managers, research biologists, and the public with a thorough discussion of the biology, ecology, conservation status, and management of certain species based on available scientific knowledge. The assessment goals limit the scope of the work to critical summaries of scientific knowledge, discussions of broad implications of that knowledge, and outlines of information needs. The assessment does not seek to develop specific management recommendations but provides the ecological background upon which management must be based. However, it does focus on the consequences of changes in the environment that result from management (i.e., management implications). Additionally, the assessment cites management recommendations proposed elsewhere and, when management recommendations have been implemented, the assessment examines the success of those implementations.

Scope and Information Sources

The *Botrychium* species assessment examines the biology, ecology, conservation status, and management of these species with specific reference to the geographic and ecological characteristics of Region 2. Although some of the literature on the species originates from field investigations outside the region, this document places that literature in the ecological and social context of the central Rocky Mountains. Similarly, this assessment is concerned with the reproductive behavior, population dynamics, and other characteristics of *B. ascendens*, *B. crenulatum*, and *B. lineare* in the context of the current environment rather than under historical conditions. The evolutionary environment of the species is considered in conducting the synthesis, but within the current context.

In producing the assessment, a comprehensive literature search was conducted to obtain all literature focusing on *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* in Region 2. We collected and reviewed refereed literature, non-refereed publications, research reports, data accumulated by resources management agencies (e.g., Natural Heritage Programs [NHP]), regulatory guidelines, and personal communications with species experts and forest botanists. We incorporated herbarium specimen label information provided by herbarium staff and available in NHP element occurrence records, but we did not visit every herbarium with specimens of this species. This assessment emphasizes refereed literature, because this is the accepted standard in science. However, non-refereed publications and reports are used extensively in this assessment, because they provided critical information unavailable elsewhere. These unpublished, non-refereed reports were regarded with greater skepticism, and we presented all information with appropriate uncertainty. In addition, we highlighted areas of current research with these species and cited if these studies were in progress, preparation, or press.

Treatment of Uncertainty

Science represents a rigorous, synthetic approach to obtaining knowledge. Competing ideas regarding how the world works are measured against observations. However, because our descriptions of the world are always incomplete and our observations are limited, science focuses on approaches for dealing with uncertainty. Sorting among alternatives may be accomplished using a variety of scientific tools (e.g., experiments, modeling, logical inference). It is difficult to conduct critical experiments in the ecological sciences and often observations, inference, good thinking, and models must be relied upon to guide the understanding of ecological relations.

In this assessment, the strength of evidence for particular ideas is noted, and alternative explanations are described when appropriate. While well-executed experiments represent a strong approach to developing knowledge, alternative approaches such as modeling, critical assessment of observations, and inference are accepted as sound approaches to understanding features of biology and are used in synthesis for this assessment.

Because of a lack of experimental research efforts concerning *Botrychium ascendens*, *B.*

crenulatum, and *B. lineare*, this assessment report relies heavily on the personal observations of botanists and resource management specialists from throughout the species' ranges. When information presented in this assessment is based on our personal communications with specialists, we cite those sources as "personal communication." In addition, much of the knowledge about the status of *B. lineare* is presented in unpublished survey reports prepared by Carpenter (1996a, 1996b), Root (1999), and Tapia (2000, personal communication 2003). Unpublished data (e.g., NHP element occurrence records) were also important in estimating the geographic distribution and describing the habitat of these species. These data required special attention because of the diversity of persons and variety of methods used to collect the data, and unverified historical information.

Because there is a paucity of knowledge specific to these species, we also incorporated information about these three *Botrychium* species from outside Region 2 and work from other closely related *Botrychium* species to formulate this assessment. These comparisons are not meant to imply that *B. ascendens*, *B. crenulatum*, and *B. lineare* are biologically identical to these other species, but they represent an effort to present possible biological characteristics of *Botrychium* species, important considerations when studying these species, and potential threats. As a result, biology, ecology, and conservation issues presented for *B. ascendens*, *B. crenulatum*, and *B. lineare* in Region 2 are based on inference from these published and unpublished sources. We clearly noted when we were making inferences based on the available knowledge to inform our understanding of *B. ascendens*, *B. crenulatum*, and *B. lineare*.

Publication of Assessment on the World Wide Web

To facilitate use of species assessments in the Species Conservation Project, they are being published on the Region 2 World Wide Web site. Placing documents on the Web makes them available to agency biologists and the public more rapidly than publishing them as reports. More importantly, it facilitates revision of the assessments, which will be accomplished based on guidelines established by Region 2.

Peer Review

Assessments developed for the Species Conservation Project have been peer reviewed prior to release on the Web. This assessment was reviewed through a process administered by the Center for Plant Conservation, employing at least two recognized experts on this or related taxa. Peer review was designed to improve the quality of communication and increase the rigor of the assessment.

Management Status and Natural History

This section discusses the special management status, existing regulatory mechanisms, and biological characteristics of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* in Region 2.

Management and Conservation Status

This section outlines the management and conservation status of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* based on federal and heritage program rankings. These rankings are summarized in **Table 1**.

The Endangered Species Act of 1973 (ESA) was passed to legally protect plant and animal species placed on the threatened or endangered list. The listing process is based on population data and is maintained and enforced by the U.S. Fish and Wildlife Service (USFWS). Within the National Forest System, a sensitive species is a plant or animal whose population viability is identified as a concern by a regional forester because of significant current or predicted downward trends in habitat capability that would reduce its distribution (U.S. Forest Service 1995). These federal rankings are associated with specific legal protection and management mandates for these species.

The Global Heritage Status Ranks and State Heritage Status Ranks represent the range-wide and state-wide conservation status of species, respectively, as published by NatureServe (2003). State NHPs collect information about the biological diversity of their respective states and maintain databases of species of concern. These heritage rankings draw attention to species potentially requiring conservation strategies for future success, but these ranks are not associated with specific legal constraints. Within Region 2, the three *Botrychium* species presented in this assessment occur in Colorado or Wyoming, so we present state heritage ranks as designated by the Colorado NHP (Colorado Natural Heritage Program 2002) and Wyoming Natural Diversity Database (WYNDD) (Fertig and Heidel 2002). In addition to heritage status ranks, WYNDD also ranks conservation priority for plant species on a three-part scale (low, medium, and high) according to their global ranking. The three *Botrychium* species discussed in this assessment are not listed (nor known to exist) in other states of Region 2 (South Dakota, Kansas, and Nebraska), although other *Botrychium* species are species of concern in those states (Kansas Natural Heritage Inventory 2002, Nebraska Natural Heritage Program 2002, South Dakota Natural Heritage Program 2002).

Status of *Botrychium ascendens*

Botrychium ascendens is not listed nor considered a candidate for the threatened or endangered species list of the ESA (U.S. Fish and Wildlife Service 1996). This species is not currently listed as a sensitive species by the Region 2 (U.S. Forest Service 2003). The Global Heritage Status Rank for *B. ascendens* is G2G3 (between imperiled and vulnerable) (NatureServe 2003). WYNDD has designated *B. ascendens* as a critically imperiled (S1) species, as a result of its small number of occurrences in the state (Wyoming Natural Diversity Database 2003). In addition, this moonwort is ranked as a "medium" conservation priority in the state of Wyoming. Medium priority species are state or regional endemics ranked G3 or of higher concern that receive some protection or have low threats, or disjunct species that are poorly protected (Wyoming Natural Diversity Database 2003). *Botrychium ascendens* is not listed as a species of special concern with other NHPs within Region 2, because it is not currently known from those states (Colorado Natural Heritage Program 2002, Kansas Natural Heritage Inventory 2002, Nebraska Natural Heritage Program 2002, South Dakota Natural Heritage Program 2002). Outside Region 2, *B. ascendens* is ranked as S1 (critically imperiled) in California, Idaho, Montana, Nevada, Alberta, Saskatchewan, and Yukon Territory; as S2 (imperiled) in Alaska, Oregon, Washington; as S2S3 (between imperiled and vulnerable) in British Columbia; and as SH (possibly extirpated) in Ontario (NatureServe 2003).

Status of *Botrychium crenulatum*

Botrychium crenulatum is not listed nor considered a candidate for the threatened or endangered species list of the ESA (U.S. Fish and Wildlife Service 1996). Currently, this species is not listed as a sensitive species by Region 2 (U.S. Forest Service 2003). The Global Heritage Status Rank for *B. crenulatum* is G3 (vulnerable) (NatureServe 2003). *Botrychium crenulatum* was previously ranked as S1 (critically imperiled) in Wyoming as a result of its small number of occurrences in the state, but this species was re-ranked to SR (state reported) in 2001. This re-ranking was necessary because the historical occurrence of *B. crenulatum* was not confirmed by a voucher (i.e., specimen verified by an expert), and the stand of plants has not been found since (W. Fertig personal communication 2002). *Botrychium crenulatum* is not listed as a species of special concern with other NHPs within Region 2 because it is not currently known from those states (Colorado Natural Heritage Program 2002, Kansas Natural Heritage Inventory 2002, Nebraska Natural Heritage Program 2002, South Dakota Natural Heritage Program 2002). Outside Region 2, *B. crenulatum* is ranked as S1 (critically imperiled) in Idaho, Nevada, Utah, and Alberta; as S2 (imperiled) in California, Montana, and Oregon; as S3 (vulnerable) in Washington; as S1S3 (between critically imperiled and vulnerable) in British Columbia; and as SH (possibly extirpated) in Arizona (NatureServe 2003).

Status of *Botrychium lineare*

A petition was filed with the USFWS in July of 1999 by the Biodiversity Legal Foundation requesting that *Botrychium lineare* be listed as an endangered or threatened species under ESA. In May of 2000, the USFWS published a 90-day petition finding concluding that the petition filed by the Biodiversity Legal Foundation presented substantial information that action may be warranted. Further review (12-month finding) of the petition by the USFWS revealed that sufficient information was available to support a finding to list *B. lineare* as a threatened species. However, the 12-month finding by the USFWS explained that although there was sufficient information to list the *B. lineare* as a threatened species, a proposed ruling on *B. lineare* was precluded by "work on other higher priority listing actions." Additionally, future funding for listing actions will likely be reserved for emergency listings only (U.S. Fish and Wildlife Service 2001). Therefore, *B. lineare* is currently listed as a candidate for threatened status under the ESA.

Currently, *Botrychium lineare* is listed as a sensitive species by Region 2 (U.S. Forest Service 2003). The Global Heritage Status Rank for *B. lineare* is G1 (critically imperiled) (NatureServe 2003). The Colorado NHP (2002) ranks this species as S1 (critically imperiled) because of its extremely low abundance within the state. *Botrychium lineare* is not listed as a species of special concern with other NHPs within Region 2, because it is not currently known from those states (Kansas Natural Heritage Inventory 2002, Nebraska Natural Heritage Program 2002, South Dakota Natural Heritage Program 2002, Wyoming Natural Diversity Database 2003). Outside

Region 2, *B. lineare* is ranked as S1 (critically imperiled) in California, Montana, Oregon, Utah, and Washington; as SH (possibly extirpated) in Idaho and Quebec; and as SU (unrankable) in New Brunswick (NatureServe 2003).

Existing Regulatory Mechanisms, Management Plans, and Conservation Practices

This section details regulatory mechanisms, management plans, and conservation strategies that currently exist for *Botrychium ascendens*, *B. crenulatum*, and *B. lineare*.

Botrychium lineare has been designated as a sensitive species by Region 2. As a result, this species may obtain some protection under various conservation strategies designed to protect plants and animals within federal lands. While managing lands for multiple use, the USFS is directed to develop and implement management practices to ensure that species do not become threatened and endangered (U.S. Forest Service 1995). The National Environmental Policy Act (U.S. Congress 1982) requires an assessment of impacts of any federal projects (e.g. USFS, National Park Service, or Bureau of Land Management) to natural environments. In addition, the National Park Service prohibits the collection of any native plants without a permit (U.S. National Park Service 2002). The USFS prohibits the collection of any sensitive plant species except by permit (U.S. Forest Service 1995). Travel management plans also protect rare species by restricting off-highway vehicle use to established roads and trails only (U.S. Forest Service and Bureau of Land Management 2000). While *B. lineare* has been identified as a candidate for listing as a threatened species under the ESA, final listing has been delayed due to budget constraints, retarding the establishment of conservation efforts at the federal level.

Colorado NHP and WYNDD have identified *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* as species of concern (**Table 1**). Natural Heritage Program databases draw attention to species potentially requiring conservation strategies for future success. In addition, based on element occurrence data, NHPs designate Potential Conservation Areas that are important to the long-term survival of targeted species and natural communities. However, these lists and Potential Conservation Areas are for planning purposes only, and they are not associated with specific legal constraints, such as restricting damage to habitats supporting these plants. The Colorado NHP has designated the Cascade Creek Potential Conservation Area in Pike-San Isabel National Forest as an area of outstanding biodiversity significance due to the presence of the largest *B. lineare* population in the world (Fayette and Grunau 1998).

Of the three species of *Botrychium* addressed in this assessment, a monitoring study has been initiated only for *B. lineare*. This monitoring program was initiated by The Nature Conservancy in 1995 to measure abundance, assess population trends, and determine the longevity of individuals for the largest population of *B. lineare* found on Pikes Peak in Colorado (Pike-San Isabel National Forest) (Carpenter 1996a, 1996b). Preliminary results from monitoring in 1995, 1996, and 2000 are detailed in the Population trends section (Carpenter 1996a, Carpenter 1996b, Tapia 2000, A. Carpenter personal communication 2003, S. Tapia personal communication 2003).

Existing laws and regulations are inadequate to conserve *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* over the long term. Although these species have been identified as taxa of special concern, there are few specific regulatory mechanisms at the federal or state level to ensure their conservation.

Biology and Ecology

Classification and description

Systematics and synonymy

The genus *Botrychium*, part of the Ophioglossaceae family within the Ophioglossales order of Division Pteridophyta in the plant kingdom, is generally divided into three subgenera in North America. These three subgenera are *Osmundopteris* (rattlesnake fern), *Sceptridium* (grapeferns), and *Botrychium* (moonworts). *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* are all species within the subgenus *Botrychium* (Lellinger 1985).

History of species

The systematics of the *Botrychium* subgenus *Botrychium* have been particularly problematic and controversial (Clausen 1938, Tryon and Tryon 1982, Wagner and Wagner 1993, Zika et al. 1995, Hauk and Haufler 1999). Using morphological features, Clausen (1938) recognized only six species in the subgenus *Botrychium*, whereas W.H. and F.S Wagner and others have raised that number to approximately 27 species, based on a combination of morphological and cytological studies (Wagner and Wagner 1981, Wagner and Wagner 1983a, Wagner et al. 1984, Wagner and Wagner 1986, Wagner and Wagner 1990, Farrar and Johnson-Groh 1991, Wagner 1993, Wagner and Wagner 1994, Hauk and Haufler 1999, Stensvold et al. 2002, Wagner and Grant 2002). Current biological, ecological, and systematics research on *Botrychium* species is ongoing (C. Johnson-Groh personal communication 2002), including a recently published study on the belowground distribution and abundance of *Botrychium* structures (Johnson-Groh et al. 2002).

W. H. Wagner and F.S. Wagner first described *Botrychium ascendens* in 1986, *B. crenulatum* in 1981, and *B. lineare* in 1994 (Wagner and Wagner 1981, 1986, 1994). The type specimens for these species are housed at the University of Michigan Herbarium (Ann Arbor, MI).

Morphological characteristics

The *Botrychium* subgenus *Botrychium* is the most species-rich subgroup of the genus, and it includes the smallest and most inconspicuous species (Hauk 1995). They are eusporangiate ferns with a short, vertical, subterranean stem that usually produces one leaf per year (McCauley et al. 1985). Species of *Botrychium* subgenus *Botrychium* are generally similar in morphology and are thus difficult to identify in the field, especially if one has only limited experience in field identification (Kolb and Spribille 2001). Zika et al. (1995) provided a list of 14 reasons why *Botrychium* species are difficult to find and identify.

A distinguishing feature of the *Botrychium* species (and the Ophioglossaceae) is that the entire aerial portion of the plant is composed of a single leaf divided into a fertile segment with a grape-like cluster of sporangia containing the spores (sporophore), and a sterile, leafy segment (trophophore) (Hauk 1995, Kolb and Spribille 2001). Essentially, a small single leaf is produced each year, ranging from 1 to 15 cm in height. This rather small, simple leaf limits distinct characteristics available for developing classifications, and has resulted in the systematics of subgenus *Botrychium* being problematic and controversial (Tryon and Tryon 1982). Many *Botrychium* species can be distinguished only by subtle differences (Wagner and Wagner 1981, Wagner 1992). *Botrychium* subgenus *Botrychium* have very similar characteristics, and these characteristics can vary depending on the environmental conditions and current developmental stage (Wagner 1992).

*Botrychium ascendens***.** This species is a low-growing herb 5 to 12 cm tall with a single leaf divided into a vegetative segment and a spore-producing segment (**Figure 1**). The vegetative portion is 1.5 to 3.5 cm long and once pinnately compound with 4 to 6 pairs of non-overlapping, upward-directed, wedge-shaped, sharply toothed, yellowish-green leaflets. The pinnately compound spore-bearing (fertile) segment diverges from the vegetative portion about midway up the leaf stalk (stipe); and is longer and narrower than the vegetative portion (Wagner and Wagner 1986, 1994). **Figure 1** includes a photograph and technical illustration of *Botrychium ascendens*.

Botrychium ascendens has characteristics similar to *B. crenulatum*, *B. lunaria*, and *B. minganense* (Wagner 1992, Fertig 2000). These species of *Botrychium* are members of the "Lunaria Group," characterized by having fan-shaped leaflets (Wagner and Wagner 1990). *Botrychium ascendens* can generally be distinguished from closely related species by its strongly ascending pinnae with sharply serrate margins (Vanderhorst 1997). In contrast to *B. ascendens*, *B. lunaria* consists of overlapping, entire-margined leaflets and spreading vegetative segments. *Botrychium crenulatum* has more rounded teeth on the leaflets than *B. ascendens* (Fertig 2001b). *Botrychium minganense* is a deep, dull green color and has thicker blade texture than *B. ascendens* (Vanderhorst 1997). *Botrychium campestre* differs from *B. ascendens*, because *B. campestre* has oblong leaflets and fertile and vegetative leaf segments of about equal length (Fertig 2000).

For additional technical descriptions, illustrations, and photographs of *Botrychium ascendens*, refer to Lellinger (1985), Wagner and Wagner (1986), Wagner and Wagner (1993), and Fertig (1994).

Botrychium crenulatum. This species is a perennial, yellowish-green fern with leaves mostly 10 cm or less (**Figure 2**). The leaf is divided into an elongated, fertile (spore-bearing) segment and a short, sterile (vegetative) segment, both arising from a common stem approximately halfway along the length of the entire leaf. The vegetative segment averages 2 cm long and 1.2 cm wide and is pinnately divided into 3 to 5 pairs of non-overlapping, wedge-shaped pinnae, with entire to round-toothed margins. The fertile fronds are once pinnate and about 1.5 to 3 times longer than the sterile fronds (Wagner and Wagner 1981, Wagner and Wagner 1993). **Figure 2** includes a photograph and line illustration of *Botrychium crenulatum*.

Botrychium crenulatum, also included in the "Lunaria Group" with fan-shaped pinnae, is very similar to *B. lunaria*, *B. minganense*, *B. ascendens*, and

A.

Photo by Mike Hays. Reprinted with permission from the Idaho Conservation Data Center.

B.

Illustration by Walter Fertig. Reprinted with permission from the Wyoming Natural Diversity Database.

Figure 1. *Botrychium ascendens* photograph in its natural habitat (A), and illustration of the vegetative and spore-bearing segments of the sporophyte (B).

A.

Photo by Linda Swartz. Reprinted with permission from the Idaho Conservation Data Center.

B.

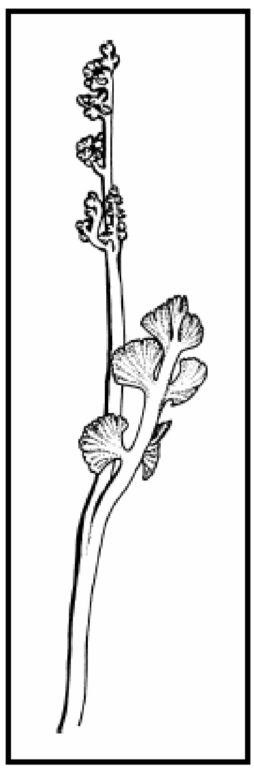


Illustration by David Wagner. Reprinted with permission from the illustrator.

Figure 2. *Botrychium crenulatum* photograph in its natural habitat (A), and illustration of the vegetative and spore-bearing segments of the sporophyte (B).

B. campestre (Wagner and Wagner 1981, Fertig 1994). *Botrychium crenulatum* can generally be distinguished from these other species by its 2 to 3 (sometimes up to 5) pairs of non-overlapping (i.e., well-separated), yellow-green pinnae. The pinnae are very thin and herbaceous (i.e., not fleshy) in texture, have scalloped margins, and the veins are easy to see when held up to the light. In contrast, *B. lunaria* is dark-green in color, and has numerous pinnae (an average of five pairs) that strongly overlap (Wagner and Wagner 1981). Additionally, spores from *B. crenulatum* average 6 micrometers larger than those from *B. lunaria*. *Botrychium ascendens* differs from *B. crenulatum* in that *B. ascendens* has wedge-shaped, upward-directed leaflets with more sharply toothed margins (Wagner and Wagner 1981). *Botrychium minganense* has greater than four sets of pinnae that are cup or wedge-shaped and do not overlap. Swartz and Brunsfeld (2002) compared *B. crenulatum* and *B. minganense* using morphometric and biochemical methods and found that these two species overlap morphologically, but differ biochemically.

For additional technical descriptions, illustrations, and photographs of *Botrychium crenulatum*, refer to Wagner and Wagner (1981), Wagner (1992), Wagner and Wagner (1993), and Williston (2001).

Botrychium lineare. This species is a perennial fern that produces a pale green leaf (trophophore), about 6 to 18 cm long including the stalk and a larger, erect, spore-bearing structure (sporophore) with a single major axis (**Figure 3**). Both arise from a common, erect, subterranean stem and can be thought of as a single, highly modified fern frond (Colorado Natural Heritage Program 2002). **Figure 3** includes a photograph and line illustration of *Botrychium lineare*.

The closest relative to *Botrychium lineare* on the Great Plains is thought to be *B. campestre*, a widespread species that typically grows at lower elevations (Wagner and Wagner 1994). It has a broader and fleshier rachis, a denser and fleshy sporangium mass, and overlapping or fused vegetative leaf segments compared to *B. lineare* (Spackman et al. 1997). In addition, *B. lineare* can also look similar to narrow forms of *B. ascendens* in the western U.S. and narrow forms of *B. matricariifolium* in eastern Canada. An unpublished key to *Botrychium* species in Colorado (including *B. lineare*) was prepared by P. Root (personal communication 2002), and a table summarizing the important features from this key is reproduced in Kolb and Spribille (2001).

For additional descriptions, illustrations, and photographs of *Botrychium lineare*, refer to Wagner and Wagner (1994) and Spackman et al. 1997.

Distribution and abundance

Global and regional distribution and abundance

North America is known to contain the greatest number and most diverse assortment of moonworts (Wagner and Wagner 1990, Hauk 1995), as there are four times as many *Botrychium* species in North America compared to the rest of the world (Wagner and Wagner 1994). Within North America, the western Rocky Mountains and Pacific Northwest harbor the greatest number of *Botrychium* species and are known as hotspots for moonworts (Wagner and Wagner 1986, Wagner 1992, Hauk 1995). The causes of this high level of speciation are not fully understood (Kolb and Spribille 2001).

Early misinterpretations of the Ophioglossales and crude taxonomies resulted in erroneous distribution inferences. Initial hypotheses concluded that hybridization, or even cross-breeding, within the species was almost impossible, which resulted in very wide species distribution descriptions (Wagner and Wagner 1986). However, more recent studies reveal that only a few species of Ophioglossales have truly broad distributions and most have narrow ranges (Wagner and Wagner 1986). While members of the *Botrychium* subgenus of *Botrychium* are known to grow on nearly every continent, distribution for most species is limited to less than half of a continent (Hauk 1995).

Botrychium species may be underdocumented because populations can be small with scattered individuals and locating stands and identifying species is generally difficult (Wagner and Wagner 1986, Farrar and Johnson-Groh 1991, Wagner and Wagner 1994). The USFWS (2001) reported that, out of the populations of *B. lineare* observed in western states, only three populations had more than 15 individuals. Plants of *B. ascendens* in the Bighorn National Forest, Wyoming were observed growing in small groups of 2 to 12 (W. Fertig personal communication 2002). Individual plants of *Botrychium* can be scarce within a stand and grow approximately 1 to 3 m apart (Wagner and Wagner 1983a). The relatively small size and low visibility of moonworts often results in identification

A.

Photo by J. Sellers. Reprinted with permission from the Colorado Natural Heritage Program.

B.

Illustration by Janet Wingate. Reprinted with permission from the Colorado Natural Heritage Program.

Figure 3. *Botrychium lineare* photograph of three living plants in their natural habitat (A), and illustration depicting the vegetative and spore-bearing segments of the sporophyte (B).

problems and causes taxonomic difficulties (Wagner 1992). It is common for several *Botrychium* species to occur together in "genus communities", a sympatric pattern of distribution where individuals of different species are growing side-by-side. This may aid in discovering populations of target species but has also caused identification problems (Wagner and Wagner 1983a, Zika 1992, U.S. Fish and Wildlife Service 2001). Further, species such as *B. crenulatum* are often found under various sedges and grasses, making it difficult to locate them (Wagner and Wagner 1981). Current knowledge of the range of *Botrychium* species may reflect the extreme difficulty in finding the plants in the field, rather than indicate their true distributions (Wagner and Wagner 1994, Fertig and Beauvais 1999, U.S. Fish and Wildlife Service 2001). The "rarity" of these species may be due primarily to their inconspicuousness, to misunderstandings of taxonomy, and to inadequate search efforts (Kolb and Spribille 2001).

However, Wagner and Wagner (1994) point out that many *Botrychium* subgenus *Botrychium* species are truly rare. Some species are known from only one specimen for each of several localities, other species have very restricted ranges and occur in only one area, and other species have large ranges with small populations in widely-separated locations. Some *Botrychium* species have been historically reported in only a few localities throughout western North America, perhaps due to poor collection efforts and identification discrepancies (Wagner and Wagner 1986). In contrast, there are several *Botrychium* species that are relatively widespread and common. For example, new inventory efforts have discovered so many populations of *B. simplex*, that it is being considered for delisting (Zika 1992). On the other hand, *Botrychium* individuals, even of common species, are frequently absent in seemingly appropriate habitats. Zika (1992) noted that *Botrychium* habitat is relatively common in the mountains of western North America, but searching for a *Botrychium* species may yield only one occupied site out of every 50 likely sites. While some *Botrychium* populations have been overlooked in the past and may be more abundant than is currently recorded, existing data document that some species are truly rare (i.e., have small abundance, few locations, and/or restricted distribution) (Zika 1992). *Botrychium ascendens* and *B. crenulatum* could be considered rare, because they have wide ranges but occur in widely scattered, relatively small populations. They can occur in large populations in some areas of their ranges. *Botrychium lineare* could be considered very rare, because it has a wide range but occurs in very small populations in disjunct locations.

Botrychium ascendens. Although *Botrychium ascendens* has a wide range in western North America, it is considered rare because it is widely scattered in disjunct populations and population numbers are usually small (Wagner and Wagner 1986, Vanderhorst 1997). Current and historical distribution of this species includes Alaska, Yukon Territory, British Columbia, Alberta, Saskatchewan, Ontario, Oregon, Washington, Idaho, California, Nevada, Montana, and Wyoming (NatureServe 2003).

Figure 4 illustrates the distribution of *Botrychium ascendens* in Region 2, where it is rare, as it is known from only four small occurrences in Wyoming. Two occurrences are in the southern Absaroka Range in Shoshone National Forest (Fremont County), and one occurrence is in the northern Wind River Range in Shoshone National Forest (Fremont County) (Fertig 1994). W. Fertig (2001a, personal communication 2002, Wyoming Natural Diversity Database 2003) also found an occurrence within Bighorn National Forest (Johnson County) in 2001, while re-visiting a *B. crenulatum* site. *Botrychium ascendens* has not been discovered in other Region 2 states (Colorado Natural Heritage Program 2002, Kansas Natural Heritage Inventory 2002, Nebraska Natural Heritage Program 2002, South Dakota Natural Heritage Program 2002).

Of the three *Botrychium ascendens* occurrences in Shoshone National Forest last observed in 1995, one occurrence had 50 plants, one occurrence had 10 to 15 plants, and the third occurrence had one spore-producing plant. Fertig (2000) suggested that populations could be larger, due to the scattered nature of this species and its microhabitats. *Botrychium ascendens* individuals in Johnson County were observed growing in small groups of 2 to 12 (W. Fertig personal communication 2002). This moonwort is abundant at some locations in the Pacific Northwest, especially in the Wallowa Mountains of Oregon and one population in Washington (Wagner 1992, Zika 1994). For example, *B. ascendens* was observed in the hundreds along Hurricane Creek Trail in Oregon (Wagner and Wagner 1986, Zika 1994). Vanderhorst (1997) recorded six individuals for the two extant populations of *B. ascendens* in Montana, and reported that the populations in Idaho have only one to two individuals. Thus, this species appears to be rarer in some parts of its range, such as Montana, Wyoming, and Idaho, compared to other areas, such as Oregon.

Botrychium crenulatum. This species is considered to be rare (Wagner and Wagner 1981) as its distribution is widely scattered over western North America, from

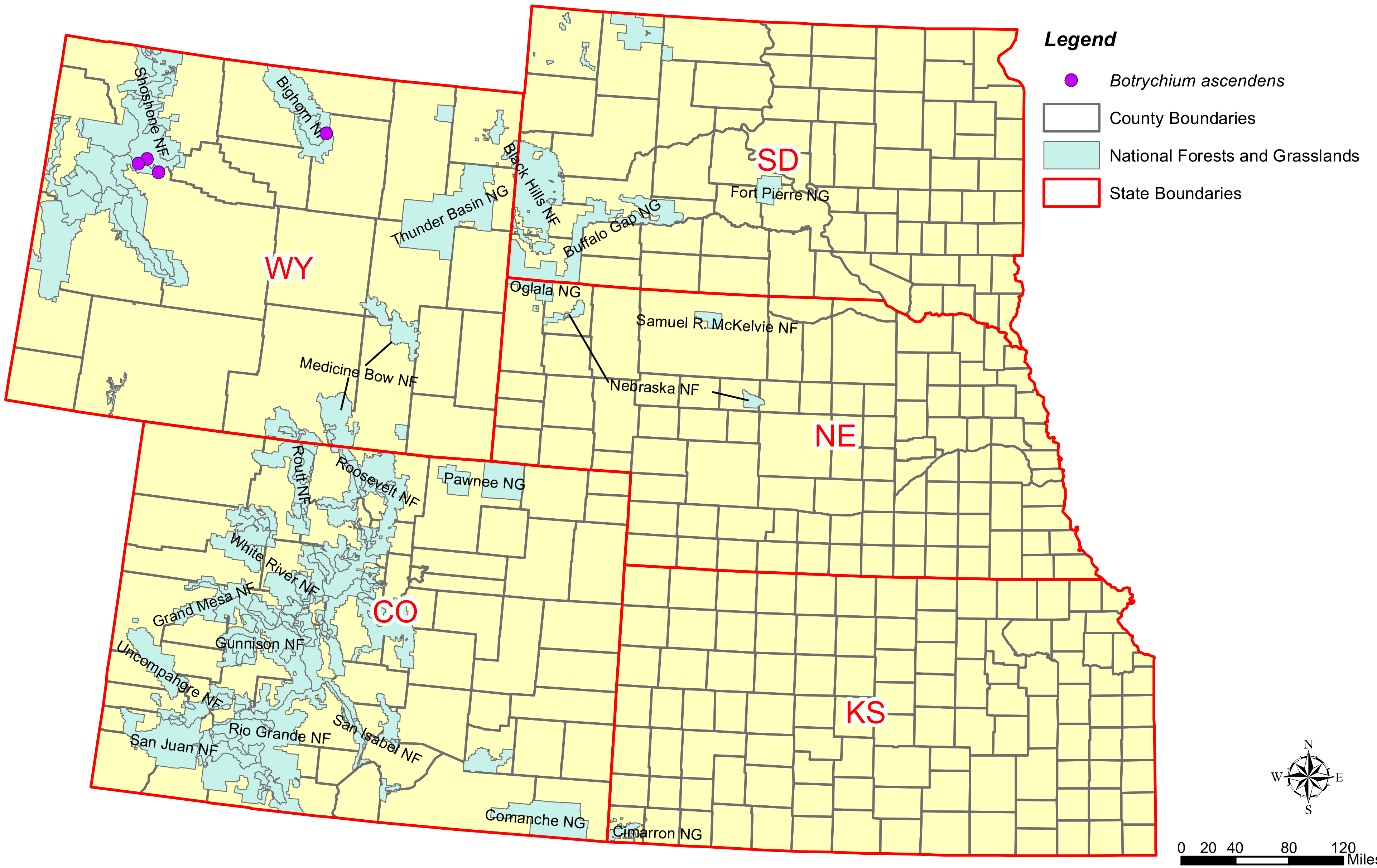


Figure 4. Map of U.S. Forest Service (USFS) Region 2 illustrating distribution of four *Botrychium ascendens* occurrences in Wyoming (Fremont and Johnson Counties). Each occurrence may include one to several populations. Additional occurrences occur outside USFS Region 2. Refer to document for abundance and distribution information. Source: Wyoming Natural Diversity Database, Laramie, Wyoming (2003).

Oregon to Arizona, with relatively small, disjunct populations (Wagner and Wagner 1993). Current and historical distribution of *Botrychium crenulatum* includes Alberta, British Columbia, Washington, Oregon, California, Idaho, Nevada, Utah, Montana, Wyoming, and Arizona (NatureServe 2003). Lellinger (1985) predicted that *B. crenulatum* would occur in the terrestrial portions of marshy places, at relatively low elevations, ranging from central and southern California to central Arizona and Montana.

Figure 5 illustrates the distribution of *Botrychium crenulatum* in Region 2, where it is extremely rare. It is known historically from only one occurrence in Bighorn National Forest (Johnson County) in Wyoming, and it has not been observed or verified since that time (Wyoming Natural Diversity Database 2003). In 1997, *B. crenulatum* was reported from this site, but not confirmed with a voucher (Fertig 2001a, personal communication 2002). Although specific directions to this population of *B. crenulatum* were recorded, it was not relocated by W. Fertig in the summer of 2001. W. Fertig (personal communication 2002) speculated that this population may actually be the closely related *B. ascendens*, which was found in abundance at that site. Wagner and Wagner (1993) also reported *B. crenulatum* in Wyoming. However, personal communication with W.H. Wagner by W. Fertig confirmed that this information was based on inference, not verified vouchers (W. Fertig personal communication 2002). Wagner suggested that since *B. crenulatum* existed in adjacent Region 2 states, the range may extend into Wyoming (W. Fertig personal communication 2002). Based on this information concerning *B. crenulatum*, the status of this species in Wyoming was changed from S1 (critically imperiled) to SR (state-reported) by WYNDD (W. Fertig personal communication 2002). There have not been any documented observations of *B. crenulatum* in any other Region 2 states (Colorado Natural Heritage Program 2002, Kansas Natural Heritage Inventory 2002, Nebraska Natural Heritage Program 2002, South Dakota Natural Heritage Program 2002). Therefore, there is no authentic (i.e., verified by an expert) record of *B. crenulatum* in any Region 2 states.

The abundance of *Botrychium crenulatum* within its range is variable. The one reported occurrence in Wyoming consisted of one plant (Fertig 2001b). In Montana, reported populations ranged from "small", to 40 individuals, to 100 to 200 individuals (Vanderhorst 1997). Some populations in Washington were considered to be "large", and one population in Idaho had "thousands of fronds" (Vanderhorst 1997). In addition, Wagner and Wagner (1981) suggested that *B. crenulatum* may occur locally in large numbers in southern California.

Botrychium lineare. New information on distribution and abundance of *Botrychium lineare* is available at http://www.fs.fed.us/r2/projects/scp/assessments/newinfo.shtml. *Botrychium lineare* is thought to be one of the rarest moonworts in North America (Root 1999); the total number of individuals estimated for all nine known populations is about 190 (U.S. Fish and Wildlife Service 2001). Current and historical distribution of this species includes Washington, Oregon, California, Idaho, Montana, Utah, Colorado, Quebec, and New Brunswick (NatureServe 2003). Information found in the literature regarding the range of *B. lineare* outside Region 2 is varied due to potential extirpation at historical sites. For example, the USFWS (2001) reported that *B. lineare* is currently known from a total of only nine populations (three in Colorado [El Paso and Lake counties]), two in Oregon [Wallowa County], three in Montana [Glacier County], and one in Washington [Ferry County]). In addition to these nine sites, the USFWS (2001) reports that there are historic sites in the United States and Canada. Populations previously known from Idaho (Boundary County), Montana (Lake County), California (Fresno County), Colorado (Boulder and Grand counties), Utah (Salt Lake County), and Canada (Quebec and New Brunswick) have not been seen for at least 20 years and may be extirpated (Wagner and Wagner 1994). However, these occurrences are still included in information presented by NHPs (Utah Division of Natural Resources 1998, Montana Natural Heritage Program 2001, U.S. Fish and Wildlife Service 2001, California Native Plant Society 2002, Colorado Natural Heritage Program 2002, Idaho Conservation Data Center 2002). Little survey work has been performed throughout the range, so it is possible that even though there are no recent confirmed sightings of *B. lineare* in these areas, populations may exist.

New information on distribution and abundance of *Botrychium lineare* is available at http://www.fs.fed.us/r2/projects/scp/assessments/newinfo.shtml. There is no documented occurrence of *B. lineare* on the White River National Forest in Colorado, and it was confirmed on the Black Hills National Forest in Wyoming in December 2003. **Figure 6** illustrates the distribution of *Botrychium lineare* in Region 2, where it is rare, historically known only from six populations in Colorado (Boulder, El Paso, Grand, and Lake counties). *Botrychium lineare* occurs at two occurrences on Pikes Peak in Pike-San Isabel National Forest (El Paso County), one occurrence near Leadville

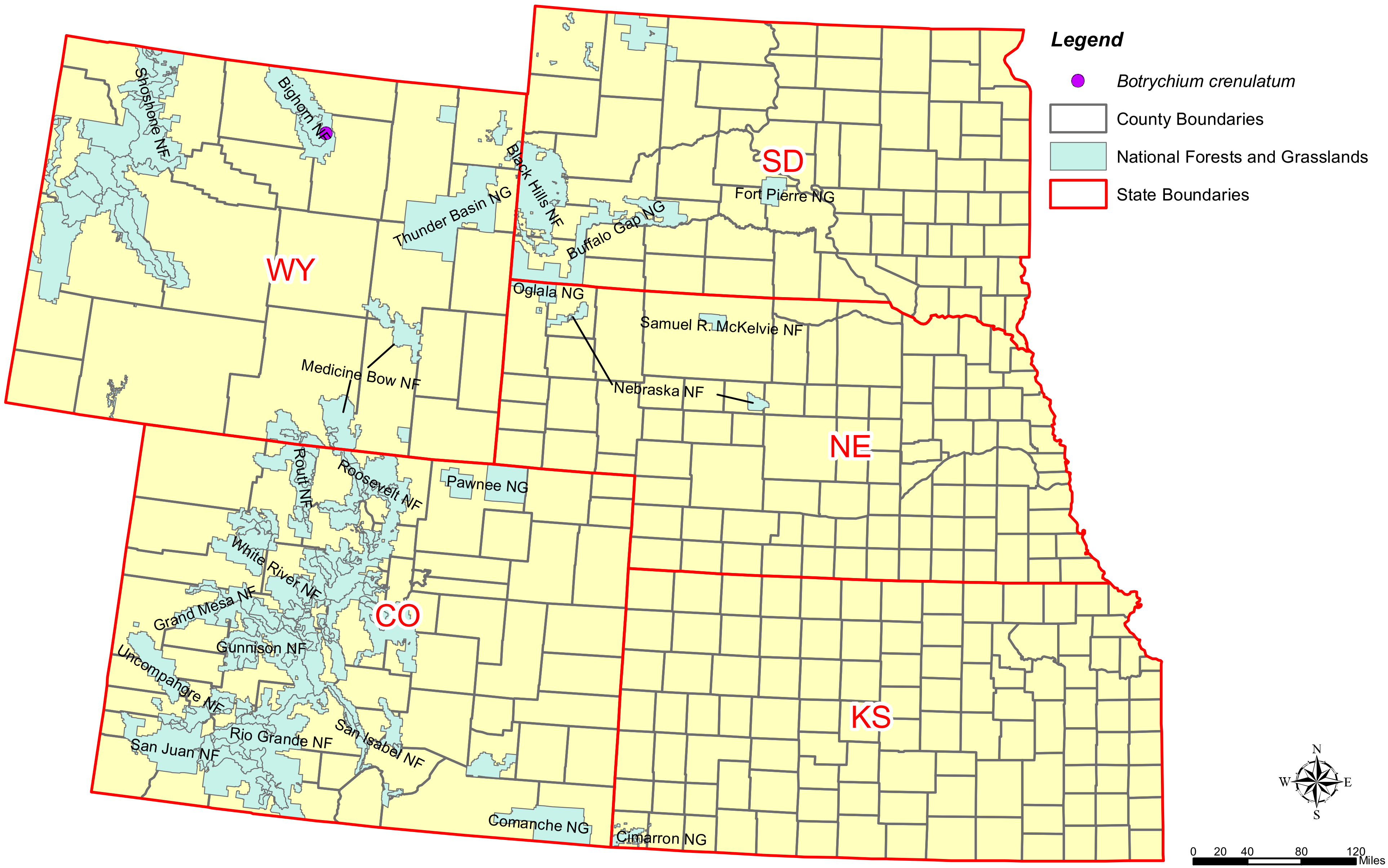


Figure 5. Map of U.S. Forest Service (USFS) Region 2 illustrating distribution of one reported occurrence of *Botrychium crenulatum* in Wyoming (Johnson County). This occurrence has not been verified by an expert. Additional occurrences occur outside USFS Region 2. Refer to document for abundance and distribution information. Source: Wyoming Natural Diversity Database, Laramie, Wyoming (2003).

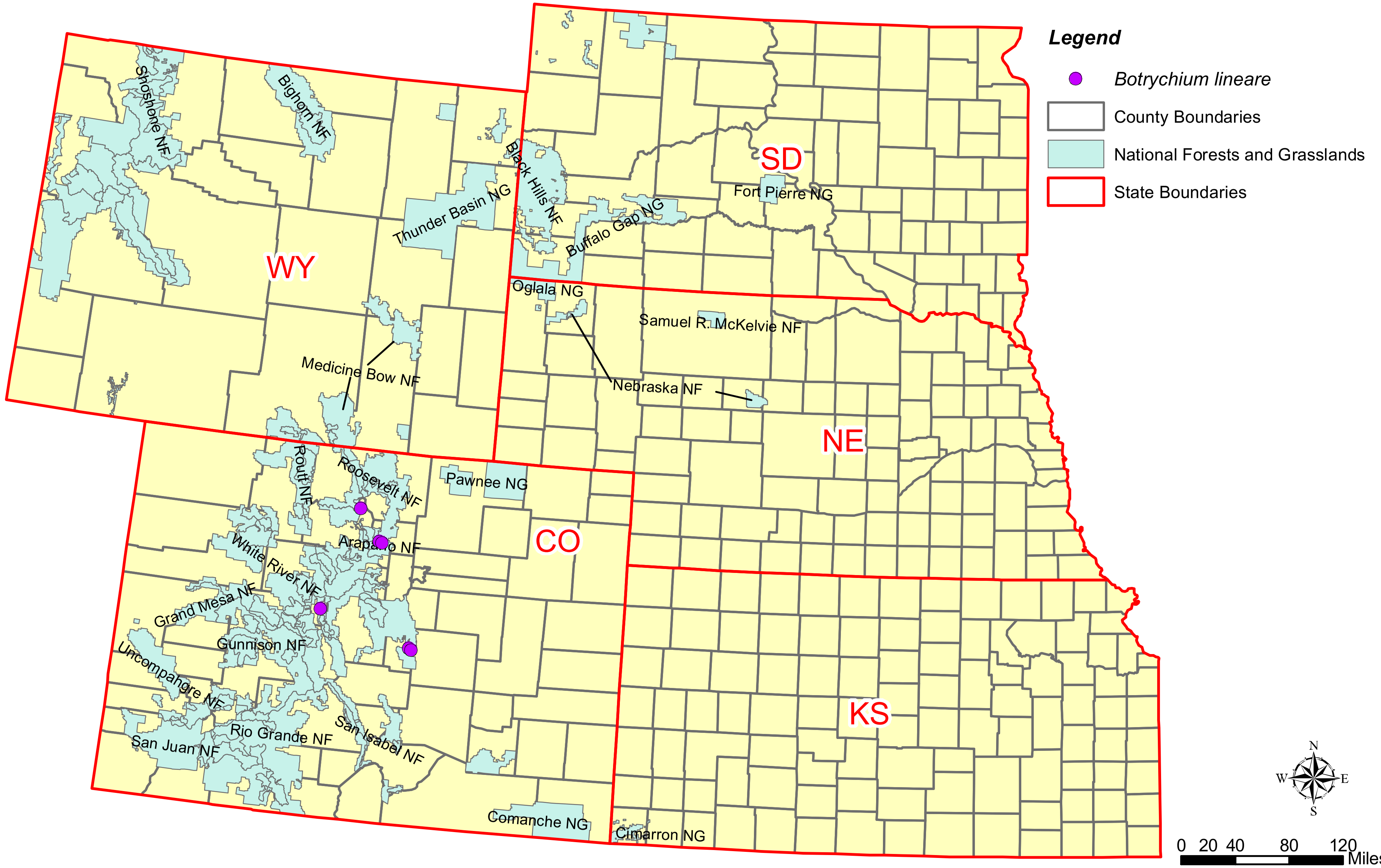


Figure 6. Map of U.S. Forest Service (USFS) Region 2 illustrating distribution of six *Botrychium lineare* occurrences in Colorado (Boulder, El Paso, Grand, and Lake Counties). New information on distribution and abundance of *Botrychium lineare* is available at http://www.fs.fed.us/r2/projects/scp/assessments/newinfo.shtml. Each occurrence may include one to several populations. Additional occurrences occur outside USFS Region 2. Refer to document for abundance and distribution information. Sources: Colorado Natural Heritage Program, Fort Collins, Colorado (2003); University of Colorado Herbarium, Boulder, Colorado (2002).

in White River National Forest (Lake County), one occurrence in Rocky Mountain National Park (Grand County), and two occurrences within the Arapaho-Roosevelt National Forest (Boulder County) (U.S. Fish and Wildlife Service 2001, Colorado Natural Heritage Program 2002, P. Root personal communication 2002, University of Colorado Herbarium 2002). There are no documented observations of *B. lineare* in any other Region 2 states (Kansas Natural Heritage Inventory 2002, Nebraska Natural Heritage Program 2002, South Dakota Natural Heritage Program 2002, Wyoming Natural Diversity Database 2003). Although *B. lineare* has not been reported in Wyoming, W. Fertig (personal communication 2002) suggested that potential habitat for this species could occur in the southern Medicine Bow Range or Sierra Madre Mountains.

In general, populations of *Botrychium lineare* can range in size from 2 to100 individuals, although only three current populations have more than 15 individuals (one population in Colorado, two populations in Montana) (U.S. Fish and Wildlife Service 2001). The population at Pikes Peak is thought to have the greatest number of individuals, with 53 individuals counted in 1996 (Wagner and Wagner 1994, Carpenter 1996b, Root 1999, Colorado Natural Heritage Program 2002). Other *B. lineare* populations in Region 2 have not been censused.

Population trend

Few data are available on population trends for *Botrychium* species within Region 2. Population monitoring has only been initiated for one population of *B. lineare*, on Pikes Peak in Colorado (Carpenter 1996a, Carpenter 1996b, S. Tapia personal communication 2003). No other long-term, rigorous, standardized data collections on local or regional populations have been performed.

For other *Botrychium* species, temporal patterns of abundance within a given population have been shown to vary from year to year. For example, in six years of monitoring by Johnson-Groh (1998), individual *B. lunaria* plants skipped years, producing no aboveground leaves in a given year, but remaining alive and producing leaves the following season. While new plants are annually recruited into the population, older plants may disappear or reappear after absences of one to three years. In addition, the populations of *B. lunaria* in some permanent monitoring plots increased, while the populations in other plots decreased.

Range-wide, regional, and local trends

In the following sections, information about population trends for the three *Botrychium* species in Region 2 is presented.

Botrychium ascendens. This species is known from four occurrences within Region 2, and there are no known data to develop population trends for this species (Wyoming Natural Diversity Database 2003).

Botrychium crenulatum. The only reported population of this species in Region 2 consisted of one individual discovered in Wyoming in 1997 (Fertig 2001b). This population was not rediscovered, despite extensive searching in 2001 (W. Fertig personal communication 2002). No population trend can be determined.

Botrychium lineare. Within Region 2, Alan Carpenter with The Nature Conservancy began monitoring the largest population of *B. lineare* on Pikes Peak in Colorado (Pike-San Isabel National Forest) in 1995 (Carpenter 1996a, Carpenter 1996b). Monitoring was planned for 10 subsequent years, and data from 1995, 1996, and 2000 are currently available (Carpenter 1996b, Tapia 2000, A. Carpenter personal communication 2003, S. Tapia personal communication 2003). It is unknown if monitoring occurred in 1998 (A. Carpenter personal communication 2003), and monitoring efforts in 2002 were re-scheduled for 2003 as a result of drought conditions (S. Tapia personal communication 2003). Apparently, enthusiasts with the Broadmoor Garden Club in Colorado Springs also observed *B. lineare* on Pikes Peak for several years, and informal observations suggest that population sizes were relatively stable from year to year (G. Cameron personal communication 2002). However, the years of these observations and specific abundance data are not available.

The goal of the ongoing monitoring project is to collect sufficient information about the large population of *Botrychium lineare* at the Pikes Peak site to determine population trends and the longevity of individual plants. A permanent monitoring plot was set up to encompass at least 90 percent of the available, apparently suitable, habitat within an area 35m long by 10m wide (Carpenter 1996b). Within this area, the monitoring team temporarily subdivides the macroplot into 35-1m wide sub-plots, and team members crawl or walk slowly to census the sub-plots for *B. lineare* plants, noting if existing live plants are tagged or new (i.e., not previously tagged). Plants are permanently marked with a numbered, aluminum tag attached to a length of rigid wire pressed into the soil near the plant. New plants have not been marked in all years, as a result of a shortage of tagging supplies (S. Tapia personal communication 2003); as of 2000, there were a total of 55 tags in the plot (Tapia 2000).

The results of monitoring efforts in 1995, 1996, and 2000 are summarized in **Table 2**. The total number of plants includes all live plants with tags and new live plants censused that year. The total number of live plants has fluctuated from 24 to 53 to 43 individuals (**Table 2**). Carpenter (1996b) pointed out that the 1996 abundance was a census of the population, while the 1995 abundance was considered to be a sample of the population. Approximate survival rates are based on the number of unambiguously tagged live plants divided by the total number of tags. Monitoring in 1996 found that individuals were tagged too far away (10 cm) in 1995 to unambiguously correlate existing individuals with tags (Carpenter 1996b). Therefore, the estimated survival rate for that year is based on a subset of unambiguously tagged individuals. The approximated survival rate was 87 percent in 1996 and 60 percent in 2000 (**Table 2**). However, these estimated survival rates are only approximations as a result of ambiguous tagging, incomplete tagging of all new plants, and the fact that some plants may be merely dormant rather than dead. Continuing data collection will help to elucidate population trends, longevity, and dormancy of *B. lineare* at this site (Carpenter 1996b).

Habitat characteristics

(New information on habitat of occurrences of *Botrychium lineare* discovered since publication of this assessment is available at http://www.fs.fed.us/r2/projects/scp/assessments/newinfo.shtml.)

Habitat characteristics for *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* within Region 2 were infrequently found in the literature, although several descriptions of habitats outside of Region 2 were found. Specific habitats and associated vegetation classifications for *Botrychium* species can be difficult to characterize since the vegetation and topography of observed sites vary greatly across their range (Wagner and Wagner 1994). Understanding habitat characteristics is also compounded by the fact that few sites have been described in detail by experts. Kolb and Spribille (2001) point out that although many *Botrychium* species are thought to be habitat generalists, because one species can be found in a range of habitats, most species likely have a suite of specific ecological requirements.

Many authors have noted that several species of *Botrychium*, including *B. ascendens*, *B. crenulatum*, and *B. lineare*, occur in open habitats and microsites with evidence of slight to moderate disturbances (Lellinger 1985, Wagner and Wagner 1993, Lesica and Ahlenslager 1996, Kolb and Spribille 2001, Williston 2001). For example, Williston (2001) reported that several *Botrychium* species in Canada, including *B. ascendens*, appear to be successful colonizers of disturbed habitat, such as the edges of

Table 2. Results of *Botrychium lineare* monitoring efforts at one population location in the Pikes Peak Ranger District, Pike-San Isabel National Forest, El Paso County, Colorado. Includes total abundance of live plants, number of tagged and new plants, and estimated survival rate based on monitoring efforts in June 1995, 1996, and 2000.

***Botrychium lineare* monitoring efforts**	**1995**	**1996**	**2000**
Total number of live plants	24	53	43
Total number of live plants with tags	0	13	33
Total number of new live plants	0	38	10
Total number of tags	0	24	55
Total number of unambiguous tags		15	55
Total number of unambiguous tags with live plants		13	33
Estimated survival rate (Live tagged plants/unambiguous tags)		0.87	0.60

Sources (Carpenter 1996a, 1996b, Tapia 2000) trails or old roads and in abandoned fields. Kolb and Spribille (2001) noted that moonworts in Summit County, Colorado were always found in non-forested and disturbed habitats, like ski runs at ski resorts, overgrown roadbeds and logging landings, and areas that had burned in the past 100 years but the canopy had not yet grown closed. Root (1999) also found several *Botrychium* species at a highly disturbed site with cut pines, evidence of fire, and old pits in coarse, decomposed granite. The moonworts were growing in small pockets of soils around the cut logs and stumps. Zika (1992) observed that rare moonworts in Oregon favor sites in old-growth riparian forests with annual, modest flooding in headwater creek floodplains.

While researchers mention that disturbances are important factors for the establishment and persistence of *Botrychium* species, they usually do not provide a detailed discussion of the type, extent, intensity, frequency, timing, or location of the disturbance in conjunction with *Botrychium* populations. While *B. ascendens*, *B. crenulatum*, and *B. lineare* may all require habitats with periodic disturbances to create early successional habitat or microsite openings in dense vegetation, there is much to be learned about the particular disturbance regime optimal for the persistence of each of these species. Natural disturbances (e.g., wildfire, floods, rock slides, erosion, small mammal activity, blowdowns, frost heaving, drought) and human-related disturbances (e.g., road and trail construction and maintenance, thinning and fuel reduction activities, timbering and harvest activities, motorized and non-motorized recreation, structure construction, prescribed burns, livestock activity) can occur at many scales and intensities and may serve to create, enhance, destroy, or degrade habitat throughout a landscape. For example, floods or landslides can create new habitat, but they can also cause physical damage to existing populations. Intense disturbances, such as those associated with structure construction or use of heavy machinery, may eliminate favorable habitats altogether and remove the likelihood of *Botrychium* establishment and growth. The existing populations of *B ascendens*, *B. crenulatum*, and *B. lineare* species in Region 2 have few individuals and cover a small area. Thus, a random, catastrophic disturbance could destroy these populations completely (U.S. Fish and Wildlife Service 2001). Some disturbances occur at small scales and high intensities (e.g., small mammal burrowing) where vegetation is removed from a small patch, while other disturbances occur at large scales and low intensities (e.g., low-intensity understory burn) where overstory vegetation is not completely denuded, and understory effects are patchy. In addition, understanding the successional processes and pathways that follow disturbances is critical for analyzing habitat characteristics of *Botrychium* species. The characteristics of disturbances important for the creation of habitat and the maintenance of habitat are likely to be different. For *Botrychium* habitat in Summit County, Colorado, the length of time since disturbance varied from site to site and ranged from 2 to 100 years. As succession proceeds and competition increases, local populations of *Botrychium* may be extirpated (Chadde and Kudray 2001). As a result, the persistence of *Botrychium* species may rely on a landscape with a mosaic of patches created by disturbances varying in frequency and intensity, where a series of local populations colonize, disperse, and disappear with the changing successional landscape (Chadde and Kudray 2001).

Throughout their ranges, including Region 2, *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* seem to prefer montane, moist, early-successional habitats (e.g., moist meadows, edges of trails, seeps and mossy openings in forests). However, the specific range of tolerance likely varies for each species. For example, where *B. crenulatum* and *B. ascendens* occurred together in Montana, *B. crenulatum* occupied slightly lower and wetter microsites, while *B. ascendens* occupied slightly higher and drier microsites (Vanderhorst 1997). The following sections present information for each species about habitat characteristics throughout the range, as this may prove useful in identifying potential habitat within Region 2. This is followed by a discussion of habitat features specifically identified within Region 2.

Botrychium ascendens. Outside Region 2, populations of *B. ascendens* have been found in a variety of habitats. Lellinger (1985) reported that *B. ascendens* was typically found in terrestrial, grassy areas from British Columbia, Alberta, and Montana to northern California and Nevada. For example, *B. ascendens* can be found in grassy fields and coniferous woods near streams, at elevations ranging from 5000 to 6000 ft (1500 to 1800 m) (Hickman 1993). The California Native Plant Society (2002) documented the occurrence of *B. ascendens* within mesic, lower montane, coniferous forests. CalFlora (2002) reported the occurrence of both *B. ascendens* and *B. crenulatum* within *Pinus jeffreyi* (yellow pine) forests, under moist conditions. Vanderhorst (1997) reported that *B. ascendens* populations in Montana were growing in mesic to moist meadows in the *Picea engelmannii* (Engelmann spruce) zone, while in Idaho and Washington, the plants were in forests of *Thuja plicata* (western red cedar), *Abies lasiocarpa* (subalpine fir), and in alpine meadow habitats. Within the Wallowa

Mountains of Oregon, *B. ascendens* was observed in the hundreds along Hurricane Creek Trail in grassy fields interspersed with spruces. Other *Botrychium* species found growing within the stand included *B. lunaria* and *B. minganense* (Wagner and Wagner 1986). Vanderhorst (1997) suggested that *B. ascendens* exists in openings of communities with high diversity of associated understory species (from mosses to shrubs), which makes this species different from other *Botrychium* species in Montana that prefer habitats with depauperate lower and middle canopies. The authors also described other associated genera within these habitats, but that information is not reproduced here, since these species may not occur within Region 2.

Williston (2001) reported that several species of *Botrychium* appear to be successful colonizers of disturbed habitat, although he did not describe details, like the time since disturbance. He reported that *B. ascendens* was often found on the edges of trails or old roads and in abandoned fields (Williston 2001). Similarly, *B. ascendens* in Montana was found along roadsides and in riparian areas, susceptible to disturbance from hydrologic changes, such as scouring and gravel deposition (Vanderhorst 1997). *Botrychium ascendens* could be found both at sites with minimal cover by trees, shrubs, and forbs, and significant ground cover by mosses; as well as sites with minimal tree cover, and significant cover from shrubs, forbs, and ferns. The creation and maintenance of suitable habitat likely depends on the intensity of flooding and scouring, the cover by flood-resistant shrubs, and successional processes following disturbance (Vanderhorst 1997). Zika (1994) also suggested that the small meadows where *B. ascendens* grows in Oregon are temporary phenomena, subject to reforestation over a time scale of several decades. While *B. ascendens* is sometimes found in the understory of forested habitat within its distribution in Oregon, most sites are in open, mesic meadows. Thus, it seems that *B. ascendens* generally prefers open or early successional habitats that might be associated with hydrologic disturbances.

Within Region 2, *Botrychium ascendens* is found within short and tall riparian willow communities with significant moss, gravel, and cobble groundcover on volcanic or granitic alluvium at 8000 to 9000 ft (2400 to 2700 m) in elevation (Wyoming Natural Diversity Database 2003). In Shoshone National Forest, *B. ascendens* plants were found about 1 to 2 ft (0.3 to 0.6 m) above and adjacent to a braided channel (Wyoming Natural Diversity Database 2003). The groundcover here is mainly comprised of mosses, cobbles, and gravel, while the canopy at these sites is 3 to 6 feet (0.6 to 1.2 m) tall, dominated by *Salix* spp. (e.g., *S. wolfii*, *S. boothii*, *S. geyeriana*) communities. The understory can include mesic graminoids, such as *Carex microptera* (smallwing sedge), *C. aquatilis* (water sedge), *C. norvegica* (Norway sedge), *Juncus balticus* (Baltic rush), *Agrostis scabra* (rough bentgrass), *Festuca saximontana* (Rocky Mountain fescue), *Danthonia intermedia* (timber oatgrass), *Elymus trachycaulus* (slender wheatgrass), *Agrostis scabra* (rough bentgrass), *Festuca saximontana* (Rocky Mountain fescue); forbs, such as *Aster foliaceus* (alpine leafybract aster), *Antennaria corymbosa* (flat-top pussytoes), *Equisetum arvense* (field horsetail), *Trifolium repens* (white clover), *Pedicularis groenlandica* (elephanthead lousewort), *Astragalus alpinus* (alpine milkvetch), *Fragaria virginiana* (wild strawberry), *Solidago multiradiata* (Rocky Mountain goldenrod), *Penstemon procerus* (littleflower penstemon); and shrubs, such as *Potentilla fruiticosa* (shrubby cinquefoil). *Botrychium ascendens* generally occurs within openings of the dense canopy cover. In Bighorn National Forest, W. Fertig (personal communication 2002) found *B. ascendens* on moist hummocks within a wetland. This swamp area is managed for a mid- to late-seral community of willows and sedges, according to the forest management plan (S. Gall personal communication 2003). The hummocks were dominated by graminoids, such as *Carex aquatilis*; forbs, such as *Trifolium repens*, *Fragaria virginiana*, and *Polygonum viviparum* (alpine bistort); and shrubs such as *Pentaphylloides floribunda* (shrubby cinquefoil) and *Salix planifolia* (diamondleaf willow). *Botrychium ascendens* individuals were at the edge or base of the hummock, in groups of 2 to 12 plants. The soil on the hummocks was moist, but drier than the flooded areas between hummocks (W. Fertig personal communication 2002).

Botrychium crenulatum. Throughout its range in the western U.S., populations of *B. crenulatum* have been found in a variety of moist, open, montane habitats, including damp meadows, boggy areas, and marshes (Wagner and Wagner 1981, Wagner and Wagner 1993, Vanderhorst 1997, CalFlora 2002, Colorado Native Plant Society 2002).

In California, CalFlora (2002) reported the occurrence of *Botrychium crenulatum* within *Pinus jeffreyi* forests, as well as in meadow, freshwater marsh, and bog/fen habitats. The California Native Plant Society (2002) identified habitat for *B. crenulatum* as bogs and fens, lower montane coniferous forests, meadows, and freshwater marshes and swamps at elevations ranging from 5000 to 11000 ft (1500 to 3280 m). More specifically, Wagner and Wagner (1981) found *B. crenulatum* in the drier portions of damp meadows, boggy areas, and marshes, either on hillsides or flat lands where there are wet banks or springy spots at approximately 3900 to 8200 ft (1200 to 2500 m). The plants were rooted in tussocks or "rises" around isolated trees or shrubs, or in depressions that dry out in the summer, or at the edges of marshes. These moonworts may occur either in sun or shade, but most frequently in partial shade. In some cases, *B. crenulatum* was found growing with other species of *Botrychium*, such as *B. simplex*, and occasionally with *B. multifidum* (Wagner and Wagner 1993). In Montana, Vanderhorst (1997) discovered *B. crenulatum* in wetlands with native trees and shrubs and wet roadsides dominated by herbaceous exotic species. Vanderhorst (1997) suggested that *B. crenulatum* exists in openings of communities with high diversity of associated understory species (from mosses to shrubs), which makes this species different from other *Botrychium* species in Montana that prefer habitats with depauperate lower and middle canopies. The microsites were usually openings in the tree or shrub canopy with a high diversity of forbs and mosses; the successional status of these sites was not known. *Botrychium crenulatum* seems to prefer the wettest habitats of all *Botrychium* species in the Kootenai National Forest, Montana. When it occurs with *B. ascendens*, it tends to occupy slightly lower, wetter microsites (Vanderhorst 1997). In Nevada, Lellinger (1985) found *B. crenulatum* growing in association with *B. lunaria*. In Oregon, Wagner and Wagner (1993) reported finding *B. crenulatum* growing in a moist area adjacent to a meadow with *B. ascendens*, *B. lunaria*, and *B. minganense* in the Wallowa Mountains. In Alberta, Williston (2001) reported that *B. crenulatum* was found in drier places within wet meadows, bogs, and marshes. In Oregon, *B. crenulatum* occurred in marshy meadows and the wettest microsites of mesic meadows (Zika 1994). *Botrychium crenulatum* was found in partial shade there, but Zika (1994) had never observed it in deep shade. The authors also described other associated genera within these habitats, but that information is not reproduced here since these species may not occur within Region 2.

Within Region 2, *Botrychium crenulatum* was reported, but not confirmed, from Bighorn National Forest in 1997 (W. Fertig personal communication 2002). The single plant observed at that time was growing on a hummock in a wetland associated with *Salix* spp. and graminoids. W. Fertig (personal communication 2002) speculated that this report may actually be of the closely related *B. ascendens*, which was found in abundance at that site. Refer to the previous *B. ascendens* section for habitat characteristics of that site in Bighorn National Forest.

Botrychium lineare. New information on habitat of occurrences of *Botrychium lineare* discovered since publication of this assessment is available at http://www.fs.fed.us/r2/projects/scp/assessments/newinfo.shtml. Typical habitat descriptions for *B. lineare* are problematic because the known sites are so different (Wagner and Wagner 1994). Wagner and Wagner (1994) reported that *B. lineare* had been observed growing along the banks of a steep woodland trail in Montana, among deep grasses and forbs or meadows in Colorado, in a grassy area under a spruce tree in Oregon, and on limestone cliffs with narrow grassy horizontal terraces in Quebec.

In California, *Botrychium lineare* is known from only one small occurrence near Piute Pass in Fresno County, in an upper montane coniferous forest at an elevation of about 8000 ft (2600 m) (California Native Plant Society 2002). Three of the known populations of *B. lineare* in Montana were found growing along roadsides (i.e., disturbed areas) in seemingly early seral, open habitat, dominated by low-growing forbs rather than shrubs or trees (U.S. Fish and Wildlife Service 2001). *Botrychium lineare* sites in Oregon occurred in grass- and forb-dominated openings in forests characterized by coniferous trees such as pine, spruce, and fir species. At these occupied sites, *B. lineare* occurred with numerous associated graminoids, including *Calamagrostis* spp. (reedgrass) and *Festuca* spp. (fescue); forbs, including *Fragaria virginiana*, *Antennaria* spp. (pussytoes), *Galium boreale* (northern bedstraw), and *Potentilla* spp. (cinquefoil); shrubs, including *Symphoricarpos albus* (snowberry), and *Vaccinium* spp. (huckleberry); and trees, including *Picea engelmannii*, *Thuja plicata*, *Pseudotsuga menziesii* (Douglas-fir), *Pinus ponderosa* (ponderosa pine), *Pinus contorta* (lodgepole pine), and *Populus tremuloides* (quaking aspen) (U.S. Fish and Wildlife Service 2001). Other *Botrychium* species often found growing with *B. lineare* included *B. ascendens*, *B. crenulatum*, *B. minganense*, *B. lunaria*, and *B. montanum*. In the Lostine River Valley, Oregon, *B. lineare* was found in association with *B. lanceolatum*, *B. pinnatum*, and *B. crenulatum*. The conspicuous trees in the area included *Abies concolor* (white fir), *Picea engelmanii*, and *Pinus contorta*; shrubs included *Symphoricarpos spp.*, *Vaccinium* spp.; forbs included *Fragaria* spp., *Gentianella* spp., *Ranunculus* spp., *Rudbeckia* spp., and *Veronica* spp.; and graminoids included *Calamagrostis* spp. (Wagner and Wagner 1994).

In Colorado, *Botrychium lineare* is reported to occur on grassy slopes, among medium-height grasses, and along edges of streamside forests, at elevations of 2400 to 2900 m (7900 to 9500 ft) (Spackman et al. 1997). On Pikes Peak, within the Pike-San Isabel National Forest, the largest population of *B. lineare* occurs at 2650 m in full sunlight in a grassy meadow adjacent to a perennial creek (Tapia 2000). This moonwort was discovered by parting the knee-high grasses to find the plants (Wagner and Wagner 1994), which suggests that this species tolerates at least partial shading. This population is a "pure" population and not a genus community with any other moonwort species (P. Root personal communication 2003). Root (1999) reported that *Erigeron* spp. (fleabane) is a common forb that occurs with the moonwort. Other associated species include graminoids, such as *Bromus inermis* (smooth brome), *Carex eleocharis* (needleleaf sedge), *Festuca arizonica* (Arizona fescue), *Festuca thurberi* (Thurber's fescue), *Koeleria macrantha* (prairie junegrass), *Phleum pretense* (timothy), and *Poa* spp. (bluegrass); forbs, such as *Achillea lanulosa* (western yarrow), *Allium cerneum* (nodding onion), *Androsace* spp. (rockjasmine), *Anemone* spp. (anemones), *Antennaria* spp., *Besseya plantaginae* (White River coraldrops), *Chenopodium* spp. (goosefoot), *Dodecatheon pulchellum* (darkthroat shootingstar), *Erysimum asperum* (sanddune wallflower), *Fragaria virginiana*, *Galium boreale*, *Geranium* spp. (geraniums), *Linaria vulgaris* (butter and eggs), *Maianthemum stellatum* (starry false lily of the valley), *Potentilla* spp., *Pseudocymopterus montanus* (alpine false springparsley), *Rosa* spp. (rose), *Rudbeckia hirta* (blackeyed Susan), *Sisyrinchium montanum* (strict blue-eyed grass), *Taraxacum officinale* (common dandelion), *Thalictrum* spp. (meadow-rue), *Tragopogon dubius* (yellow salsify), and *Viola* spp.(violet); shrubs, such as *Arctostaphylos uva-ursi* (kinnikinnick), *Artemisia frigida* (prairie sagewort), *Artemisia ludoviciana* (white sagebrush), and *Pentaphylloides floribunda*; and trees, such as *Populus tremuloides* and *Salix* spp. (Tapia 2000).

Nearby meadows were also searched by P. Root for *Botrychium lineare*, with negative results. However, other searchers did find another population about one mile down the slope at a similar level area by a creek (Root 1999). Meadows without *B. lineare* tended to be more abruptly sloped toward the creek bed with dense stands of aspens at the base of the slope (Root 1999).

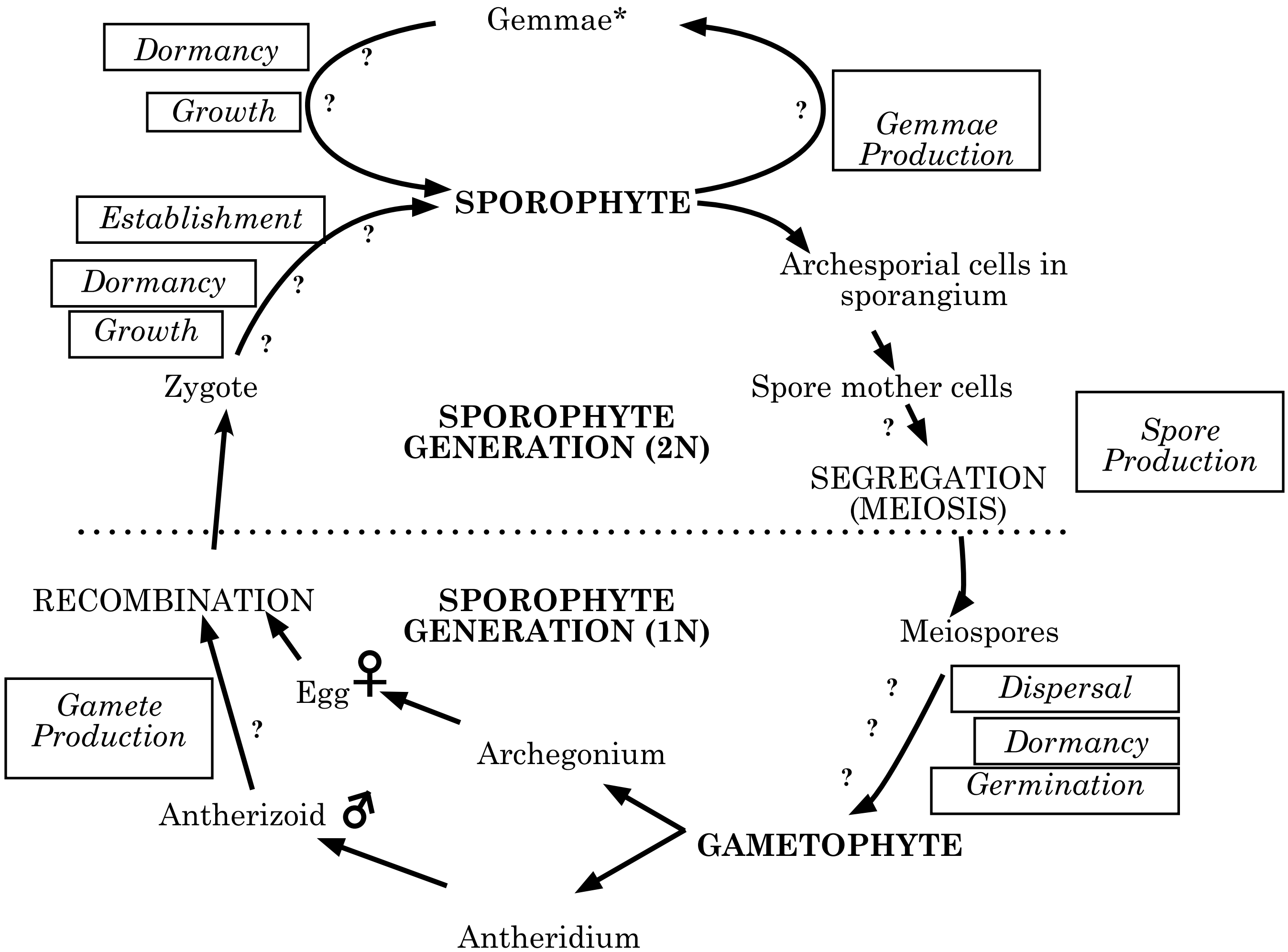
Reproductive biology and autecology

Reproduction

All three species of *Botrychium* discussed in this assessment (*B. ascendens*, *B. crenulatum*, and *B. lineare*) belong to the subgenus *Botrychium* (moonworts) of the genus *Botrychium* within the Division Pteridophyta (ferns). In North America, there is also subgenus *Osmundopteris* (rattlesnake fern) and subgenus *Sceptridium* (grapeferns) (Lellinger 1985, Wagner and Wagner 1993). The life cycle of all three subgenera is similar (Lesica and Ahlenslager 1996), and a generalized diagram of the *Botrychium* life cycle is depicted in **Figure 7** (Lellinger 1985, Johnson-Groh et al. 2002). Although details of reproduction in *B. ascendens*, *B. crenulatum*, or *B. lineare* have not been studied, we present information from other *Botrychium* species in an effort to elucidate potential reproductive mechanisms for the three species covered in this assessment. For example, although Johnson-Groh et al. (2002) studied belowground structures for seven *Botrychium* species other than *B. ascendens*, *B. crenulatum*, or *B. lineare*, it is possible that many of these characteristics apply to other *Botrychium* species as well.

The life history of ferns includes a distinct alternation of generations, represented by separate and unlike plants (Lellinger 1985, Johnson-Groh et al. 2002) (**Figure 7**). The diploid sporophyte stage of *Botrychium*, which is the familiar, visible portion consisting of the aboveground structures, produces spores in sporangia borne on specialized portions of the *Botrychium* frond. The spores are haploid, with half the number of chromosomes of the parent sporophyte. These spores are dispersed, filter into the soil, germinate underground, and develop into the haploid gametophyte stage (Farrar 1998, Johnson-Groh et al. 2002). The haploid gametophyte is a small, achlorophyllous, irregularly-shaped structure, usually less than 1 mm in size (Johnson-Groh et al. 2002). This underground gametophyte bears rhizoids and sexual structures (i.e., male antheridia and female archegonia) producing sperm and eggs. When mature, sperm are released and swim to an egg with which they fuse to create a zygote and to initiate the next sporophyte (diploid) generation. The juvenile sporophytes of *Botrychium* are also underground structures, bearing a short rhizome, a leaf-producing bud, and mycorrhizal roots. It may take several years for the single leaf of the juvenile sporophyte to develop and emerge aboveground (Johnson-Groh et al. 2002).

Some *Botrychium* species, including *B. ascendens*, *B. pumicola*, *B. pallidum*, *B. echo*, *B. gallicomontanum*, and *B. campestre*, can also asexually reproduce by creating minute, spheric structures called gemmae on the rhizome (Camacho and Liston 2001, Johnson-Groh et al. 2002), but there is no evidence that *B. crenulatum* or *B. lineare* have gemmae. Johnson-Groh et al. (2002) report that *B. ascendens* produces numerous gemmae.



Adapted from: Lellinger, D.B. 1985. *A Field Manual of the Ferns and Fern Allies of the United States and Canada*. Smithsonian Institution Press: Washington, D.C.; Grime, J.P. 1979. *Plant Strategies and Vegetation Processes*. John Wiley & Sons, Ltd: Bath, U.K.; Colorado Natural Heritage Program, Biological Conservation Database, Fort Collins, Colorado; Johnson-Groh, C., C. Riedel, L. Schoessler, and K. Skogen. 2002. Belowground distribution and abundance of *Botrychium gametophytes* and juvenile sporophytes. *American Fern Journal* 92(2): 80-92.

Figure 7. Schematic representation of the hypothesized life cycle of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare*. Note the alternation of generations between the diploid (2n) sporophyte and the haploid (1n) gametophyte. Gametophytes, juvenile sporophytes, and gemmae are belowground structures, and sporophytes emerge aboveground (Johnson-Groh et al. 2002). Rates of growth, dispersal, germination, recruitment, spore and gamete production, and survival are unknown for these species and are indicated by "?".

* Only *B. ascendens* is reported in the literature to produce gemmae (Johnson-Groh et al. 2002); gemmae were not reported for *B. crenulatum* or *B. lineare*. The rate of gemmae production is unknown for *B. ascendens* and is indicated by "**?**".

These gemmae are small (0.5 to 1 mm) and can give rise to an independent plant when detached from the parent plant.

Reproductive timing

The current understanding of reproductive timing for *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* are discussed below.

Botrychium ascendens. In Region 2, *Botrychium ascendens* produces spores from July through August (Fertig 1994, Fertig and Beauvais 1999).

Botrychium crenulatum. Outside Region 2, *Botrychium crenulatum* is reported to release spores from June to July in California (California Native Plant Society 2002). Within Region 2, the *B. crenulatum* spore-producing period extends from July through August in Wyoming (Fertig 1994). The fronds on *B. crenulatum* appear from mid- to late spring and dry out in late summer (Fertig 1994).

Botrychium lineare. *Botrychium lineare* spores have been reported to mature in late June and July (U.S. Fish and Wildlife Service 2001). *Botrychium lineare* may be different from lower-elevation moonworts because it is a subalpine species with a shorter available growing season (P. Root personal communication 2002). The plants in the Pikes Peak population emerge from the ground in late June and return to dormancy by mid-July.

Life history and strategy

The reproductive biology of *Botrychium* has implications for the life history and strategies of these species, including (but not restricted to) issues related to dispersal, genetic variability, colonizing ability, habitat needs, distribution, and long-term persistence. The life history of ferns differs from flowering plants in that ferns have a distinct alternation of generations and reproduce by spores rather than seeds (Jones and Luchsinger 1979). The familiar, aboveground, leafy part of the plant is the diploid sporophyte phase of the life cycle, when haploid spores are produced and dispersed. The spore germinates to form an independent alternate generation known as the haploid gametophyte plant. The underground gametophyte fern plant produces sperm and eggs on the antheridia and archegonia, respectively. When sperm from the antheridia swim toward the archegonia, the union of sperm and egg gives rise again to a sporophyte (**Figure 7**).

Self-fertilization may be the primary form of reproduction in *Botrychium*, because the sperm can more easily reach the archegonium (eggs) of its own gametophyte, just a few millimeters away, rather than the archegonium of another more distant gametophyte (McCauley et al. 1985, Farrar 1998, Camacho and Liston 2001). Uniting a genetically identical sperm and egg yields a sporophyte with no genetic heterozygosity. Thus, with no means of maintaining or generating genetic variability (except by a new mutation), sexual reproduction through self-fertilization results in a clone-like population (Farrar 1998). In over 2,000 individual *Botrychium* plants examined electrophoretically, less than one percent showed heterozygosity from outcrossing. This may have implications for dispersal and colonizing abilities. For example, a single spore could theoretically disperse, colonize suitable habitat, and establish a new population through self-fertilization (Farrar 1998, Johnson-Groh et al. 2002).

The belowground structures of *Botrychium* (i.e., gametophytes, juvenile sporophytes, gemmae, and spores) may help these species to survive aboveground perturbations and be more resilient (Johnson-Groh et al. 2002). In addition, species that can reproduce asexually, like *B. ascendens*, may be able to take advantage of existing conditions (e.g., mycorrhizal associations with parent) and rapidly colonize a small area with a cluster of multiple sporophytes derived from gemmae (Johnson-Groh et al. 2002). Gemmae reproduction may be a short-term advantage in favorable habitats, while spore-gametophyte reproduction may provide a long-term advantage by allowing dispersal to other sites (Johnson-Groh et al. 2002). It is unknown to what extent or under what conditions *B. ascendens* reproduces asexually or sexually. However, Johnson-Groh et al. (2002) noted that species producing numerous gemmae tended to yield fewer gametophytes.

Based on the current understanding of the habitat characteristics and reproductive mechanisms of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare*, all three species evaluated in this report could likely be considered r-selected species because they can colonize dynamic or open environments (Grime 1979, Barbour et al. 1987). Evidence for the preference of *B. ascendens*, *B. crenulatum*, and *B. lineare* for open, early-successional areas is presented in the Habitat Characteristics section. In temporary habitats (e.g., unpredictable habitats or habitats that exist for only a short period of time), rapid and abundant production of propagules and short life-span are adaptive (Grime 1979). Further, these *Botrychium* species seem to have characteristics of stress-tolerant ruderals because they are short-lived perennial species with mycorrhizal associations and the ability to be dormant during times of stress or disturbance (Grime 1979, California Native Plant Society 2002, Johnson-Groh et al. 2002).

Pollinators and pollination ecology

Pollination is not an issue with *Botrychium* species, as they reproduce by producing independent gametophytes and not by flowers. Many flowering plant species rely on a pollination mechanism (e.g., insects, wind) to move the gametes (pollen) of one flower to the receptive structure (ovary) of another flower to facilitate sexual reproduction. In the case of *Botrychium* species, the gametes are produced by underground gametophytes. The sperm produced by one gametophytic structure (antheridia) need to swim to the eggs within another structure (archegonia) in order for sexual reproduction to occur. Sexual reproduction in this case is facilitated by the presence of groundwater, not pollinators.

Dispersal mechanisms

Botrychium species disperse to new locations by disseminating spores. Although stages in the *Botrychium* life cycle include gametophytes, juvenile sporophytes, and gemmae, these structures generally do not play a role in propagating or disseminating the species to new habitats because they exist underground with limited means for movement.

The spore cases of *Botrychium* species are the largest of all known ferns, and they are filled with thousands of spores (Wagner 1998). Details of dispersal mechanisms in *B. ascendens*, *B. crenulatum*, and *B. lineare* are not well known, but dispersal of *Botrychium* spores probably occurs by gravity or wind (Vanderhorst 1997, Wagner 1998, Johnson-Groh et al. 2002), and may possibly occur by animal activities (Wagner et al. 1985, Zika 1992, Kolb and Spribille 2001, U.S. Fish and Wildlife Service 2001, Johnson-Groh et al. 2002).

The largest spore banks for *Botrychium virginianum* occurred right below the parent plants and decreased significantly at 2 m away (Johnson-Groh et al. 2002), suggesting that most dispersal occurs by gravity over short distances. In addition, *Botrychium* spores are small and light (they look like yellow dust), and they can theoretically be carried long distances by air currents, particularly if the sporophyte emerges above the herbaceous layer (Vanderhorst 1997, Wagner 1998, U.S. Fish and Wildlife Service 2001). If the sporophyte remains within the herbaceous layer, however, spores may potentially travel only a few centimeters from the parent plant (Casson et al. 1998). The capability of moonworts for long distance spore dispersal, coupled with the tendency to self-fertilize and the ability to establish a population with one spore, could potentially lessen the negative impacts of habitat disconnectivity, isolation, and fragmentation (Kolb and Spribille 2001). In contrast, other rare plants that have minimal capabilities for long-term dispersal and requirements for gamete dispersal may be more affected by habitat fragmentation (Kolb and Spribille 2001). However, effective long distance spore dispersal still requires necessary conditions: (1) intact, suitable habitat on both ends, including mycorrhizae; and (2) a large enough initial population to produce an immense number of spores for the odds to favor a few spores to find distant, appropriate habitat. Thus, isolation, habitat fragmentation, loss of habitat, and small *Botrychium* populations are likely still important factors in the success of long distance colonization.

Botrychium spores may also be distributed by the activities of animals, like ungulates (e.g., deer, elk, cattle) and small mammals (e.g., voles, gophers, pikas, rabbits). The spore case walls of *Botrychium* are exceptionally thick for ferns, which may aid in their movement through animal guts (Wagner and Wagner 1993). Apparently one researcher fed grape fern spores to a vole and observed intact spores in the droppings (P. Root personal communication 2003). The Wagners are reported to have observed ungulates and small mammals grazing the sporophores of moonworts (Kolb and Spribille 2001), and to have seen grape ferns distributed along old deer trails in forests (P. Root personal communication 2003). Apparently the Wagners also noted that rabbits and rodents selectively nipped off the sporophores and left the leaf blades intact (Wagner et al. 1985, Zika 1992). It is possible that if small mammals clip the sporophores and bring them to underground burrows, they could give rise to underground gametophytes.

Spore viability and germination requirements

Botrychium species can produce thousands of spores per spore case, which is probably the highest known for vascular plants (Wagner 1998). However, very little information is available regarding spore fertility, viability, germination needs, or extent of a spore “bank” for *B. ascendens*, *B. crenulatum*, and *B. lineare*. Studies summarized by Johnson-Groh et al. (2002) have found that fern spores persist in the soil for many years, and the long-term accumulation of these spores typically leads to a high abundance of spores in the soil. *Botrychium* species may also have spores with similar longevity in an extensive spore bank, where the size and flux of the spore bank is affected by additions (i.e., variable annual input) and losses (i.e., predation, loss of viability, environmental perturbations) of spores (Johnson-Groh et al. 2002). Annual spore production varied greatly for species studied by Johnson-Groh et al. (2002). In addition, the researchers discovered that only 55 percent of aboveground *B. gallicomontanum* plants actually produced spores that year; the remainder of the plants senesced before spore production. The factors influencing spore production in *B. ascendens*, *B. crenulatum*, and *B. lineare* have not been studied.

For *Botrychium* species, the underground structure "bank" can also include gametophytes and juvenile sporophytes (and gemmae for some species) in addition to spores (Johnson-Groh et al. 2002). Johnson-Groh et al. (2002) discovered that the density of belowground structures greatly exceeded the aboveground population for all species, and the abundance of belowground structures varied greatly between species. The differences between species may be an artifact of sampling patchy populations, or it may reflect biological differences in spore production.

The germination needs of *Botrychium* spores are not well known, but it is likely that spores are dormant until the appropriate light, moisture, and mycorrhizal conditions exist (Whittier 1973, Whittier 1981, Johnson-Groh et al. 2002). Once *Botrychium* spores are dispersed, in order for the spores to germinate and form the sexual gametophytes, it is necessary for them to infiltrate down into the soil one to several inches where they can germinate (Wagner 1998). It is likely that spores filter into porous soil with rainwater percolation or drop into small cracks or holes created by soil fauna or other soil disturbances (Whittier 1972). To prevent premature germination, spores will not germinate in the light (Whittier 1973, Wagner 1998). They must penetrate into the lower layers of soil, where it is completely dark, before they will germinate and develop into gametophytes. Whittier (1973) found that spores previously ungerminable in the light would germinate when placed in the dark. In addition, germination of spores in research cultures can be inhibited by nitrate and promoted by ammonium, and growth of the gametophyte can be promoted by the addition of sugar (Whittier 2000). The subterranean gametophyte needs to be infected by an endophytic fungus in an obligate association; otherwise, without this external carbon source, they will not develop past the two- to three-cell stage (Whittier 1973). The mortality between the spore and gametophyte stages is unknown, but it is hypothesized to be high, especially as gametophytes require mycorrhizal infection (Johnson-Groh et al. 2002). Gametophytes and sporophytes of *Botrychium* species may exist underground for many years before an aboveground plant develops (Muller 1993).

The belowground biology and ecology of *Botrychium* structures undoubtedly plays a huge role in the population dynamics of these species (Johnson-Groh et al. 2002). This is an area requiring additional study to develop an understanding of factors affecting survival and reproduction in *Botrychium* species in Region 2.

Cryptic phases

The life history of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* includes cryptic phases. As previously discussed, *Botrychium* species demonstrate a distinct alternation of generations, with gametophytes, juvenile sporophytes, and gemmae being completely subterranean (Johnson-Groh et al. 2002). Juvenile sporophytes can be dormant for one or more years, and cannot be identified with certainty in their immature stages (U.S. Fish and Wildlife Service 2001). However, the extent of the spore bank or dormancy of these three *Botrychium* species is still unknown.

Phenotypic plasticity

Phenotypic plasticity is demonstrated when members of a species vary in height, leaf size, flowering (or spore-producing) time, or other attributes, with change in light intensity, latitude, elevation, or other site characteristics. No information specifically regarding phenotypic plasticity in *Botrychium* species was found in the literature reviewed. Obtaining technical descriptions of one species from different regions is difficult, because regional floras are often based on the original species description and are not updated with the most current *Botrychium* research. However, some generalizations can be made. First, the three moonworts evaluated in this report are all small, inconspicuous plants, ranging in height from 5 to 18 cm. The propensity towards self-fertilization potentially results in clone-like populations of genetically and presumably phenotypically similar individuals. Morphologically, the appearance of *B. lineare* varies little from one location to another (Wagner 1998). Further study is required to ascertain whether phenotypic expressions of the genotype, either within or between populations, vary according to environmental influences.

Mycorrhizal relationships

All *Botrychium* species are believed to be obligately dependent on mycorrhizal relationships (the symbiotic association of a fungus with the roots of a vascular plant) in all life stages (Wagner and Wagner 1981, Gifford and Foster 1989, Zika 1992, Johnson-Groh et al. 2002). There are no reports of successful completion of the *Botrychium* life cycle without mycorrhizal fungi (U.S. Fish and Wildlife Service 2001). *Botrychium* species most likely depend on mycorrhizal associations during gametophyte and sporophyte development as a source of carbohydrates, minerals, and water (Johnson-Groh et al. 2002). Johnson-Groh (1998) suggested that mycorrhizal health may be a contributing factor to the appearance or absence of *Botrychium* plants in a given growing season. The identity of the mycorrhizal fungi forming associations with *Botrychium* species has not been determined, but they are most likely a group of common fungi belonging to relatively few species that are broadly non-specific with regard to their associated plant species (Farrar 1998). *Botrychium* mycorrhizae have been described as the vesicular-arbuscular type by Berch and Kendrick (1982) and Schmid and Oberwinkler (1994). This type of endophytic fungus can penetrate inside the plant cells of both the roots and the gametophytes without causing disease. The specific characteristics of mycorrhizal associations with *B. ascendens*, *B. crenulatum*, and *B. lineare* have not been studied.

Botrychium plants have a few, thick, fleshy roots, lacking root hairs for extracting water and minerals from the soil (Farrar 1998). The roots are infested with fungi whose mycorrhizal filaments extend from the roots into the surrounding soil. In these kinds of associations, it is generally believed that the role of the fungus is to take up water and mineral nutrients and to transport these to the root of the host plant. It has also been demonstrated that mycorrhizae attached to two separate host plants can transfer carbohydrates between the plants (mycotrophism) (Farrar 1998). The mycotrophic condition is important to the ecology of *Botrychium* species in several ways. Studies by Johnson-Groh (1998) on *B. mormo* suggest that, at least for this species, photosynthesis is not critical, and the mycorrhizae are more important in the overall energy budget. *Botrychium mormo* probably depends relatively little on leaves for photosynthesis, since the leaves can be white or pale yellow and frequently do not emerge above the litter layer. The role of mycorrhizae in the nutritional needs of *B. ascendens*, *B. crenulatum*, and *B. lineare* has not been studied.

Intimate associations of *Botrychium* species with mycorrhizae may assist these moonworts in coping with environmental change, especially because mycorrhizal fungi are quite ubiquitous in terrestrial habitats and presumably adaptable to environmental change (Farrar 1998). Nutrition supplied by fungal associations may allow the moonworts to withstand repeated herbivory, prolonged dormancy underground (i.e., juvenile sporophytes), or growth in dense shade (Montgomery 1990, Kelly 1994, Johnson-Groh et al. 2002). The mycorrhizal relationship may also have implications for the occurrence of genus communities, the distribution of the species across the landscape, and associations with particular vascular plants. For example, close associations have been observed between certain moonworts and strawberries (*Fragaria* spp.) (Zika 1992, 1994), and between grapeferns (*Botrychium* subgenus *Sceptridium*) and Rosaceous fruit trees (Lellinger 1985). These associations could possibly result from shared mycorrhizal associations between these plant species and the moonworts, but this has not been studied.

Mycorrhizae are probably the most important limiting factor for *Botrychium* establishment, distribution, and abundance (Johnson-Groh 1998, Johnson-Groh et al. 2002), but there is little information regarding habitat requirements for these fungi that are associated with moonworts (U.S. Fish and Wildlife Service 2001). Adequate soil moisture is probably one of the main factors affecting mycorrhizal health (Johnson-Groh 1998).

Hybridization

Many differences and misunderstandings pertaining to the Ophioglossales resulted in inaccurate historical conclusions on hybridization (Wagner and Wagner 1986). Initial theories on hybridization within the *Botrychium* subgenus *Botrychium* species, based on the subterranean gametophyte, concluded that hybridization or even cross-breeding within the species was almost impossible (Wagner and Wagner 1986). Conversely, in more recent studies, Wagner and Wagner (1986) reported that they found no evidence that the subterranean gametophyte inhibited hybridization and outcrossing. Wagner and Wagner (1986) stated that 12 hybrids are known in the *Botrychium* genus, and the authors presented original species descriptions for two additional *Botrychium* hybrids, all with subterranean gametophytes. Wagner (1998) explained that while self-fertilization may be the predominant form of reproduction, under moist conditions, sperm can swim from one gametophyte to the gametophyte of another species of *Botrychium*, potentially forming an interspecific hybrid. Those few hybrids that do form are intermediate in characteristics between the parents and apparently sterile; the spores they produce are abortive and unable to initiate populations. Hybridization is likely an important feature in the evolution and speciation of moonworts, especially where hybrids overcome initial sterility through polyploidy or reproducing via unreduced spores (Flora of North America 2003).

In their original description for *Botrychium ascendens*, Wagner and Wagner (1986) report that a single hybrid between *B. ascendens* and *B. crenulatum* was found at the type locality for *B. ascendens*. Morphological characteristics of the hybrid individual were intermediate between the two species. The hybrid pinnae were slightly wider than those found on *B. ascendens*, approaching the width of *B. crenulatum*. Additionally, the lowest pinna on the hybrid was fan-shaped, similar to *B. crenulatum*, while the remaining pinnae were observed to be cuneate, as found in *B. ascendens*. Spores from the observed hybrid turned out to be abortive. With the current knowledge of chromosomes in these plants, Wagner and Wagner (1986) concluded that the hybrid plant would prove to be a sterile triploid.

Demography

Although some demographic research has been done in Minnesota by Johnson-Groh (1998), there is very little currently known about population demographics in moonworts (Kolb and Spribille 2001).

Life history characteristics

There is little information regarding the life span, recruitment, survival, or age at which individuals become reproductive. The information that is available indicates that *Botrychium* species have short sporophytic life spans (i.e., half-life). For example, Lesica and Ahlenslager (1996) reported an aboveground life span of three years in their monitoring study of *B. hesperium*, *B. paradoxum*, and *B. x watertonense* in Waterton Lakes National Park in Alberta. From the Vosges Region of France, Muller (1993) reported a life span of two years for B. matricariifolium once it had begun reproducing. Other *Botrychium* species are estimated to have an average life span of about five to six years after the first aboveground appearance (Kolb and Spribille 2001). The life spans of sporophytes and gametophytes of *B. ascendens*, *B. crenulatum*, and *B. lineare* have not been studied.

Detailed long-term monitoring studies are required to obtain demographic information. It is difficult to measure simple demographics such as recruitment and mortality, even with individually tagged plants (Johnson-Groh 1998). One of the longest monitoring studies on *Botrychium* species was conducted by Johnson-Groh (1998) on *B. mormo* in Minnesota. Based on monitoring of five plots over three years, the average recruitment (i.e., first appearance of a leaf in a new location within the plot) of *B. mormo* was 35 percent of the population. There was a large year-to-year variability in numbers of individuals occurring in a given population. It was discovered that part of the explanation for this phenomenon is that individual plants may skip years, producing no aboveground leaves in a given year, but remaining alive and producing leaves the following season. While new plants are annually recruited into the population, older plants may disappear or reappear after absences of one to three years. Because of the variation from year to year in the individuals that emerge aboveground and the potential that a new plant could emerge from the location where a different plant was tagged, this estimate may not reflect actual field conditions.

Similar results have been documented in USFS Region 2 during the monitoring of the *Botrychium lineare* population on Pikes Peak (Pike-San Isabel National Forest) (Carpenter 1996a, Carpenter 1996b, Tapia 2000) (**Table 2**). Monitoring data from 1996 and 2000 have revealed that approximately 75 percent of the individuals for that year survived from a previous year (i.e., tagged plants), and approximately 25 percent represented new individuals (Carpenter 1996b, Tapia 2000) (**Table 2**). This is an area requiring additional study in future monitoring programs.

Life cycle diagram and demographic matrix. Demographic parameters have not been recorded for these *Botrychium* species, so there is no definitive data regarding the vital rates that contribute to species fitness. Although stage-based models based on population matrices and transition probabilities can be used to assess population viability (Caswell 2001), there is not adequate demographic data about *B. ascendens*, *B. crenulatum*, and *B. lineare* to create a similar life history model. For these *Botrychium* species, the stages that could potentially be incorporated into a demographic matrix include: spore bank, gametophyte, juvenile belowground sporophyte, adult aboveground sporophyte, and adult belowground sporophyte (Johnson-Groh 1998, Johnson-Groh et al. 2002) (**Figure 7**). From information input to the matrix, a corresponding life cycle diagram could be constructed. The life cycle diagram is a series of nodes that represent the different life stages, connected by various arrows that represent the vital rates (i.e., survival rate, fecundity). Casson et al. (1998) constructed a population model for *B. mormo*, and this work could be used as an example when sufficient demographic data have been collected for *B. ascendens*, *B. crenulatum*, and *B. lineare*.

Population viability analysis. There is inadequate data to create a population viability analysis for *Botrychium ascendens*, *B. crenulatum*, or *B. lineare*. While demographic monitoring has been initiated on Pikes Peak for *B. lineare*, monitoring studies for *B. ascendens* and *B. crenulatum* have not been conducted. Due to the paucity of demographic information for *Botrychium* species, it is difficult at this time to assess population viability for *Botrychium* species in Region 2. This is an area that requires additional study before assessments of population viability can be successfully completed.

Recruitment

Recruitment in plant populations is generally defined as survival by juvenile plants to reproductive maturity. In the limited population monitoring studies that have been conducted (i.e., for *Botrychium lineare* [Carpenter 1996b, Tapia 2000], and for other *Botrychium* species [Johnson-Groh 1998]), the numbers of aboveground individuals varied substantially from year to year. In a study where individual *B. mormo* plants were tagged, Johnson-Groh (1998) found that an individual plant may emerge one year, but remain underground the next year. The plants in the population studied represented new recruits, as well as individuals that emerged the previous year, or had been underground for one season or more. In the population of *B. lineare* monitored on Pikes Peak, of the approximately 50 individuals found, 25 percent (i.e., about 12 to 14 plants) were probably new individuals (Carpenter 1996b).

Gametophytes and sporophytes of *Botrychium* species may exist underground for many years before an aboveground plant develops (Muller 1993). In the study of belowground structures for a variety of *Botrychium* species (not including *B. ascendens*, *B. crenulatum*, or *B. lineare*) by Johnson-Groh et al. (2002), the researchers found that there is high mortality between the gametophyte and juvenile sporophyte stages (up to 73 percent) and between the juvenile sporophyte and emergent sporophyte stages (up to 93 percent) (Johnson-Groh et al. 2002). The rates of recruitment have not been studied for *B. ascendens*, *B. crenulatum*, or *B. lineare*.

Ecological influences on survival and reproduction

Spore germination, gametophyte and sporophyte growth, spore production, and long-term persistence of *Botrychium* species most likely depend on a range of ecological influences over many years, including environmental fluctuations (e.g., precipitation), microsite conditions (e.g., soil suitability, moisture levels), associated species (e.g., mycorrhizal relationships), disturbance patterns (e.g., intensity and frequency), interspecific competition (e.g., successional processes, invasive plants), and herbivory (e.g., dispersal, spore predation). The establishment of new populations of *B. ascendens*, *B. crenulatum*, and *B. lineare* most likely depends on factors related to dispersal and the availability of suitable germination sites and conditions.

Both the gametophytic and the sporophytic life stages are equally important for reproductive success in *Botrychium* species. The gametophyte must develop an underground mycorrhizal relationship in order to survive and to successfully reproduce and produce the sporophyte. The sporophyte must mature for the spores to be disseminated to favorable habitat and start the new gametophytic life stage. For the gametophyte life stage, soil moisture, soil suitability, and the presence of mycorrhizal fungi are possible limiting ecological factors. The gametophyte produces both male and female gametangia, and fertilization only occurs under wet conditions, when sperm move through soil water to reach eggs (Lesica and Ahlenslager 1996).

In a study by Johnson-Groh (1998) on the population biology of *Botrychium mormo*, results showed that aboveground populations of this species fluctuated independently among plots at any given site and that some populations seemed to be increasing while others were decreasing. Such variation could be the result of microsite differences, such as soil moisture, distribution of mycorrhizae, herbivory, or other factors (Johnson-Groh 1998). The distribution of mycorrhizae is likely the most limiting factor for *Botrychium* establishment, distribution, and abundance (Johnson-Groh 1998). As a result, environmental factors that may affect mycorrhizae, such as reduced water availability or soil compaction, may have significant impacts on moonworts (Johnson-Groh 1998). For example, numbers of visible, aboveground moonwort populations may be greatly reduced during drought years.

As off-site colonizers, moonworts seem to be adapted to colonization of new habitats and the bridging of habitats due to wind dispersal of spores. As discussed previously, light to moderate habitat disturbances may be needed to create habitats and microsites for some *Botrychium* species (including *B. ascendens*, *B. crenulatum*, and *B. lineare*), especially those that occur in meadows and openings in densely vegetated communities (Lellinger 1985, Zika 1992, Wagner and Wagner 1993, Lesica and Ahlenslager 1996, Root 1999, Kolb and Spribille 2001, Williston 2001). Future populations of *B. ascendens*, *B. crenulatum*, and *B. lineare* may rely on disturbances to create habitat, while existing populations could be extirpated by a disturbance event. For example, scoured streamside habitats provide appropriate substrate for *B. ascendens*, but flooding is believed to have extirpated one population in Montana (Vanderhorst 1997). As a result, the persistence of *Botrychium* species may rely on a landscape with a mosaic of patches, where a series of local populations exist, disperse, and disappear with the changing successional landscape (Chadde and Kudray 2001). Details concerning the type, size, intensity, or frequency of disturbance regime important for the establishment and growth of these three *Botrychium* species are not well known.

Spatial characteristics

Botrychium species are often patchily distributed at both local and regional scales (Johnson-Groh et al. 2002). The spatial distribution of *Botrychium* individuals most likely depends on the availability of macrohabitat and landscape heterogeneity, microsite characteristics, dispersal factors, environmental stochasticity, survivorship, soil bank of underground structures, relationships with associated species, gene flow between populations, and disturbance patterns. At a small scale, Carpenter (1996b) observed that within a *B. lineare* monitoring plot, the individuals were clumped in small groups that were scattered across the plot. Wagner et al. (1985) observed hundreds of *B. mormo* gametophytes densely distributed over 1 square foot of ground. The authors also observed browsed sporophores of this species and hypothesized that if spores are dispersed by animal vectors, then a pile of feces could lead to a cluster of gametophytes. In addition, reproduction by gemmae (as seen in *B. ascendens*) could also lead to clustering of sporophytes. The patchiness of disturbances could also affect *Botrychium* distribution. K. Ahlenslager (personal communication 2003) noted that *Botrychium* species are often found near old gopher holes, but not fresh gopher disturbances. On a larger scale, *Botrychium* spores are small and light enough to be carried by air currents. This dispersal mechanism may help explain the broad and often disjunct distribution patterns exhibited by moonworts at the regional scale (U.S. Fish and Wildlife Service 2001).

Many of these factors have not been detailed sufficiently to understand the spatial distribution of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* in Region 2.

Genetic characteristics and concerns

Botrychium species are characterized by unique reproductive strategies, such as belowground gametophytes, propensity for self-fertilization, possibility for outcrossing and hybridization, long distance spore dispersal, and colonization of mildly to moderately disturbed habitats. These strategies need to be considered when evaluating genetic concerns. Over the last decade, procedures for starch gel electrophoresis have become standardized and applied widely across plant taxa as a method to detect genetic variation (Farrar 1998). Genetics studies of several *Botrychium* species have revealed very low intraspecific variation, likely the result of inbreeding as the primary mode of reproduction. Typically, low genetic variation in vascular plant species or populations is regarded as a negative attribute, in that the species is less capable of adapting to meet the needs of a changeable environment (Farrar 1998). The concern with continuous inbreeding is the loss of genes from the gene pool, leading to lower variation and reduced capacity to respond to environmental change. Furthermore, inbreeding can lead to reduced vigor resulting from expression of deleterious alleles (inbreeding depression). However, Kolb and Spribille (2001) suggest that inbreeding depression, which can be an issue in the conservation of isolated or small populations of rare plants, is not an issue with *Botrychium* species. They propose that the deleterious genes that cause inbreeding depression in outcrossing species are quickly eliminated from the gene pool in habitually selfing species. Thus, low genetic variability and homozygosity may not be a negative attribute for the persistence of Botrychium, either at the species or population level.

Although *Botrychium* species seem to have extremely low genetic variability, the range of many species spans several states (Farrar 1998). The capacity for self-fertilization is likely an advantageous feature in dispersal, because it ensures the production of sporophytes and more spores, each of which is capable of producing a colony of plants over time if it reaches a suitable habitat (Farrar 1998). This is especially important for long distance colonization where widely dispersed spores are likely to be isolated from one another. Using this reproductive strategy, if a lightweight spore is carried to a distant suitable habitat, there is a better chance of it producing a new colony of plants than if the species was not capable of self-fertilization (Farrar 1998, Kolb and Spribille 2001).

Genetic variability in *Botrychium* may not be as important to the long-term viability of the species as other factors, such as self-fertilization and mycorrhizal associations (Farrar 1998). As discussed, the establishment of mycorrhizal associations in the life cycle of *Botrychium* species may result in the ability of moonworts to tolerate or adapt to environmental changes. Self-fertilization and asexual reproduction (in some species, like *B. ascendens*) may, in fact, facilitate the establishment of mycorrhizal associations in new sporophytes by taking advantage of associations established with the gametophyte or parent plant (Johnson-Groh et al. 2002). In addition, genetic stability may actually play a role in assuring that the host *Botrychium* remains attractive to mycorrhizal fungi (Farrar 1998).

Cindy Johnson-Groh and Donald Farrar are currently involved in further studies on the genetic characteristics of *Botrychium* (C. Johnson-Groh personal communication 2002).

Community ecology

New information on *Botrychium lineare* is available at http://www.fs.fed.us/r2/projects/scp/assessments/newinfo.shtml.

Herbivores and relationship to habitat

Herbivores may have an impact on *Botrychium* species directly by affecting existing plants (e.g., grazing, trampling, dispersing spores) or indirectly by influencing habitat (e.g., compacting soil, facilitating spread of invasive plants, changing plant community and/or microsite). In Montana, herbivory of *B. ascendens* and *B. crenulatum* was not observed, but herbivory on the sporophytes of other species of moonworts was noted (Vanderhorst 1997). Not only are *Botrychium* species presumably susceptible to herbivory and habitat change by native herbivores (e.g., ungulates, small mammals, ants, insects), but they are also susceptible to the effects of livestock activity where they occur with permitted grazing. The extent and effects of native herbivory on *B. ascendens*, *B. crenulatum*, and *B. lineare* and their habitat in USFS Region 2 have not been studied. It is possible that native herbivores, such as fossorial mammals, may play an important role in the maintenance of suitable habitat, creation of open sites for colonization, and spore dispersal. They can also graze or damage existing individual plants. There is no cattle grazing at *B. lineare* sites in Region 2, but there is elk activity in this habitat (Carpenter 1996b). Low to moderate grazing by cattle occurs at *B. ascendens* sites in Shoshone National Forest (K. Houston personal communication 2003, Wyoming Natural Diversity Database 2003), and light to moderate cattle grazing and extensive elk and moose activity has been noted at the Bighorn National Forest site with *B. ascendens* (and maybe *B. crenulatum*) (S. Gall personal communication 2003). In Montana, *B. crenulatum* exists along disturbed roadsides with the presence of cattle grazing (Vanderhorst 1997).

The positive or negative effects of livestock and other herbivores on *Botrychium* populations and habitat quality presumably depend on *Botrychium* biology (e.g., response to herbivory), type of grazer (e.g., cattle, sheep), timing of grazing (e.g., season), grazing intensity (e.g., stocking density), habitat type (e.g., meadow or forest), and site conditions (e.g., topography, moisture, invasive plants).

The repeated removal of *Botrychium* aboveground leaf tissue may have little effect on viability (Montgomery 1990, Wagner 1992, Johnson-Groh 1998, Hoefferle 1999, Johnson-Groh et al. 2002). Initially, it was believed that the loss of the one aboveground leaf would decrease photosynthetic output and therefore decrease the overall vigor of the plant. However, moonworts may obtain much of their energy from mycorrhizal associations, which would lessen their reliance on photosynthetic tissues (Johnson-Groh et al. 2002). In addition, *Botrychium* species keep the primordia for next year's trophophore protected by the base of the current leaf (Wagner 1992). Pinching or pulling off this year's leaf could damage the primordia, but clipping the plant above the base would leave the underground tissues intact. Thus, herbivory by sheep, where plants are uprooted, could potentially be more detrimental than herbivory by cattle. Several studies have found that the loss of aboveground biomass either through herbivory, fire, or plant collection seems to have no effect on the subsequent return of the plant the following year (Montgomery 1990, Wagner 1992, Johnson-Groh 1998, Hoefferle 1999, Johnson-Groh et al. 2002). *Botrychium dissectum* individuals can survive the removal of leaves for many years (Montgomery 1990). In contrast, a study of two *Botrychium* species in Missouri found that if leaves were removed after spore release (i.e., significant energy expenditure), the plant could not recover enough energy, and this resulted in significantly smaller leaves the following year (Hoefferle 1999). If leaves were removed before spore production and maturation, however, then the size of the following year's leaves was not affected because the individual presumably retained enough energy. The study, however, incorporated only two years of data and may have been influenced by climatic conditions (e.g., dry conditions).

The sources of spore loss for *Botrychium* species is not well-documented but could occur with grazing by livestock or wildlife (U.S. Fish and Wildlife Service 2001). If grazing were to repeatedly occur prior to the maturation and release of spores, the capacity for successful reproduction over the long-term could be reduced (U.S. Fish and Wildlife Service 2001). While removing aboveground leaf tissue before sporulation may not affect individual survival, it would undoubtedly reduce spore output and potentially result in less long distance dispersal. Moonwort spores may possibly be dispersed by the activities of ungulates (e.g., deer, elk, cattle) and small mammals (e.g., voles, gophers, pikas, rabbits) (Wagner et al. 1985, Zika 1992).

Chadde and Kudray (2001) suggested that *Botrychium* species are more likely to be damaged by incidental trampling than by grazing, because of their small size. In Bighorn National Forest, the swamp area with *B. ascendens* (and maybe *B. crenulatum*) is a small portion of a grazing allotment (S. Gall personal communication 2003). Cattle occasionally visit the area, as it is a source of water, but they do not tend to concentrate there heavily. Cattle use of the area probably varies from year to year, depending on moisture conditions and the condition of the surrounding pasture areas. There is also substantial use of the area by elk and moose, and these large herbivores could also incidentally trample *Botrychium* individuals (S. Gall personal communication 2003). Grazing by cattle and sheep could have different consequences, as sheep tend to browse more forbs and uproot plants, while cattle tend to prefer grasses and may have more trampling impacts. In Washington, K. Ahlenslager (personal communication 2003) observed clipped *Botrychium* sporophytes (including *B. lineare* and *B. crenulatum*), although the identity of the herbivore (i.e., cattle, gophers) was unknown. She believes that although cattle likely do not target *Botrychium* species, they inevitably might eat *Botrychium* individuals. She also noted that *Botrychium* species are often found near old gopher holes (not fresh gopher disturbances), which can be scattered throughout an area.

Livestock grazing and its effect on habitats is a complex interaction of many factors, including plant community composition, evolution of the habitat type with grazing, site conditions, and grazing intensity. Depending on these factors, livestock activity can cause direct and indirect changes in plant composition, abundance, and structure; alter net primary productivity; influence the overall patchiness of edaphic characteristics and biotic resources; alter litter dynamics and microclimate; affect soil microbial activity and nutrient availability; change seed bank dynamics; increase soil compaction and decrease water infiltration; and increase soil erosion and create bare spots (Archer and Smeins 1991, Frank and McNaughton 1998, Belsky and Gelbard 2000). It is possible that light to moderate grazing could create suitable *Botrychium* habitat by reducing competition with other plants, maintaining open grassy meadows, and opening up suitable microsites for colonization. Heavy grazing could cause substantial trampling of existing *Botrychium* individuals, facilitate the spread of invasive plants, and negatively effect edaphic conditions by compacting soils, changing mycorrhizal communities, and reducing moisture availability.

At *Botrychium* sites within the Colville National Forest in Washington, land managers are experimenting with the effects of fencing on *Botrychium* species (K. Ahlenslager personal communication 2003). A small *B. lineare* population along a small rivulet in cedar-forested habitat has been fenced to protect the 8 or 9 individuals from livestock grazing (U.S. Fish and Wildlife Service 2001); the enclosure is approximately 6m by 15m and was erected about 5 years ago. K. Ahlenslager (personal communication 2003) believed that grazing in this habitat type was not beneficial to *B. lineare* and trampling threatened this small population. Although no report is available, annual monitoring of the individually tagged plants has demonstrated that most of these individuals have been reappearing every year. In this case, K. Ahlenslager (personal communication 2003) believes that the scouring from the rivulet is suitable disturbance to maintain habitat for this population. In addition, several sites in a grassy meadow (old meadows cleared by homesteaders and plowed) have been fenced within approximately 16m by 16m plots to study the effects of grazing on *Botrychium* species (including *B. crenulatum*, *B. paradoxum*, and *B. montanum*) in this habitat type. The *Botrychium* species are monitored within the exclosures and at control sites outside the exclosure using a variety of tagging and transect sampling techniques. Although no report is available, preliminary observations suggest that grazing in this habitat may play an important role in minimizing the growth of the grass canopy and maintaining suitable habitat and microsites for these *Botrychium* species.

Similar herbivore exclusion studies have not been performed at *Botrychium* occurrences in Region 2. It is possible that cattle grazing and moose activity may also play an important role in maintaining small openings suitable for *B. ascendens* and *B. crenulatum* in Bighorn National Forest (S. Gall personal communication 2003).

The specific effects of herbivory and livestock activity on *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* are not known. Herbivore activity could be beneficial if it reduced competition of palatable species with Botrychium, opened up suitable microsites for *Botrychium* colonization, reduced shrubby encroachment onto suitable grassland habitat, or aided in the dispersal of *Botrychium* spores. Herbivore activity could be detrimental if it repeatedly caused the loss of spores, caused soil compaction or soil erosion, altered community dynamics to favor an unsuitable community type, facilitated weed invasion, or trampled individuals and disturbed belowground structures.

Competitors and relationship to habitat

Many moonworts grow in the sparsely-vegetated understory of forested habitats where competition from forb or grass species is much reduced, as well as grassy meadows and riparian areas where competition may be greater. *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* are also found in open habitats and microsites, probably prefer early successional patches, and may rely on disturbances to create and maintain suitable openings. Thus, as succession proceeds and competition increases, local populations of *Botrychium* may be extirpated (Chadde and Kudray 2001). For example, *B. crenulatum* growing in an area with dense shrub cover was pale green and probably had reduced photosynthetic capabilities; individuals in full sun were more robust (Vanderhorst 1997). *Botrychium ascendens* tends to grow in moist microsites and *B. crenulatum* in wetter microsites, with significant cover by shrubs, forbs, and mosses (Vanderhorst 1997). In these habitats, aboveground competition may appear greater, but periodic flooding may reduce root zone competition (Vanderhorst 1997). The successional processes of *B. lineare* habitat on Pikes Peak (Pike-San Isabel National Forest) and the extent of interspecific competition with the moonwort are not known (A. Carpenter personal communication 2003, S. Tapia personal communication 2003). The role of fire in habitats with *B. ascendens*, *B. crenulatum*, and *B. lineare* has not been described. It is possible that fire may be more important in maintaining habitat in forested areas, and may be less important in wetter areas or areas with lower fuel loads. S. Gall (personal communication 2003) reported that a 15,000 acre fire occurred in Bighorn National Forest near a population of *B. ascendens* (and maybe *B. crenulatum*), but the fire passed around the wet, swampy areas. As discussed previously, *B. ascendens*, *B. crenulatum*, and *B. lineare* each probably rely on a slightly different disturbance regime to create and maintain habitat and reduce competition from associated species.

Botrychium species also tend to grow in "genus communities" with other species of Botrychium, perhaps as result of similar mycorrhizal associations. In Montana, *B. ascendens* was often found with *B. crenulatum* (Vanderhorst 1997). However, the populations of these two species grew in low densities and occupied slightly different microsites, with *B. crenulatum* in the lower-lying, wetter areas and *B. ascendens* on the uphill edges of the habitat. Thus, direct interspecific competition for resources is probably not a significant issue in these circumstances.

Introduction of exotic species can be a secondary effect of disturbances such as grazing or trail construction (Fayette and Grunau 1998, Belsky and Gelbard 2000). In some instances, exotic species can outcompete native plants for light, space, nutrients, or water. Consequently, exotic species may threaten existing and/or future habitats and populations of these rare *Botrychium* species. In Colorado, Montana, Oregon, and Washington, exotic species were found in habitats with *B. lineare*, although specific details were not discussed (U.S. Fish and Wildlife Service 2001). In Montana, Vanderhorst (1997) recorded *Cirsium arvense* (Canada thistle) and *Poa pratensis* (Kentucky bluegrass) in *B. crenulatum* plots. Specific interactions between *Botrychium* and these exotic species were not discussed, but both of these species are perennial, rhizomatous invaders that can form monotypic stands, compete for soil and water resources, and eliminate native vegetation. Fayette and Grunau (1998) recorded the presence of *Linaria vulgaris* (yellow toadflax) on the Pikes Peak tollway. Although this noxious invader was present on the side of the road, it had not moved into the natural vegetation. W. Jennings (personal communication 2002) reported that he did not observe any infestations by weeds on the Colorado noxious weed list with *Botrychium* populations in Colorado, despite evidence of disturbances in the past. However, S. Tapia (personal communication 2003) reported that *Linaria vulgaris* has spread significantly and is now encroaching within 10 m of the large *B. lineare* population on Pikes Peak. Other potential montane and subalpine invaders in Colorado include *Cirsium arvense*, *Carduus nutans* (musk thistle), *Chrysanthemum leucanthemum* (ox-eye daisy), or *Matricaria perforata* (scentless chamomile)

(W. Jennings personal communication 2002). *Taraxacum officinale* and *Trifolium repens* are frequent non-native associates of moonworts in Colorado (P. Root personal communication 2003). The threat of exotic species most likely differs depending on geographic location, elevation, distance from weed hotspots (e.g., roads and trails), interactions with grazing, and other factors related to disturbance factors.

There were no reports of any extensive weed control tactics (herbicides, hand-pulling, etc.) near *Botrychium* populations and the implications of those actions in Region 2. S. Tapia (personal communication 2003) has hand-pulled *Linaria vulgaris* where it occurred near the *B. lineare* monitoring site. Herbicide spraying along roads is a potential threat to *B. crenulatum* and *B. ascendens* populations in Kootenai National Forest in Montana (Vanderhorst 1997). On the Blackfeet Indian Reservation in Montana, herbicide spraying along a roadside with *B. lineare* apparently killed much of the vegetation (U.S. Fish and Wildlife Service 2001), but the current status of *B. lineare* is not known (M. Weatherwax personal communication 2003). *Botrychium lineare* was present in the year following herbicide spraying, but the population has not been monitored since that time, and herbicide spraying is presumed to occur annually (M. Weatherwax personal communication 2003). In Glacier National Park, Montana, herbicide spraying and mowing occur along the road with a *B. lineare* population (T. Williams personal communication 2003). Weed managers avoid the moonwort area while spraying, and mowing is presumed to have little effect on *B. lineare* as the mower blades are set above the height of the moonwort (T. Williams personal communication 2003). In Washington, K. Ahlenslager (personal communication 2003) monitored abundance of *B. hesperium* on a privately-owned field for three years after it was sprayed with herbicide once. She observed that *Botrychium* individuals continued to reappear every year. However, it is possible that sites with repeated spraying could be more negatively affected.

Parasites and disease

Ferns, in general, are not susceptible to insect predation but can be vulnerable to fungal decay (Lellinger 1985). A lack of discussion in the literature on the effects of parasites and diseases on all three *Botrychium* species indicates that either these threats have not been studied or do not appear to be problematic. Neither disease nor parasites are known to be a threat to *Botrychium lineare* (U.S. Fish and Wildlife Service 2001). In addition, the effects of parasites or diseases on the mycorrhizae associated with *Botrychium* species are not known.

Symbiotic and mutualistic interactions

Mycorrhizal relationships are necessary for *Botrychium* species to complete their life cycle (U.S. Fish and Wildlife Service 2001). A detailed account of this symbiotic relationship is explained in the section on mycorrhizal relationships. In the conservation assessment of sensitive moonworts in Montana, Vanderhorst (1997) observed a noticeably higher diversity of mushrooms (genera *Cortinarius*, *Hebloma*, *Hygrophorus*, *Russula*, and *Suillus*) in moonwort habitats than in surrounding habitat without moonworts in a western red cedar and western hemlock forest. He hypothesized that this occurrence was most likely a shared requirement for moist microhabitats but also could be related to low levels of root zone competition, substrate characteristics, or mycorrhizal relationships. Additionally, 28 taxa of mosses were identified as growing with sensitive *Botrychium* species in Montana (Vanderhorst 1997). The closest and most consistent group of moss associates found was in the Mniaceae. These mosses are also hypothesized to be adapted to decomposing duff and organic soils with high moisture levels (Vanderhorst 1997), possibly similar to *Botrychium* species' preferences.

Habitat influences

As discussed in previous sections, the establishment of *Botrychium* species could be affected by environmental fluctuations (e.g., precipitation), microsite conditions (e.g., soil suitability, moisture levels), associated species (e.g., mycorrhizal relationships), disturbance patterns (e.g., intensity and frequency), interspecific competition (e.g., successional processes, invasive plants), and herbivory (e.g., dispersal, spore predation).

CONSERVATION

This section discusses conservation information for the three *Botrychium* species associated with this report, including what is known about threats to the persistence of these species, current conservation status, management considerations, and existing data gaps.

Threats

Threats to the long-term persistence of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* in Region 2 are mostly unknown because of the lack of species understanding and specific research. The few studies on the threats or effects of disturbances on these *Botrychium* species concluded that there are no immediate threats to species, although certain populations may be at more risk than others (Carpenter 1996b, Vanderhorst 1997, Fayette and Grunau 1998, U.S. Fish and Wildlife Service 2001, A. Kratz personal communication 2002, S. Tapia personal communication 2003). However, existing populations of *B. ascendens*, *B. crenulatum*, and *B. lineare* in Region 2 have few individuals and cover a small area; therefore, a random, catastrophic disturbance could destroy these populations completely (U.S. Fish and Wildlife Service 2001). Information about the threats or effects of disturbance on *Botrychium* species is based on field research of these species from Region 2 and other USFS regions, as well as speculative information from land managers. Possible threats to these species include: changes to natural disturbance regimes (e.g., fire suppression), road and trail construction and maintenance, structure construction, herbicide application, recreational activities, grazing and trampling by wildlife/livestock, non-native species competition, habitat fragmentation, timber harvest, and climate change (U.S. Fish and Wildlife Service 2001). *Botrychium crenulatum*, *B. ascendens*, and *B. lineare* share many of the same biological characteristics and ecological needs, but they may not all be equally affected by these potential threats. This section synthesizes the available information, with an emphasis on threats present in Region 2.

Threats influencing habitat quality or individual populations

Throughout their ranges, including Region 2, *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* seem to prefer montane, moist, early-successional habitats (e.g., moist meadows, edges of trails, seeps and mossy openings in forests), although the range of ecological tolerances is probably slightly different for each species. Natural and man-made disturbances (e.g., fire, floods, rock or snow slides, road and trail maintenance, thinning and fuel reduction activities, blowdowns, storms, etc.) that create these open habitats could either threaten or enhance populations of these *Botrychium* individuals, depending on the extent, intensity, frequency, timing, or location of the disturbance. For example, floods, landslides, or fire could create new habitat, but they could also cause physical damage to existing aboveground plant structures. If damage occurs to the individuals during the growing season or prior to the maturation and release of spores, the capacity for sexual reproduction could be compromised (U.S. Fish and Wildlife Service 2001).

There were few reports describing the extent or effects of specific management (e.g., prescribed fires or timber harvest) and other human-related activities (e.g., road construction) on existing *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* habitat in Region 2. Intense disturbances, such as those associated with structure construction or use of heavy machinery, may eliminate favorable habitats and decrease the likelihood of *Botrychium* establishment and growth. Foothills, montane, and even subalpine environments in Region 2 are at risk for home construction and other development. While some habitats are protected within USFS National Forests, activities like construction and expansion at ski areas in Region 2 can affect some of these lands. Timber harvest and thinning activities can also be associated with the use of heavy machinery and road construction. Several populations of *B. crenulatum* in Montana have been extirpated or threatened by log skidding, decking, and road widening associated with logging activities (Vanderhorst 1997). On the other hand, thinning and creating ski runs may open up areas of suitable habitat for *Botrychium* species. *Botrychium ascendens* and *B. lineare* are both found in riparian habitats in Region 2 (Carpenter 1996b, Fertig 2000), so management activities occurring upstream could impact downstream populations (e.g., siltation, pesticide use). Within Region 2, an occurrence of *B. lineare* collected in 1992 from Leadville, Colorado was revisited in 2000 (A. Kratz personal communication 2002). The researchers found *B. lunaria* and *B. minganense* occupying this site but not *B. lineare*. This area was located at a highly disturbed site with excavations for a concrete conduit and construction of an asphalt bike trail. It is unknown if the *B. lineare* individuals were destroyed by intense disturbance, dormant, dried up, or otherwise unseen. In Montana, there are two *B. lineare* sites found on roadsides, and these populations may be affected by road maintenance activities, herbicide spraying, mowing, or vehicles that pull onto the side of the road to view wildlife (U.S. Fish and Wildlife Service 2001, M. Weatherwax personal communication 2003, T. Williams personal communication 2003). Vanderhorst (1997) also reported that roadside populations of *B. crenulatum* are threatened by traffic, road maintenance, and herbicide spraying activities. Road maintenance activities that occur prior to spore maturation and dispersal could adversely affect the reproduction of *Botrychium* species. A large population of *B. lineare* in Region 2 occurs in a meadow with a utility pole, located approximately 100 m from the Pikes Peak toll road. Maintenance of the utility pole could potentially threaten the *B. lineare* populations directly and indirectly, by compacting soil or changing hydrologic flow. This *B. lineare* location is separated from the road by a small creek and is probably far enough from the road that salts and other pollutants are not an imminent threat (Carpenter 1996b). Recreational activities, such as off-trail trampling and camping, can also threaten *Botrychium* populations. A *B. lineare* population within the Wallowa-Whitman National Forest in Oregon is close to a trailhead, and campfire rings have been observed in the area (U.S. Fish and Wildlife Service 2001). The largest threat to the *B. lineare* population on Pikes Peak is trampling from off-road/off-trail recreational activity (Fayette and Grunau 1998). It is likely that some populations of *Botrychium* in suitable recreational habitat (e.g., *B. lineare* in a subalpine meadow in Pike-San Isabel National Forest) are more susceptible to impact from recreational uses than populations in less-suitable recreational habitat (e.g., *B. crenulatum* in a hummocky swamp in Bighorn National Forest).

It is well understood that the presence of mycorrhizal fungi is important to these *Botrychium* species at all life stages. Disturbances caused by activities and events such as recreation, logging, trampling, flooding, fire fighting, and fuel reduction in areas where populations exist, may strip habitats of necessary organic matter, shade, or soil moisture. Altering these conditions may disrupt root/mycorrhizal fungi interaction and threaten *Botrychium* individuals. For example, controlled burns or wildfires can create high ground temperatures that may sterilize the soil and eliminate fungal species that are necessary for the survival of these moonworts. Research efforts have not ascertained the effect of fire or other disturbances on the mycorrhizae associated with *Botrychium* (Zika 1992). The belowground structures of *Botrychium* (i.e., gametophytes, juvenile sprophytes, gemmae, and spores) and mycorrhizal interactions may help *Botrychium* species to survive low-intensity aboveground perturbations (Johnson-Groh et al. 2002).

Herbivory (e.g., livestock grazing) may impact *Botrychium* species directly by affecting individuals (e.g., grazing, dispersal, trampling) or indirectly by influencing habitat (e.g., soil compaction, facilitating spread of invasive plants, plant community changes). Grazing may not pose a threat to *Botrychium* species because nutrition may be supplied through interactions with mycorrhizal fungi, which may allow *Botrychium* individuals to withstand repeated removal of leaf tissue by herbivores. Removing the current year's growth may not affect future years' growth, unless the primordia is damaged or significant energy cannot be reclaimed by the plant (Montgomery 1990, Wagner 1992, Hoefferle 1999). There is speculation that herbivory of the spore-bearing sporangia may actually facilitate dispersal (Wagner et al. 1985, Zika 1992, P. Root personal communication 2002). In addition, eliminating natural grazing could be adverse for these *Botrychium* species, since grazing may help keep habitats and microsites "open" and suitable for *Botrychium* species by reducing competition from native and non-native plants. However, livestock activity also has the potential to introduce non-native exotic weeds. In addition, excessive trampling by livestock can physically damage plants, cause soil compaction, and alter moisture availability. The population of *B. lineare* on Pikes Peak (Pike-San Isabel National Forest) is not threatened by livestock grazing (P. Root personal communication 2002), but other populations of *Botrychium* throughout Region 2 may be.

Vegetation encroachment, caused by invasion of exotic species, natural succession, or fire suppression, could also cause local extirpations of *Botrychium* species (Chadde and Kudray 2001, U.S. Fish and Wildlife Service 2001). Exotic species frequently colonize ruderal habitats similar to where *Botrychium* species are found. Invasive species with rhizomes may compete with other species, including *Botrychium* species, for soil resources. The threat of exotic species most likely differs depending on geographic location, elevation, and the distance from weed hotspots (e.g., roads and trails). The effects of exotic, invasive plant species may be particularly serious for native taxa that have extremely small population sizes, such as *B. lineare*. In Montana, herbicide spraying along roadsides with *B. lineare* killed much of the vegetation, but the direct effects on *B. lineare* are currently unknown (U.S. Fish and Wildlife Service 2001).

For some species, harvesting plants for commercial, recreational, scientific, or educational purposes can lead to over-utilization and extirpation of populations, especially if the species has low abundance or is found only in one area. According to the USFWS (2001), harvesting of this kind has not taken place, and thus does not appear to pose a threat to *Botrychium*. Collection techniques described by Wagner (1992) and Zika (1992) emphasize strategies to minimize impact on the population by collecting after spore dispersal and collecting only the aboveground portion of the plant.

The long-term effects of wildfires, hydrologic stochasticity, climatic fluctuations, succession, flooding, erosion, and other disturbance regimes on populations and habitat of *Botrychium crenulatum*, *B. ascendens*, and *B. lineare* have not been studied. Global climate changes could possibly cause changes to existing climatic and precipitation patterns (U.S. Environmental

Protection Agency 1997) and could affect these species, the associated mycorrhizal interactions, or their habitat availability. The factors determining long-term population stability are not yet well understood; therefore, it is difficulty to accurately predict the optimal successional stage, frequency, intensity, timing, or size of disturbance for their persistence.

Given the current understanding, the primary threats to existing populations of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* in Region 2 are: road, trail, or structure construction and maintenance; trampling by wildlife, livestock, or off-trail recreational activities; competition from non-native plant species; natural habitat succession or fire suppression; and changes in hydrology affecting soil moisture or mycorrhizal existence. Specific populations could be at a greater risk than other populations, depending on the landscape context, characteristics of the natural and human disturbance regimes, and biological characteristics of each species. For example, *B. crenulatum* tends to be found in wetter habitats, and some populations could potentially be less threatened by damage from recreationalists than *B. ascendens* or *B. lineare*.

Conservation Status of the Species in Region 2

Not enough data are available to conclude if *Botrychium crenulatum*, *B. ascendens*, and *B. lineare* populations are increasing, decreasing, or remaining stable throughout Region 2. These *Botrychium* species have few documented locations, have relatively small abundances, and can be difficult to relocate due to dormancy during drought or otherwise unsuitable conditions. The lack of demographic data and population monitoring makes it impossible to determine the number of individuals required to maintain population viability for these species. Without additional species-specific surveys or census data, it is difficult to determine the population trends, distribution, viability, vulnerability, or abundance of these *Botrychium* species within their range in Region 2.

Status of *Botrychium ascendens*

There are only four documented occurrences of *Botrychium ascendens* in Region 2; three in Shoshone National Forest and one in Bighorn National Forest. Although the abundances of these populations range from 2 to 50 individuals, population fluctuations have not been monitored and recorded, and the minimum viable population size for this species is not known. This species may be more abundant in Wyoming than presently known as a result of its scattered distribution and small size (Fertig 2000). Risks to these populations from land management have not been evaluated (W. Fertig personal communication 2002). Based on the available information, *B. ascendens* is potentially vulnerable to stochastic events, especially since it has been documented in only a few locations. It is difficult to assess risk as a consequence of life history vulnerability or land management for this species in Region 2 based on the current information.

Status of *Botrychium crenulatum*

The only population of *Botrychium crenulatum* in Region 2 was recorded in 1997 in Bighorn National Forest, and it has not been reconfirmed despite a search in 2001 (W. Fertig personal communication 2002, Wyoming Natural Diversity Database 2003). This suggests that the population of this species in the region is extirpated, dormant, or most likely misidentified, since *B. ascendens* occurs there (W. Fertig personal communication 2002, Wyoming Natural Diversity Database 2003). Although suitable habitats (riparian forests, meadows, seeps, bogs, fens, and/or streams in grasslands) probably exist for *B. crenulatum* in Region 2, there are no confirmed populations of this species. Extremely low to no evidence exists to assess risk as a consequence of life history vulnerability or land management.

Status of *Botrychium lineare*

(New information on the distribution and abundance of *Botrychium lineare*, with impacts to conservation status evaluation, are available at http://www.fs.fed.us/r2/projects/scp/assessments/newinfo.shtml. There is no documented occurrence on the White River National Forest, Colorado, and an occurrence was confirmed in December 2003 on the Black Hills National Forest, Wyoming.)

There are only six documented occurrences of *Botrychium lineare* in Region 2. While the two populations on Pikes Peak (Pike-San Isabel National Forest) have been observed in the last five years, the other populations of *B. lineare* (i.e., Arapahoe-Roosevelt National Forest, White River National Forest, and Rocky Mountain National Park) have not been re-observed in recent years (P. Root personal communication 2002). Preliminary abundance data of the largest population of *B. lineare* censused the population at 53 individuals in 1996 and 43 individuals in 2000 (Carpenter 1996b, Tapia 2000) (**Table 2**), but additional data will be helpful in ascertaining population trends. P. Root (1999) surveyed extensively for additional populations in similar habitats on Pikes Peak but was largely unsuccessful. This species does not appear to be at immediate risk from current management in Region 2, but potential hazards exist from recreational or catastrophic disturbances. The overall viability of this species within Region 2 is unknown based on current information, and this species could be considered vulnerable mainly because it is known to exist in small populations in only a few locations. In addition, the number of populations of this species has declined throughout its range in North America (NatureServe 2003).

Potential Management of the Species in USFS Region 2

Management implications

There is no specific information concerning the responses of *Botrychium crenulatum*, *B. ascendens*, or *B. lineare* populations to land management strategies within Region 2. The type, size, intensity, or frequency of disturbance regime important for the establishment and growth of these three *Botrychium* species in Region 2 are not well known. It is likely that each species needs light to moderate disturbances in their habitats to maintain slightly open microsites for establishment and growth. Intense disturbances, both natural and human, in and around these occupied habitats may threaten existing populations. However, management strategies and other disturbances that potentially pose a threat to existing *Botrychium* populations (e.g., timber harvest, road and trail creation) could also create suitable habitat for future populations (Chadde and Kudray 2001). For example, Vanderhorst (1997) found that the creation of a road formed wet areas with gravelly substrate suitable for *B. crenulatum*. However, widening the road and log skidding and decking subsequently threatened that population with siltation and washout. Little is known about the effects of prescribed fires, fire suppression, or habitat succession to the persistence of *B. crenulatum*, *B. ascendens*, or *B. lineare* populations in Region 2. Moderate cattle grazing could be beneficial or detrimental, depending on the timing, location, habitat, and other related issues. Minimizing concentrated livestock activity and other intense disturbances may help reduce the risk of soil compaction, direct trampling, and repeated sporangia removal. Some occurrences (e.g., *B. lineare* in Pike-San Isabel National Forest) may need invasive weed management to reduce potential threats from encroachment.

These moonwort species are probably closely associated with mycorrhizal fungi at all life stages, often inhabit early successional habitats or open microsites, and may rely on light to moderate disturbances. Environmental changes that could have detrimental effects on the abundance and distribution of these *Botrychium* species include: decreased soil moisture, loss of shade or darkness, barriers to spore dispersal, soil compaction, reduced organic matter, duff layer, or soil nutrients, or changes to conditions necessary to sustain mycorrhizal fungi. In a study of *B. mormo* in Minnesota, researchers concluded that many forestry practices (e.g., clearcutting, thinnings, single tree selection, group selection, salvage sales, roads and skid trails, inappropriate rotation age, and conversion to aspen) could threaten this species by affecting key ecological needs (Sather et al. 1998). It is not known how *B. crenulatum*, *B. ascendens*, or *B. lineare* populations respond to passive management (e.g., grazing cessation, fire suppression) or active management (e.g., grazing, fencing, weed removal, brush cutting, thinning, mowing, prescribed fires) techniques.

Potential conservation elements

Specific details concerning the life history characteristics and habitat relationships of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* within Region 2 are largely unknown. Formulating conservation elements for these sensitive species is inferred from personal communications with resource management specialists and information about *Botrychium* species within and outside Region 2. Surveying high probability habitat for new populations, protecting and buffering existing populations from direct damage, documenting and monitoring the effects of current management activities, preserving landscape diversity to facilitate establishment of additional populations, and studying mycorrhizal relationships, the effects of trophophore and sporophore removal, spore longevity and dispersal, microsite requirements, and effects of environmental fluctuations are possible key conservation elements for *B. ascendens*, *B. crenulatum*, and *B. lineare*. Additional information on the disturbance ecology, threats, habitat needs, and responses to forest practices for these species is needed to ensure viability on multiple use lands.

Botrychium species are especially dependent on relationships with mycorrhizae, and these relationships may be the key limiting factor for their establishment, distribution, and abundance. Maintaining the health of mycorrhizae may be an important conservation element for the persistence of *Botrychium* species (Johnson-Groh and Farrar 1996). Mycorrhizae may be affected by moisture levels, soil substrates, soil conditions, and disturbances, among other factors. In addition, the process of fertilization in *Botrychium* and subsequent growth requires groundwater for the sperm to swim between the antheridia and archegonia structures on belowground gametophytes. Thus, disturbances that dry out the soil or intensely disturb the soil (e.g., trail construction that alters hydrologic flow) could have a negative impact on *Botrychium* populations (Chadde and Kudray 2001).

Minimizing sporophore removal and maximizing spore dispersal is essential for *Botrychium* population establishment. *Botrychium* individuals may be able to tolerate removal of aboveground structures, but timing may be a key element for conservation of moonwort populations. Removing the sporophore and trophophore before spore dispersal may not affect the longevity or vigor of that individual (Johnson-Groh and Farrar 1996). However, removing the sporangia before spore dispersal could reduce the reproductive potential of the population over time by reducing the number of spores available to colonize new sites and produce gametophytes. The extent and longevity of the spore bank or the necessary conditions for spore germination for *B. ascendens*, *B. crenulatum*, and *B. lineare* are still unknown. In addition, the extent of reproduction by gemmae in *B. ascendens* is also poorly known.

It is unclear how *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* react to successional changes over time or how sensitive they are to changes in their habitat. Johnson-Groh and Farrar (1996) hypothesized that *Botrychium* species rely on nutrition gained from mycorrhizae, but populations of *B. ascendens* were more robust in full sun than sites with a high percentage of groundcover (Vanderhorst 1997). Because many of these species live in stochastic environments, maintaining a landscape with a mosaic of patches may provide opportunities for dispersal and establishment (Chadde and Kudray 2001). It is unknown how far *Botrychium* spores can travel. Spores can travel great distances (Wagner and Wagner 1993), but most probably drop close to the parent plant, so buffer areas around populations may provide a place to expand. Incorporating these key issues of *Botrychium* biology and ecology into management plans will aid conservation efforts.

Tools and practices

Several researchers have provided protocols for monitoring *Botrychium* species, and there are a few examples of ongoing monitoring projects. Wagner (1992) described detailed protocols for documenting, collecting, and monitoring *Botrychium* species. Ostlie (1990), Carpenter (1996b), Johnson-Groh (1998), and USFS (2000) have implemented basic census and demographic monitoring programs and provided helpful considerations when designing monitoring plans. For example, surveying for moonworts requires identifying potential habit on a large scale and then performing fine-scale searching, often on hands and knees (Zika 1992). P. Root (personal communication 2002) suggested that more habitat surveys are needed, because *B. lineare* occurs in a wide variety of habitats, and that the classification, distribution, and habitat preferences of *Botrychium* are still not understood. Habitat surveys should also include human-disturbed areas like roadsides, ditches, pastures, rock quarries, and trails in typical habitats such as meadows, seeps, bogs and fens. Annual censuses of sporophytes in known populations and regular revisits to search for individuals in historical locations would provide basic population trend data for all of the populations in a region. For example, *B. lineare* was absent at the historical Leadville, Colorado site in 2000, but it may have been dormant that year and a revisit to this site may locate it again. Monitoring individually-tagged plants within permanently-marked, long-term plots is a useful survey system to monitor population change and individual patterns of emergence, dormancy, and longevity (Wagner 1992, Carpenter 1996). Assessing species viability should also incorporate mycorrhizae identification and underground searches for dormant gametophytes and sporophytes.

Information Needs and Research Priorities

Based on the literature collected and reviewed, many data gaps in understanding the biology, ecology, conservation, and management strategies for *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* exist, especially pertaining to populations of these species in Region 2. Information needed to address these data gaps can be obtained through surveys and inventories, long-term monitoring plans, and extended research programs. Surveys and inventories are useful in the short-term to locate populations and to determine population sizes and distributions of populations within the region. Populations that are located by surveys or inventories can be immediately protected, if threats to the population are imminent, and can subsequently be targeted for long-term research or monitoring programs. Long-term monitoring programs are a useful component of conservation planning, as they help determine what information is needed to preserve populations, communities, and habitats. The resulting information can be used to direct management actions and to initiate adaptive management practices as the project proceeds over time. Long-term research studies can supplement the current biological knowledge of the species and may be useful in providing feedback for use in long-term monitoring programs.

The following sections identify areas where more information is needed to help understand the species, to develop management objectives, and to initiate monitoring and research programs.

Species distribution and habitat

(New information on distribution and abundance of *Botrychium lineare* is available at http://www.fs.fed.us/r2/projects/scp/assessments/newinfo.shtml. *Botrychium lineare* was confirmed in Wyoming in December 2003.)

The distribution of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* are not understood in sufficient detail to formulate regional conservation strategies. For each of the three species, extensive inventories are needed to document the occurrence of populations within Region 2. For example, *B. ascendens* is known to be widely scattered in western North America, with a concentration of known populations in the Pacific Northwest. The current known distribution within Region 2 is limited to four locations in Wyoming. For *B. crenulatum*, there have been no confirmed observations in any of the states in Region 2. For *B. lineare*, populations have been located at six sites in Colorado, and experts have suggested that appropriate habitat exists in Wyoming, although the species has not been observed there.

All three *Botrychium* species are considered to be very rare in Region 2 due to small abundances, few documented occurrences, and disjunct distributions. However, because of the inconspicuous nature and small size of the plants, if populations have gone unnoticed, then the species' distribution and abundance are underestimated. The reliance on current reports of species distribution will probably not provide sufficient information to develop conservation strategies for these three species of *Botrychium* in Region 2. The development of a survey and inventory protocol within Region 2 will supplement the knowledge regarding current distribution of these species. Once located, population sites could be recorded using global positioning system technology, and mapped for future reference. This would allow the identification of populations in areas slated for various management, maintenance, or disturbance activities, and it would also allow the selection of populations for future study.

For each of the three *Botrychium* species addressed in this report, habitat associations have been described for locations outside Region 2, but very little information was found for locations within Region 2. While observed habitat associations can be a useful starting point for identifying survey areas within Region 2, specific habitats are difficult to characterize, because sites where species of *Botrychium* have been observed vary greatly across the range of the species (Wagner and Wagner 1994). This fact underlines the importance of documenting preferred habitats within Region 2. The development of a survey and inventory protocol within Region 2 will supplement the knowledge regarding habitat associations for these species. Knowledge of preferred habitat associations will provide conservation biologists with information to conduct surveys in similar habitats, to identify potential habitat that may be slated for development, and to develop land management strategies for conservation of the species.

Life cycle, population trends, and demography

The life cycle of *Botrychium* species is understood as an alternation between sporophyte and gametophyte generations, typical of ferns. However, certain aspects of the life cycle for the three species evaluated in this report are less understood. For example, information is lacking on spore fertility and longevity, threats resulting in spore loss, and actual spore dispersal methods. The role of animals in the dispersal of spores is an area for further study. In general, *Botrychium* species are known to be obligately dependent on mycorrhizal fungi in both the gametophyte and the sporophyte life stages. It is also generally suggested that there is no specific species of fungus that preferentially associates with a given species of *Botrychium*. Information about species-specific associations with mycorrhizal fungi would be useful in developing mitigation and restoration strategies. Whether a particular taxonomic group of fungus is predominant is unknown. It would also be important to determine whether there are limiting factors (e.g., parasites, diseases, moisture, soil conditions) that affect fungal species, which in turn would affect the success of the three *Botrychium* species. The rate of dispersal and colonization of mycorrhizal species to recently created habitats also warrants research. Lastly, it is important to ascertain the contribution of mycorrhizae to the overall energy budget of the *Botrychium* plant, as the mycorrhizae may be an important limiting factor in the recruitment and survival of individuals.

Another important question is whether the demography of the species is understood sufficiently to analyze persistence at local or regional scales. Certain aspects of demography are a priority in order to provide basic population information, and these are indicated by the first two bullets below. The remaining bullets identify information that is also of interest in understanding the species, if it can be obtained.

- ❖ How does the number of individuals within a population vary from year to year?
- ❖ What are the population trends over ten years?
- ❖ Are individual *Botrychium* plants reproducing? Are mycorrhizae reproducing?
- ❖ What are the rates of survival, longevity, and recruitment? And what factors affect these rates?
- ❖ What is the age at which individuals become reproductive?
- ❖ What is the age structure of the population?

Little data are available on demography and population trends for these three *Botrychium* species within Region 2. Long-term monitoring has only been initiated for one population of *B. lineare* on Pikes Peak, but the study has not been ongoing for a long enough period to detect trends. An annual census of all known populations is a basic method to determine population trends for *B. ascendens*, *B. crenulatum*, and *B. lineare* in Region 2. Long-term monitoring studies and population viability analyses will yield helpful information, such as population trends, temporal patterns of abundance and dormancy, environmental factors that influence abundance, and the minimum number of plants necessary to perpetuate the species. Ostlie (1990), Wagner (1992), Zika (1992), Carpenter (1996b), Johnson-Groh (1998), and USFS (2000) have provided helpful considerations when designing successful long-term monitoring programs on other species of *Botrychium*. Those studies and general protocols for designing monitoring plans for rare plant species (Hutchings 1994, Elzinga et al. 1998, Austin et al. 1999, Bonham et al. 2001) should be reviewed in the development of a monitoring strategy for *Botrychium* populations within Region 2. In addition, population matrix models that measure individual fitness and population growth provide flexible and powerful metrics for evaluating habitat quality and identifying the most critical feature of the species' life history (Hayward and McDonald 1997). Deterministic demographic models of single populations are the simplest analyses and are used as powerful tools in making decisions for managing threatened and endangered species (Beissinger and Westphal 1998).

Species response to habitat changes and management

The responses of these *Botrychium* species to fine- and broad-scale changes in habitat are not understood in sufficient detail to evaluate effects of management or changes in natural disturbance patterns. While intense disturbances, such as structure construction, will likely destroy habitat for *Botrychium* species, moderate disturbances could likely create new habitat. As discussed above, much of the information regarding reproduction, dispersal, establishment, relationship with herbivores, and competition with introduced species is somewhat known for the genus, but there is little or no species-specific information available. Research studies to evaluate these phenomena would provide valuable input to the development of conservation strategies and management programs. Two important questions are: 1) How do the *Botrychium* species being evaluated and their associated mycorrhizae respond to changes in soil moisture? and 2) How do the *Botrychium* species being evaluated and their associated mycorrhizae respond to natural succession? Resource managers developing management activities for a region would benefit from knowing percent canopy preference and the level of competition tolerated by *Botrychium* species (Ostlie 1990). *Botrychium* species may benefit from a landscape with a variety of successional habitat patches. Creating and maintaining patches could be accomplished with a variety of management techniques, but it is currently unknown which method (e.g., prescribed fire, thinning, mowing, grazing), combination of methods, or with what timing would be most effective and compatible with *Botrychium* species.

The types of monitoring studies required to answer all of these questions could take decades, although initial correlations and patterns could occur after several years. As an example, consider snowpack levels in Colorado for the last 34 years (Colorado Climate Center 2002). In 1997, snowpack was 156 percent of normal, then only slightly above average for 1998 and 1999. In 2000 and 2001, snowpack was about 80 percent of normal. In 2002, snowpack was the lowest in recorded history. If monitoring began in 1997 and continued to the present, during a persistent downward trend in available soil moisture, it would seem likely that populations would show a decline in the number of sporophytes appearing aboveground. On the other hand, monitoring studies beginning in the late 1980s (low snowpack years) and continuing to 1997 would likely show an increase, since this period represented an upward trend in available moisture. If these trends were graphed, it would appear that studies dating as far back as 1977 would have experienced two up and two down periods in snowpack accumulation. This may be the minimum amount of time to determine the influence of just soil moisture (i.e., only one environmental parameter) on the population dynamics.

The importance of identifying study objectives up front cannot be emphasized enough. For example, it may also be that the *Botrychium* species are not influenced directly by the soil moisture, but "lag behind," because it is the mycorrhizal fungi that need to respond first, then the *Botrychium*. So, such a 25-year study may show that the *Botrychium* species do best in the closing years of a wet cycle, because they build up "strength" from the mycorrhizae that flourished during the wet years. Long-term monitoring studies should be developed based on information needs that became apparent during literature reviews regarding the three *Botrychium* species.

A second factor that needs additional evaluation is the species' response to natural succession. For example, several populations of various *Botrychium* species have been found growing in disturbed habitats and have been characterized as having r-selected life strategies (i.e., ruderal). They are found growing in old roadbeds, old house foundations, along roadsides, and on recently cleared ski slopes. Studies could be conducted to determine if light disturbance (e.g., low intensity fire, light mowing or grazing) is necessary to maintain a population, and whether natural succession and encroaching vegetation would result in extirpation of a population. Although populations are few, these studies could use paired control sites to help determine if it is the disturbance or some other factor that is causing the change. At present, when *Botrychium* species are found in a disturbed area, it is unknown if the spores are long-lived, ubiquitous in the environment, and were just waiting for the proper disturbance to occur, or whether the spores are short-lived and originated locally, entering and colonizing the site after the disturbance occurred.

Other studies that could be conducted include temporal effects of disturbance on population reproductive success. For example, if plants are grazed or trampled while producing spores, does this have more of an adverse effect on the population than if plants are grazed or trampled prior to or after the spore-bearing season? Because *Botrychium ascendens*, *B. crenulatum*, and *B. lineare* are rare species with a small number of known individuals, studies could perhaps focus on related species that are more abundant. Hoefferle (1999) performed initial experiments with leaf removal before and after sporulation, but the results were based on two years of data and patterns could be related to moisture availability. Additional studies are warranted to replicate the results and understand the processes behind the patterns. Data concerning the affects of aboveground leaf removal could provide information to conservation planners regarding the isolation of populations for certain parts of the year, or regarding the identification of construction windows that would not adversely affect the population.

Availability of reliable restoration methods

Are there reliable restoration methods available for the habitats and populations of *Botrychium ascendens*, *B. crenulatum*, and *B. lineare*? Current information regarding restoration of habitat for the three *Botrychium* species was not found. There are still too many unknowns regarding habitat preferences and basic population dynamics to know what factors are critical in restoring habitat for these species. For example, critical questions that need to be answered include the role of disturbance in habitat preferences for the three species. It is currently not known what types, intensities, or frequencies of disturbance are suitable for creating or maintaining habitat for *Botrychium* species. Similarly, if a separate restoration is being implemented (e.g., reforestation), the reforestation may be successful in itself, but the preferred seral stage of the *Botrychium* species may be bypassed. No information was found regarding the potential to freeze spores (or other stages) of *B. ascendens*, *B. crenulatum*, and *B. lineare* for future use in a restoration program, or the success of spore germination at restored sites. The success of planting *Botrychium* species probably relies heavily on the establishment of mycorrhizal communities. In short, the distribution and location of populations, and information regarding the basic biology of these species are needed before restoration methods can be considered.

Identification of research priorities in Region 2

There is so little known about *Botrychium* species in Region 2, that there are a large number of research projects that could be implemented. The location of populations and identification of habitat preferences and mycorrhizal relationships are of primary importance to further the understanding of these species in Region 2.

The following types of studies would supplement basic knowledge regarding these three species.

- Additional surveys to locate existing populations within Region 2
- Population distribution, abundance, density, and habitat characterization
- Relationships with mycorrhizae in the soil
- Threats affecting population establishment, growth, and reproduction
- Effects of habitat fragmentation or isolation
- Population trends and patterns, correlations between climate (e.g., precipitation) and abundance
- Demographics (age structure of population, recruitment, age of breeding, fertility, annual rate of survival)
- Minimum number of plants and populations necessary to perpetuate the species
- Patterns of population size, and spore fertility, dormancy, and viability
- Patterns of genetic variability

Other studies, as discussed earlier in this section, would provide valuable information, but have a lower priority in terms of immediate management needs.

Research and data needs that may be useful, but not incorporated into this assessment

One important aspect of long-term monitoring and data gathering is how to manage the data for efficient use. Data acquired during surveys, inventories, monitoring programs, and research projects are most easily accessible if they are entered into an automated relational database. Such a database should be integrated with geographic information system and allow queries and activities such as the following:

- Efficient incorporation of data in the field
- Generation of location and habitat maps
- Identification of population locations
- Characterization of associated habitat types
- Identification of population trends over time
- Identification of data gaps that require further information gathering
- Monitoring of success indicators
- Easy modification as additional information becomes available

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DEFINITIONS

Achlorophyllous – Lacking chlorophyll; appearing without a green color.

Alternation of generations – Alternation of diploid (2n) sporophyte and haploid (n) gametophyte generations in ferns.

Antheridium – Male sperm-bearing organ in plants other than seed plants.

Archegonium – Female egg-bearing organ in plants other than seed plants.

Bifid – Two-parted or two-cleft.

Chartaceous – Having a papery or tissue-like appearance.

Crenulate – With small, rounded, marginal teeth.

Cross-breeding – A breeding system in which sexual reproduction involves the mating and union of gametes of different individuals.

Cuneate – Wedge-shaped.

Demographics – The study of fecundity and mortality parameters that are used to predict population changes.

Dentate – With coarse, sharp teeth that are directed outward from the margin, rather than forward.

Denticulate – Minutely toothed, finely dentate.

Disjunct – A geographically isolated population or species outside of the range of other similar populations or species.

Electrophoresis – The separation of proteins on the basis of size and electrical charge, by measuring their rate of movement through a starch gel with an electrical field.

Endangered – Defined in the Endangered Species Act as any species which is in danger of extinction throughout all or a significant portion of its range.

Endemic – A population or species with narrow physiological constraints or other restrictions, which limit it to a special habitat or a very restricted geographic range, or both.

Endophytic fungi – Fungi that live within the tissues of a host plant without causing disease.

Eusporangiate – Having a thick-walled sporangium originating from several epidermal cells.

Exotic species – Species accidentally or purposely propagated in an area by man.

Fertility – Reproductive capacity of an organism.

Fitness – Success in producing viable and fertile offspring.

Gametophyte – The inconspicuous, non-vascular structure that bears male and female sex organs and sex cells (gametes).

Gemmae – Asexual reproductive structures located on the underground stem of some *Botrychium* sporophytes. These groups of cells can develop into mature sporophytes.

Genotype – Assemblage of genes in an organism.

Habitat fragmentation – The break-up of a continuous landscape containing large patches into smaller, usually more numerous, and less connected patches. Can result in genetic isolation.

Habitat isolation - When two or more habitats are separated (i.e., geographically) to an extent to prevent cross-breeding, thereby genetically isolating two parts of a once continuous population.

Herbaceous – An annual or perennial plant which dies back to the ground at the end of the growing season because it lacks the firmness resulting from secondary growth.

Heterozygous – Having different alleles of a gene present in the same cell or organism; for instance, a tall pea plant with alleles for tallness (T) and dwarfness (t).

Homosporous – Having spores of a single size.

Homozygous – Having the same alleles of a gene present in the same cell or organism (e.g., AA)

Hybridization – The result of a cross between two interspecific taxa.

In-breeding – A breeding system in which sexual reproduction involves self-fertilization.

In-breeding depression – Decreased individual vigor and viability resulting from increased homozygosity and accumulation of deleterious genes.

Metropolis – Principle center of a population.

Midrib – Main nerve of a leaf.

Mycorrhiza – Symbiotic association between a fungus and the root of a higher plant.

Mycotrophism – An association whereby a non-photosynthesizing plant obtains nutrients via a mycorrhizal fungus attached to the root of another plant.

Phenotype – The external visible appearance of an organism.

Phenotypic plasticity – When members of a species vary in height, leaf size or shape, flowering (or spore-producing time), or other attributes, with changes in light intensity, latitude, elevation, or other site characteristics.

Pinna – A stalked or sessile primary division of a compound lamina that is narrowed at the base.

Pinnate – With leaflets arranged on both sides of a common axis.

Population Viability Analysis – An evaluation to determine the minimum number of plants needed to perpetuate a species into the future, the factors that affect that number, and current population trends for the species being evaluated.

Primordium – Undifferentiated meristematic cells at the tip of a shoot, from which leaves develop.

Prothallus – In ferns, the haploid gametophyte structure.

Proximal – Toward the base in position.

Rachis – The main axis of a fern frond.

Recruitment – The addition of new individuals to a population by reproduction.

Ruderal habitat – Temporary or frequently disturbed habitats.

Ruderal species – Species that can exploit low stress, high disturbance environments.

Sessile – Borne without a stipe, petiole, or stalk.

Spatulate – Shaped like a spoon.

Sporophore – A leaf bearing sporangia.

Sporophyte – In alternation of generations, the diploid stage which produces haploid spores through meiosis occurs.

Threatened – Defined in the Endangered Species Act as any species which is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range.

Trophophore – The photosynthetic lamina of the aboveground parts in species of *Botrychium*.

Vesicular - arbuscular mycorrhizae (VAM) – Mycorrhizae that form storage structures (vesicles) and finely-branched, modified roots (arbuscles) that penetrate plant root cells and may function in nutrient exchange.

Viability – The capability for living or continuing to develop.

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