

Species Report of *Asplenium diellaciniatum* (no common name)
Version 1.0



Asplenium diellaciniatum in the montane mesic forest of Kaua'i. Photo by Michelle Clark,
USFWS

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Asplenium diellaciniatum Species Report, Final Version 1.0

This document was prepared by the staff at the Pacific Islands Fish and Wildlife Office, Honolulu, Hawai‘i. We received valuable input and assistance from the following experts: Ruth Agurauja, Senior Researcher, of the Tallinn Botanical Garden, David H. Lorence, Senior Research Botanist, and Kenneth R. Wood, Research Biologist, of the National Tropical Botanical Garden and the Hawai‘i Plant Extinction Prevention Program. We greatly appreciate their guidance and support, which resulted in a more robust report.

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EXECUTIVE SUMMARY

This document presents the Species Report for *Asplenium diellaciniatum* to present the overall viability of the species. To assess viability, we used the three conservation biology principles of resiliency, representation, and redundancy. We identified the species' ecological requirements for survival and reproduction at the individual, population, and species levels, and described risk factors influencing the species' current condition.

Asplenium diellaciniatum is one of six endemic Hawaiian fern species which belonged to the monophyletic genus formerly known as *Diellia*. *Asplenium diellaciniatum* is a medium sized terrestrial fern which grow in soil and can sometimes be found growing on rocks. Recent molecular-based phylogenetic studies have shown that *Diellia* belongs to the current genus, *Asplenium*. *Asplenium diellaciniatum* is highly variable, and there are currently three morphotypes recognized, *alexandri*, *erecta* and *knudsenii*. Study of the populations in the field and herbarium collections reveals that these morphotypes are neither consistent nor stable and intergrade with each other.

Threats to *Asplenium deillacinatum* include habitat degradation and predation by introduced ungulates, including feral pigs (*Sus scrofa*), goats (*Capra hircus*), and black-tailed deer (*Odocoileus hemionus columbianus*), habitat degradation and competition with nonnative plants, consequences of small population size, inadequate regulatory mechanisms and climate change.

We measure resiliency for *Asplenium diellaciniatum* by population trends over time and population size and structure. Populations which have abundant individuals in which all size classes can be found, have a more probable outcome for survival. We evaluate redundancy for *Asplenium diellaciniatum* based on the metric of the number of populations and their distribution across the known range of the species. We define representation by the number of resilient populations expressing the full morphological variation of *Asplenium diellaciniatum*.

Currently there are 40 sporleings, 65 premature and 97 mature individuals in two sub-populations. Historically, the species was known from Halemanu on Kaua'i. Recent surveys have not relocated the species at Halemanu. There is only one population (population unit A) consisting of two sub-populations at Kawai'iki and Kaluahulu. The population at Kaluahulu is protected by an ungulate proof fence. The larger of the two sub-populations at Kawai'iki remains unprotected. Thirty-nine propagated individuals from the Kawai'iki sub-population were planted in the Po'omau enclosure.

The current condition of *Asplenium diellaciniatum* is described as having one population on Kaua'i with 162 individuals (excluding 40 sporelings) total. Overall, individuals in populations have generally been decreasing due to existing threats and habitat degradation. Some redundancy and representation is maintained in *ex situ* seed storage and greenhouses, and reintroduced individuals, and some recruitment has been documented as a result of these reintroductions. As this species has low resiliency, very low redundancy, and low representation in the current condition, the overall viability of this species is very low.

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INTRODUCTION

Asplenium diellaciniatum is a medium sized terrestrial fern which grows in soil but can sometimes be found growing on rocks. It belongs to the spleenwort family, Aspleniaceae, and is an endemic species, found only on Kaua‘i (Palmer 2003, p. 117). Currently this fern is restricted to the montane mesic forests of northwest Kaua‘i at Kawai‘iki and Kaluahaulu Ridge in two sub-populations. There are approximately 162 (excluding 40 sporelings) individuals remaining in the wild (Aguraiuja, pers. comm. 2020). Population studies have revealed a high level of variation in leaf morphology and the two sub-populations have leaf morphotypes (varieties or forms) which are neither consistent nor stable and intergrade with each other (Lorence et al. 2013, p. 167).

Species Report Overview

This Species Report summarizes the biology and current status of *Asplenium diellaciniatum* and was conducted by Pacific Islands Fish and Wildlife Office. It is a biological report that provides an in-depth review of the species’ biology, factors influencing viability (threats and conservation actions), and an evaluation of its current status and viability.

The intent is for the Species Report to be easily updated as new information becomes available, and to support the functions of the U.S. Fish and Wildlife Service’s Endangered Species Program. As such, the Species Report will be a living document and biological foundation for other documents such as recovery plans, status in biological opinions, and 5-year reviews.

Regulatory History

Asplenium diellaciniatum was listed as an endangered species on September 30, 2016 (81 FR 67786, U.S. Fish and Wildlife Service [USFWS] 2016). No critical habitat has been designated for this species. A recovery outline was completed on July 30, 2020 and a recovery plan is currently in preparation (USFWS 2020a).

Methodology

We used the best scientific available to us, including peer-reviewed literature, grey literature (government and academic reports), and expert elicitation. To the best of our ability we used the current taxonomy at the time this report was drafted.

To assess the current status and viability of *Asplenium diellaciniatum*, we identified population units. The classic definition of a population is a self-reproducing group of conspecific individuals that occupies a defined area over a span of evolutionary time, an assemblage of genes (the gene pool) of its own, and has its own ecological niche. However, due to information gaps, we could not assess the viability of *A. diellaciniatum* using this definition. The Hawai‘i and Pacific Plants Recovery Coordinating Committee revised its recovery objectives guidelines in 2011 and included a working definition of a population for plants: “a group of conspecific individuals that are in close spatial proximity to each other (i.e., less than 1,000 meters apart), and are presumed to be genetically similar and capable of sexual (recombinant) reproduction” (HPPRCC 2011, p. 1).

Based on this working definition, maps were created to display population units. In an effort to protect the sensitivity of species data, we created maps with symbol markers rather than

displaying species points or polygons. We created the symbols in steps. First, we added a 500-meter buffer around each individual species point and polygon. We then dissolved all buffer areas intersecting each other into a single shape. Next, we created a centroid (i.e., point representing the center of a polygon) within each dissolved buffer area. The symbol marker represents the centroid. Finally, the Disperse Marker tool in ArcGIS Pro was used shift symbol markers that were overlapping so they would all be visible at the scale of the map. All points and polygons were used in this process, regardless of observation date or current status (historical, current, extant, or extirpated), to represent the known range of the species.

Species Viability

The Species Report assesses the ability of *Asplenium diellaciniatum* to maintain viability over time. Viability is the ability or likelihood of the species to maintain populations over time, (i.e., likelihood of avoiding extinction). To assess the viability of *A. diellaciniatum*, we used the three conservation biology principles of resiliency, redundancy, and representation, or the “3Rs” (Figure 1; USFWS 2016, entire). We will evaluate the viability of a species by describing what the species needs to be resilient, redundant, and represented, and compare that to the status of the species based on the most recent information available to us.

Definitions

Resiliency is the capacity of a population or a species to withstand the more extreme limits of normal year-to-year variation in environmental conditions such as temperature and rainfall extremes, and unpredictable but seasonally frequent perturbations such as fire, flooding, and storms (i.e., environmental stochasticity). Quantitative information on the resiliency of a population or species is often unavailable. However, in the most general sense, a population or species that can be found within a known area over an extended period of time (e.g., seasons or years) is likely to be resilient to current environmental stochasticity. If quantitative information is available, a resilient population or species will show enough reproduction and recruitment to maintain or increase the numbers of individuals in the population or species, and possibly expand the range of occupancy. Thus, resiliency is positively related to population size and growth rate, and may also influence the connectivity among populations.

Redundancy is having more than one resilient population distributed across the landscape, thereby minimizing the risk of extinction of the species. To be effective at achieving redundancy, the distribution of redundant populations across the geographic range should exceed the area of impact of a catastrophic event that would otherwise overwhelm the resilient capacity of the populations of a species. In the report, catastrophic events are distinguished from environmental stochasticity in that they are relatively unpredictable and infrequent events that exceed the more extreme limits of normal year-to-year variation in environmental conditions (i.e., environmental stochasticity), and thus expose populations or species to an elevated extinction risk within the area of impact of the catastrophic event. Redundancy is conferred upon a species when the geographic range of the species exceeds the area of impact of any anticipated catastrophic event. In general, a wider range of habitat types, a greater geographic distribution, and connectivity across the geographic range will increase the redundancy of a species and its ability to survive a catastrophic event.

Representation is having more than one population of a species occupying the full range of habitat types used by the species. Alternatively, representation can be viewed as maintaining the breadth of genetic diversity within and among populations, in order to allow the species to adapt to changing environmental conditions over time. The diversity of habitat types, or the breadth of the genetic diversity of a species, is strongly influenced by the current and historic biogeographical range of the species. Conserving this range should take into account historic latitudinal and longitudinal ranges, elevation gradients, climatic gradients, soil types, habitat types, seasonal condition, etc. Connectivity among populations and habitats is also an important consideration in evaluating representation.

The viability of a species is derived from the combined effects of the 3Rs. A species is considered viable when there are a sufficient number of self-sustaining populations (resiliency) distributed over a large enough area across the range of the species (redundancy) and occupying a range of habitats to maintain environmental and genetic diversity (representation) to allow the species to persist indefinitely when faced with annual environmental stochasticity and infrequent catastrophic events. Common ecological features are part of each of the 3Rs. This is especially true of connectivity among habitats across the range of the species. Connectivity sustains dispersal of individuals, which in turn greatly affects genetic diversity within and among populations. Connectivity also sustains access to the full range of habitats normally used by the species, and is essential for re-establishing occupancy of habitats following severe environmental stochasticity or catastrophic events (see Figure 1 for more examples of overlap among the 3Rs). Another way the three principles are inter-related is through the foundation of population resiliency. Resiliency is assessed at the population level, while redundancy and representation are assessed at the species level. Resilient populations are the necessary foundation needed to attain sustained or increasing representation and redundancy within the species. For example, a species cannot have high redundancy if the populations have low resiliency. The assessment of viability is not binary, in which a species is either viable or not, but rather on a continual scale of degrees of viability, from low to high. The health, number and distribution of populations were analyzed to determine the 3Rs and viability. In broad terms, the more resilient, represented, and redundant a species is, the more viable the species is. The current understanding of factors, including threats and conservation actions, will influence how the 3Rs and viability are interpreted for *Asplenium diellaciniatum*.

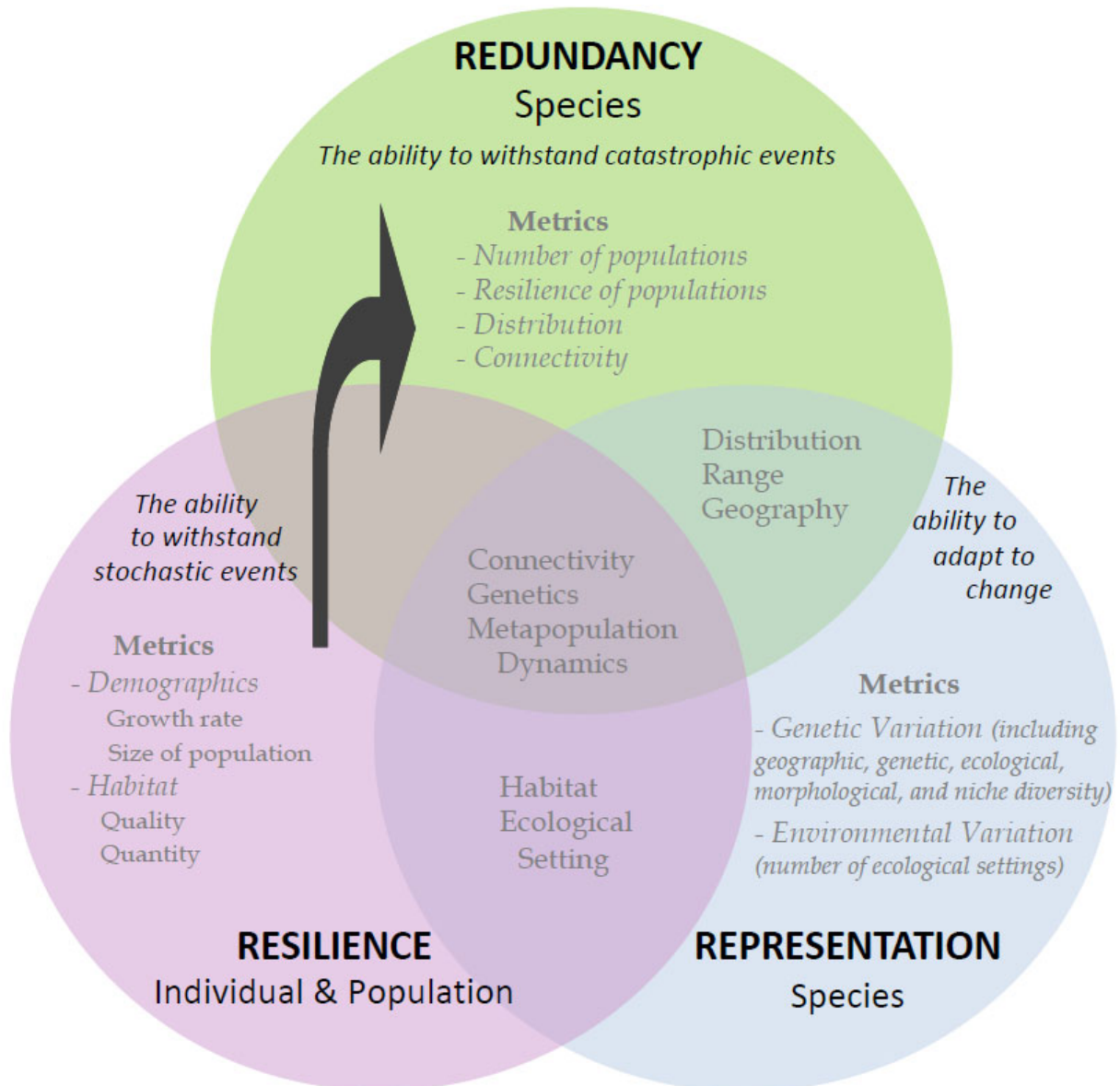


Figure 1. The three conservation biology principles of resiliency, redundancy, and representation, or the “3Rs”.

SPECIES ECOLOGY

Species Description

Asplenium diellaciniatum is one of six endemic Hawaiian fern species which belonged to the monophyletic genus formerly known as *Diellia* (Lorence et al. 2013, p.168, Palmer 2003, p.116). Recent molecular-based phylogenetic studies have shown that *Diellia* belongs to the current genus, *Asplenium* (Schneider et al. 2004, p. 265). There are two other species of diellioid ferns which are endemic to the mesic forests of Kaua‘i, *Asplenium dielmannii* and *Asplenium dielpallidum*, both are endangered. The sub-clade of diellioid ferns have an estimation of divergence time to be ca. 24.3 million years ago (Myr) and rapid radiation of the five extant

species at two Myr ago suggesting that the ancestor of the diellioid lineage may have been among the first successful island hopping, colonists of the Hawaiian Islands (Schneider et al. 2005, p. 456). All species, in the diellioid clade are now considered very rare, listed as endangered or already extinct and have very small population sizes (Palmer 2003, p. 116).

Diellioid ferns are small to medium-sized, terrestrial and sometimes epipetric (growing on rocks). Their rhizomes (creeping root-stalks) are short-creeping and vary from growing along the surface to being erect. *Asplenium diellaciniatum* has stipes (stalks) and rachis (stems) which are black or purple-black to maroon, and shiny. The fern blades are one to two (sometimes three)-pinnate, meaning the leaflets are arranged on either side of the stem, in pairs opposite each other. The primary division of the fern leaf (pinnae) is entire to shallowly or deeply divided but not all the way down to the stipe. They are mostly free of veins and which rarely join together. Sori (the cluster of spore bearing structure of ferns) are at the tips of expanded lobes (rounded section of the fern blade) (Palmer 2003, p. 116) (see below, Figure 2). The peak of spore production for diellioid ferns is usually in the months of April and May (R. Agurauja 2020, pers. comm).



Figure 2. Sori on lobes of *Asplenium diellaciniatum* at Kaluahaulu Ridge. Photo by Michelle Clark, USFWS.

Diellioid ferns have gone through several taxonomic revisions. They have been placed at one time or another in association with no less than four different assemblages of fern groups which are now interpreted as belonging to four separate families (Wagner 1953, p. 34). Population studies of *Asplenium diellaciniatum* have revealed a striking range of variation in leaf morphology and in the degree of lobing. Pictured below (Figure 3) are two morphotypes (*erecta* and *alexandri*) from the Kaluahaulu ridge sub-population and a third morphotype (*knudsenii*) from the Kawaiʻiki ridge sub-population (Lorence et al. 2013, p. 167).





Figure 3. *Asplenium diellaciniatum* at Kaluahaulu (A and B) and Kawai‘iki (C). Photo A is of the morphotype referred to as *alexandri*, photo B is morphotype *erecta* and photo C is morphotype *knudsenii*. Photos by Ruth Agurauja, Tallinn Botanical Garden.

Studies of the two subpopulations at Kawai‘iki and Kaluahaulu and additional herbarium collections indicate that these morphotypes are neither consistent nor stable and intergrade with each other. *Asplenium diellaciniatum*, is represented with highly variable frond morphology and dissection, its phenology depends on numerous factors including, age, stage of development of the plant, and also microhabitat. A single plant may even possess fronds representing several morphotypes (Lorence et al. 2013, p. 167). The high level of variation within and between subpopulations of *A. diellaciniatum* warrants further taxonomic investigation of the species (R. Agurauja 2020, pers. comm).

Asplenium diellaciniatum has been in cultivation since 2011. Germination trials using spores were initiated at Tallinn Botanical Garden in Estonia. The three subpopulations were kept separate during the trials. Average germination rates varied from 30 to 35 days. The growth and development of the gametophytes (generative phase of the ferns) was much slower. Full maturity of gametophytes was reached 3.5 to 4 months after the sowing and the full cycle from the spore to mature spore-producing individuals took 4 to 5 years in cultivation (Agurauja 2017, p. 38).

Individual Needs

Individuals of *Asplenium diellaciniatum* need light, water, nutrients, and space to grow and reproduce. Germination trials were conducted for the three dielliod species which occur on Kaua‘i. The trials concluded that the full cycle from the spore to mature spore-producing individuals took four to five years in cultivation (Agurauja 2017, p. 38). The life cycle of ferns

consists of several developmental stages: they begin as spores which are released from the sori and grow into gametophytes. When the gametophytes mature archegones (female reproductive structure) and antherids (male reproductive structures) are formed and sexual reproduction occurs on the gametophytes. A sporeling is formed after fertilization. The sporeling matures into a sporophyte (fern fronds) which then produce sori. The sori are clumps of sporangia (receptacle in which asexual spores are formed). The sporangia release the spores and they cycle begins again if the conditions are conducive to reproduction (i.e., sufficient moisture or rainfall and favorable habitat conditions) (Raven et al. 2005, pp. 396–397, Agurauja 2020, pers. comm.).

The current populations of *Asplenium diellaciniatum* are growing in montane mesic forest at around 3,582 to 4,429 feet (ft) (1,092 to 1,350 meters [m]) elevation (USFWS 2020, unpublished data). Wagner (1953) described the habitat of dielliod ferns to occur in arid localities where annual rainfall ranges from about 35 to 80 inches. Temperature estimates range from 70 to 62 degrees Fahrenheit, and temperatures become cooler with increasing elevation. He also noted that within these relatively arid regions, the ferns are almost always confined to steep gulch sides and the soils are somewhat rocky. Plants are commonly found growing directly on rock surfaces in crevices or ledges, or on open soil. Soil pH ranged from 7.0 to 8.0. When the plants were found growing directly on soil, the soil is always loose, dry, and granular (i.e., tends to hold together in small lump and appears to be well aerated) with quick drainage (Wagner 1953, p.27). Nitrogen fixation may play a particularly important role in providing nutrients to the soil which are essential for growth and regeneration of plants including dielliod ferns. The nitrogen-fixing, *Acacia koa* (koa), is a dominant tree in montane mesic forests (Lowe et al. 2020, p. 2). Canopy cover from trees and other plants provide shade for dielliod ferns. *Aleurites moluccana* (kukui), a Polynesian introduction, *Metrosideros polymorpha* (‘ōhi‘a), *Myrsine lanaiensis* (kolea), and *A. koa*, are commonly associated with dielliod ferns (Wagner 1953, p. 27). Understory plants associated with *A. diellaciniatum* include *Kadua terminalis* (manono), *Dodonaea viscosa* (a‘ali‘i), *Microlepia strigosa* (palapali), *Dryopteris wallichiana* (laukahi), *Dryopteris fusco-atra* (‘l‘i), *Dubautia laevigata* (na‘ena‘e), *Dianella sandwicensis* (uki uki), *Poa sandwicensis*, *Bidens cosmoides* (po‘olā nui), *Peperomia membramea* (‘ala‘ala wai nui), *Doodia kunthia* (pāmoho), *Asplenium macraei* (‘iwa ‘iwa lau li‘i) (NTBG 2008a, 2009a, Wagner 1953, p. 31).

Population Needs

Agurauja et al. (2004) conducted a study on using population stage structure to assess the viability of dielliod fern populations. The following stages of fern development were distinguished; gametophyte; sporeling (young sporophyte); premature (pre-reproductive sporophyte); mature (reproductive sporophyte); dormant and (or) dead. It is unknown whether or not the ferns go dormant. The study focused on the stages of sporophyte generation, sporeling, premature and mature. Gametophytes are extremely important for recruitment of new populations, however they are difficult to count in the field due to their small size.

Populations were classified as “dynamic” (sporelings predominate), “normal” (a relative high proportion of adults but still a considerable number of young individuals), or “regressive” (mature plants predominate). The three types of populations reflect different colonization or local extinction of populations and individuals. Dynamic populations represent a relatively new colonization event, with low local extinction. Normal populations represent a balance between recruitment of new individuals and local extinction. Regressive populations are populations in

which local extinction is higher than recruitment (Agurauja et al. 2004, p.1,441). Such data is a useful tool to evaluate conservation management priorities and understand regional dynamics in conditions for diellioid ferns as quantitative demographic data is unavailable. Using these data gathered on life-stage structure and population size of remaining diellioid ferns, Agurauja and others have concluded that the whole sub-clade is critically endangered and threatened with extinction unless conservation measures continue to be implemented (Agurauja et al. 2004, pp. 1,442–1,444).

The germination tests initiated at Tallinn Botanic Garden in 2011 for three species of diellioid ferns including, *Asplenium diellaciniatum*, revealed that single individual gametophyte cultures were unable to yield sporophytes. This indicates that these species are obligatory out-crossers (Agurauja 2017, p. 38). Therefore, spores must be produced from different individuals in order for individuals to reproduce. Small population size may have a significant influence on colonization of suitable habitats and may be one reason for the rarity of the species (Agurauja et al. 2004, p. 1,444).

In fact, a follow up multi-island study conducted by Agurauja et al. (2008) in which a second census of the extant populations of diellioid ferns was conducted, showed that the size of the population during the first census was strongly correlated with population status during the second census. Dynamic populations increased in size, however sporelings and premature individuals decreased, and regressive populations were stable or decreased. Four regressive populations which had the smallest number of individuals during the first census became locally extinct (Agurauja et al. 2008, p. 14).

Habitats supply the basic needs (food, air, water, space to grow and shelter) of populations. *Asplenium diellaciniatum* populations occur in montane mesic forest. Montane mesic forests are found in regions that lack sufficient rainfall to support a wet forest, however they do not suffer moisture deficits for prolonged periods. Water can be a major limiting factor in the growth of plants, including ferns (Agurauja et al. 2004), in montane mesic forest habitat (Gagne and Cuddihy, 1999, Lowe et al. 2020, p. 3). Diellioid ferns grow in relatively arid sites, lack of moisture may cause ferns to go dormant and inhibit sexual reproduction. Reproductive success, juvenile survival and persistence of mature individuals may depend on years with higher precipitation (Agurauja et al. 2004, p.1,444).

A healthy population consists of abundant individuals within habitat patches of adequate area and quality to maintain survival and reproduction in spite of disturbance. Populations of *Asplenium diellaciniatum* need habitats in which the degree of threats are at a low enough level that the habitat is able to continue to be suitable and supply the basic needs of the *A. diellaciniatum* populations. Resiliency is the capacity of a population (or a species) to withstand stochastic disturbance events, we measure resiliency by population trends over time and population size and structure. Populations which have abundant individuals in which all size classes can be found, have a more probable outcome for survival.

Species Needs

Species need resilient populations that are redundant and represented.

Redundancy is the ability of *Asplenium diellaciniatum* to withstand catastrophic events and is measured by the number of populations (redundancy/duplication), distribution of the populations across the landscape, and connectivity among populations. In order to achieve redundancy, the distribution of *A. diellaciniatum* populations across the geographic range should exceed the area of impact of a catastrophic event that would otherwise overwhelm the resilient capacity of the populations. Essentially, the more populations of *A. diellaciniatum* and the broader the distribution of those populations, the more redundancy the species will exhibit, thereby increasing its ability to survive a catastrophic event. Currently *A. diellaciniatum* is known from a single population consisting of two sub-populations at Kawaiʻiki and Kaluahaulu in montane mesic forest (PEPP 2019, Aguraiuja pers. comm. 2020).

The distance between the two sub-populations at Kawaiʻiki and Kaluahaulu on different ridges is not great enough to exclude gene flow between sub-populations. Pteridophytes (spore producing plants such as ferns and fern allies) are known to disperse widely because of their small spores. Studies of pteridophyte distribution report that habitat availability may be the limiting factor for the distribution (Guo et al. 2003, p. 129). As such, *Asplenium diellaciniatum* may be capable of dispersal within or even among islands. However, considering the prominent direction of the winds or other factors, there could be isolation which causes the morphological differences among the sub-populations (Aguraiuja pers. comm. 2020). Further molecular studies are needed to understand the cause of variation among and within the sub-populations of *A. diellaciniatum*. Efforts are underway to introduce this species in protected suitable habitat (PEPP 2019, Aguraiuja pers. comm. 2020). For *A. diellaciniatum*, redundancy requires the presence of multiple, stable to increasing populations distributed across montane mesic forests on Kauaʻi.

Habitat degradation by feral ungulates such as pigs, goats and deer, negatively impact the survival of diellioids including *Asplenium diellaciniatum*. Aguraiuja et al. (2008) found of the 19 populations assessed on the basis of stage structure as ‘dynamic’ (2 populations), ‘normal’ (8 populations), or ‘regressive’ (9 populations), population size was negatively dependent on soil disturbance by alien animals (Aguraiuja et al. 2008, pp. 8–13). Invasive plants also negatively impact habitat by competing with *Diellia* populations for resources and space. Evidence on the impact of alien plant species is less clear, and may become more evident over longer time scales (Aguraiuja et al. 2008, p. 15).

Representation is the ability of *Asplenium diellaciniatum* to adapt to changing environmental conditions over time and can be measured by having one or more populations of a species occupying the full range of suitable habitat used by the species. Alternatively, representation can be viewed as maintaining the breadth of genetic diversity within and among populations, in order to allow the species to adapt to changing environmental conditions over time. Unique traits exist within and between sub-populations. We measure representation by the number of resilient populations expressing the morphological variation of *A. diellaciniatum*. Given the morphological variation that *A. diellaciniatum* displays, it was likely more widespread across the montane mesic forests of Kauaʻi. The holotype of *A. diellaciniatum* was collected by Kndusen in 1875 at Halemanu (Wagner 1935, p. 29). Subsequent surveys in this area have not relocated *A.*

diellaciniatum (Lorence et al. 2013, p.167, Agurauja and Wood 2003, p. 155). We assume that as populations decline and disappear, genetic diversity decreases. We have limited information on the connectivity of sub-populations which would support genetic exchange and representation. However, connectivity decreases with habitat loss and fragmentation, thus we can assume that genetic diversity has likely decreased in the species over time. Representation is maintained in the *A. diellaciniatum* species by having abundant individuals in stable to increasing populations that represent the existing full genetic diversity and morphological variation within the species, dispersed throughout the montane mesic forests of Kaua‘i.

FACTORS INFLUENCING VIABILITY

Threats

Threats to *Asplenium deillacinatum* include habitat degradation and predation by introduced ungulates, including feral pigs (*Sus scrofa*), goats (*Capra hircus*), and black-tailed deer (*Odocoileus hemionus columbianus*), habitat degradation and competition with nonnative plants, consequences of small population size, inadequate regulatory mechanisms and climate change (USFWS 2016, p. 67,792).

The mild climate of the islands, combined with the lack of competitors or predators, led to the successful establishment of large populations of these invasive mammals, to the detriment of native Hawaiian species and ecosystems. Montane mesic forests where *Asplenium diellaciniatum* occurs are exposed to both direct and indirect negative impacts of invasive ungulates (Lowe et al. 2020, p. 15). The effects of ungulates include the destruction of vegetative cover; trampling of plants and seedlings; direct consumption of native vegetation, including *A. diellaciniatum*; soil disturbance; dispersal of invasive plant seeds on hooves and coats, and through the spread of seeds in feces; and creation of open disturbed areas conducive to further invasion by invasive plant species. All of these impacts can lead to the subsequent conversion of a native plant community to one dominated by invasive species (USFWS 2016, p. 67,827). Feral ungulates threaten *A. diellaciniatum* throughout the species range. Only sub-populations within ungulate-proof enclosures are considered to be protected. In addition to these direct effects, because ungulates inhabit terrain that is often steep and remote, foraging and trampling contributes to severe erosion of watersheds. Ungulates accelerate erosion which can cause landslides and dislodging stones from ledges which result in rock falls and landslides that damage or destroy native vegetation below (Cuddihy and Stone 1990, pp. 63–64).

Nonnative plants are a threat to *Asplenium diellaciniatum* as they compete for the same resources (water, space, nutrients, and light) that *Asplenium diellaciniatum* individuals need to survive. Invasive plants adversely affect microhabitat in the forest by modifying availability of light and nutrient cycling processes, and by altering soil-water regimes (Smith, 1985). Some invasive plants may release chemicals that inhibit growth of other plants. These competitive advantages allow invasive plants to convert native-dominated plant communities to nonnative plant communities. Nonnative plants which threaten *Asplenium diellaciniatum* include; *Adiantum hispidulum* (rough maidenhair fern), *Blechnum appendiculatum* (no common name), *Erigeron karvinskianus* (daisy fleabane), and *Rubus argutus* (prickly Florida blackberry), *Bryophyllum pinnatum* (air plant), *Psidium cattleianum* (strawberry guava), *Grevillea robusta* (silk oak), *Myrica faya* (fire tree), *Mariscus meyenianus* (Meyen’s flat sedge), *Passiflora mollissima*

(passion fruit), *Lantana camara* (lantana), *Setaria parviflora* (bristle grass), *Ehrharta stipoides* (meadow rice grass) (NTBG 2008a; USFWS 2016, p. 67,792).

Because there are so few individuals left in the wild, *Asplenium diellaciniatum*, has limited species' adaptability to environmental changes (USFWS 2016, p. 67,793). Small populations of *Asplenium diellaciniatum* are more prone to extinction. Agurauja et al. (2008) found that the four populations which had the fewest number of individuals of diellioid ferns went extinct in between to census surveys occurring in 1999 and 2005 (Agurauja et al. 2008, p.15).

As environmental conditions are altered by climate change, *Asplenium diellaciniatum* is unlikely to tolerate or adapt to projected changes in temperature and moisture, and is unlikely to be able to move to areas with more suitable climatic conditions (Fortini et al. 2013, p. 18). Although we cannot predict the timing, extent, or magnitude of specific impacts, we do expect the effects of climate change to exacerbate the threats to *A. diellaciniatum* described above (USFWS 2016, p. 67,792).

Inadequate regulatory mechanisms threaten *Asplenium diellaciniatum*. Nonnative feral ungulates pose threat to *A. diellaciniatum* through destruction and degradation of the species' habitat and herbivory but regulatory mechanisms are inadequate to address this threat (USFWS 2016). The State of Hawai'i provides game mammal (feral pigs and goats and black-tailed deer) hunting opportunities on 17 State-designated public hunting areas on the island of Kaua'i (HDLNR 2015, pp. 19–21 and 66–77). However, the State's management objectives for game animals range from maximizing public hunting opportunities (e.g., "sustained yield") in some areas to removal by State staff, or their designees, in other areas (HDLNR 2015, entire).

Introduction of Nonnative Plants and Insects: Currently, four agencies are responsible for inspection of goods arriving in Hawai'i (USFWS 2016). The Hawai'i Department of Agriculture (HDOA) inspects domestic cargo and vessels and focuses on pests of concern to Hawai'i, especially insects or plant diseases. The U.S. Department of Homeland Security-Customs and Border Protection (CBP) is responsible for inspecting commercial, private, and military vessels and aircraft and related cargo and passengers arriving from foreign locations (USFWS 2016). The U.S. Department of Agriculture-Animal and Plant Health Inspection Service-Plant Protection and Quarantine (USDA-APHIS-PPQ) inspects propagative plant material, provides identification services for arriving plants and pests, and conducts pest risk assessments among other activities (HDOA 2009, p. 1). The Service inspects arriving wildlife products, enforces the injurious wildlife provisions of the Lacey Act (18 U.S.C. 42; 16 U.S.C. 3371 *et seq.*), and prosecutes CITES (Convention on International Trade in Wild Fauna and Flora) violations. The State of Hawai'i allows the importation of most plant taxa, with limited exceptions (USFWS 2016). It is likely that the introduction of most nonnative invertebrate pests to the State has been and continues to be accidental and incidental to other intentional and permitted activities. Many invasive weeds established on Hawai'i have currently limited but expanding ranges. Resources available to reduce the spread of these species and counter their negative ecological effects are limited. Control of established pests is largely focused on a few invasive species that cause significant economic or environmental damage to public and private lands, and comprehensive control of an array of invasive pests remains limited in scope (USFWS 2016).

Conservation Actions

The Kaluahulu sub-population consists of two patches of ferns which are close together (less than 100 meters apart). The larger patch consisting of approximately 30 individuals was protected by an ungulate-proof fence in 2009 and the smaller patch consisting of 7 individuals was fenced in 2010 (KRCP 2013a). Annual fence maintenance, weed control and population surveys have continued since the fences were initially constructed. The sub-population at Kawaiʻiki was found in 2010, this population is not currently fenced and ungulate damage has been observed (KRCP 2013b).

Agurauja, in partnership with the Kōkeʻe Resource Conservation Program, Department of Land and Natural Resources, Division of Forestry and Wildlife (DLNR DOFAW) and National Tropical Botanical Garden (NTBG), has conducted annual census counts of *Asplenium diellaciniatum* since 2002 (Agurauja 2020, pers. comm.). Census counts include data on population structure, survivorship, mortality, life stage transitions and sexual reproduction.

Agurauja began germination trials for *Asplenium diellaciniatum* at Tallinn Botanical Garden in Estonia in 2011. The three morphotypes (*alexandri*, *erecta* and *knudsenii*) were kept separate during the tests. The results of the trials led to trays of densely filled gametophytes which were cultivated over the years, establishing an *ex-situ* collection at Tallinn Botanical Garden. Agurauja found that the older gametophytes remained capable of vegetative and regenerative growth and therefore gametophyte cultures can be stored for ex situ conservation purposes given the appropriate growing conditions.

In 2015 the ferns were shipped from Tallinn Botanical Garden in Estonia to the NTBG on Kauaʻi for their eventual translocation back into the wild. Gametophyte cultures as well as 477 individuals of *Asplenium diellaciniatum* were returned to Kauaʻi. The ferns were replanted into pots and then relocated to the DLNR DOFAW, Kōkeʻe Rare Plant Facility for acclimation before reintroduction (Agurauja 2017, p. 39).

Outplanting began in 2016, 19 individuals were reintroduced into a the Poʻomau enclosure in the Nā Pali Kona Forest Reserve and all remained alive after one year. In 2017, an additional 60 individuals were planted. All ferns outplanted at this site are from the Kawaiʻiki sub-population (morphotype *knudsenii*.) At the last census in 2020, five of the 2016 individuals remain and 31 of the 2017 individuals remain. Natural regeneration was observed in 2019 (see Figure 4 below).



Figure 4. First born individual from reintroduced *Asplenium diellaciniatum* within enclosure at Nā Pali Kona Forest Reserve. Photo by R. Agurauja, Tallinn Botanical Garden.

The University of Hawai‘i’s Lyon Arboretum currently has 10,000 spores of *Asplenium diellaciniatum* in storage from two different accessions, both from Kawai‘iki (Lyon Arboretum 2019). The National Tropical Botanical Garden has spores in storage from six accessions and three gametophyte trays all from Kawai‘iki (NTBG 2019). The DLNR DOFAW Kōke‘e Rare Plant Facility has 26 plants from three different accessions, all from Kawai‘iki (HDLNR DOFAW 2019).

Endangered Species Act: Regulatory actions such as the Endangered Species Act provide conservation benefits for *Asplenium diellaciniatum*. The Service determined endangered status under the Endangered Species Act of 1973 (Act), as amended, for 39 plants and 10 animals on October 31, 2016 including *Asplenium diellaciniatum* (USFWS 2016). The primary purpose of the Act is the conservation of endangered and threatened species and the ecosystems upon which they depend. The ultimate goal of such conservation efforts is the recovery of these listed species, so that they no longer need the protective measures of the Act. Conservation measures provided to species listed as endangered or threatened under the Act include recognition of threatened or endangered status, recovery planning, requirements for Federal protection, and prohibitions against certain activities. The Act encourages cooperation with the States and requires that recovery actions be carried out for all listed species. The Act and its implementing

regulations in addition set forth a series of general prohibitions and exceptions that apply to all endangered wildlife and plants. For plants listed as endangered, the Act prohibits the malicious damage or destruction on areas under Federal jurisdiction and the removal, cutting, digging up, or damaging or destroying of such plants in knowing violation of any State law or regulation, including State criminal trespass law. Certain exceptions to the prohibitions apply to agents of the Service and State conservation agencies. The Service may issue permits to carry out otherwise prohibited activities involving endangered or threatened wildlife and plant species under certain circumstances. With regard to endangered plants, a permit must be issued for scientific purposes or for the enhancement of propagation or survival. For federally listed species unauthorized collecting, handling, possessing, selling, delivering, carrying, or transporting, including import or export across State lines and international boundaries, except for properly documented antique specimens of these taxa at least 100 years old, as defined by section 10(h)(1) of the Act, is prohibited. Damaging or destroying any of the listed plants in addition is violation of the Hawai‘i State law prohibiting the take of listed species. The State of Hawai‘i’s endangered species law (HRS, Section 195-D) is automatically invoked when a species is Federally listed, and provides supplemental protection, including prohibiting take of listed species and encouraging conservation by State government agencies. *Asplenium diellaciniatum* occurs only non-Federal lands. Critical habitat has not been designated for this species.

CURRENT CONDITION

Historical Condition

Pre-human Habitat Distribution and Description

Montane mesic forests on Kaua‘i occurred above the ‘ōhi‘a lowland wet forest and below montane wet forests. Dominant trees included koa and ‘ōhi‘a and the understory was consisted of many native sedges, ferns, and shrubs. Large expanses of the koa / ‘ōhi‘a montane mesic forest were converted to cattle ranches after logging of koa. Browsing by feral ungulates, fires, and clearing of land for commercial tree planting and harvest have increased the spread of invasive grasses and reduced the quality and extent of the mesic forests and (Lowe et al. 2020, pp. 7–8). It was estimated in pre-human times that montane mesic forest covered over 8,000 acres (3,237 hectares) on Kaua‘i, currently only a few thousand acres remain (Reeves and Amidon 2018, Lowe et al. 2020, p. 11).

Historic Trends and Current Condition of *Asplenium diellaciniatum*

Asplenium diellaciniatum was first collected by Valdemar Knudsen sometime between 1871 and 1875 at Halemanu in Kōke‘e, Kaua‘i, see Figure 5 below for island location (Wagner 1935, p. 29, Lorence et al 2013, p. 167). The specimens were sent to W. Hillebrand who described the species in 1888 and deposited the specimens at the herbarium of the Botanical Garden and Botanical Museum Berlin-Dahlem. The species was not seen again for over 100 years. In 2001 K.R. Wood and S. Perlman (NTBG) discovered the Kaluahaulu sub-population of approximately 49 mature fertile individuals, and 10 young sporophytes (Wood 2006, p. 16). M. O’Sullivan (DLNR DOFAW) discovered the sub-population of approximately 57 mature individuals and eight sporophytes at Kawai‘iki in 2010 (PEPP 2019), see Figure 6 for population unit.

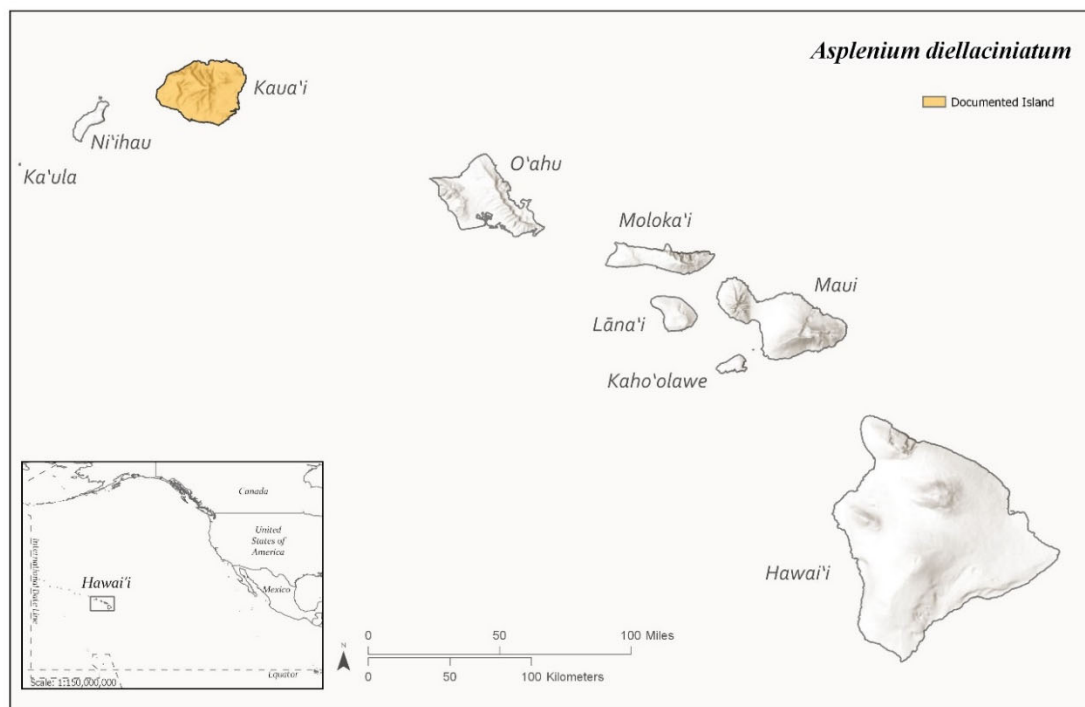


Figure 5. Historical island range of *Asplenium diellaciniatum*.

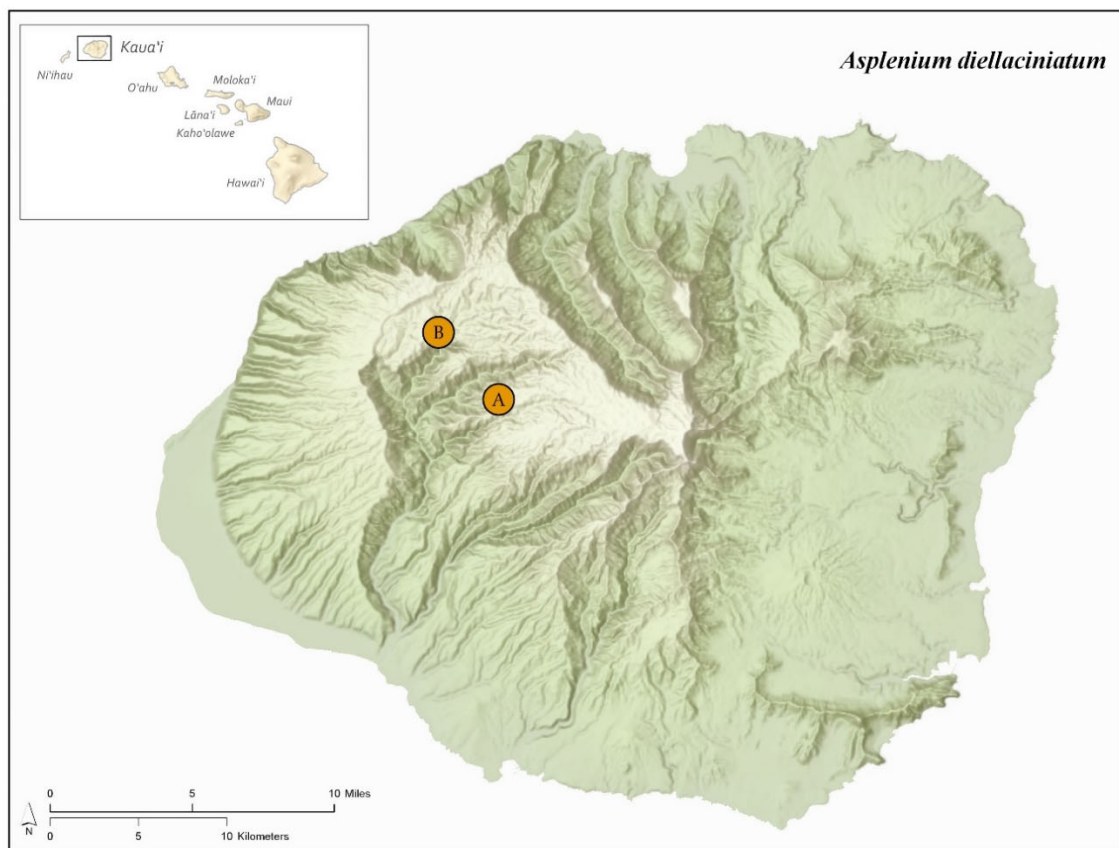


Figure 6. Distribution map of population units for *Asplenium diellaciniatum*.

Annual census counts (except for in 2014 and 2015) have been conducted since both sub-populations were discovered. There are currently three sporelings, seven premature and 43 mature individuals at Kaluahulu and 37 sporelings, 58 premature and 54 individuals at Kawai‘iki (Aguraiuja 2020, pers. comm.). See Table 1 below for a summary of the population units.

Table 1. Current Population Units of *Asplenium diellaciniatum*.

Population Unit Name	Kaluahulu	Kawai‘iki	Po‘omau Exclosure
Population Unit Letter	A	A	B
Habitat Type	Montane mesic forest	Montane mesic forest	Montane mesic forest
Morphotype	<i>alexandri</i> and <i>erecta</i>	<i>knudsenii</i>	<i>knudsenii</i>
Last Observation Date	2020	2020	2020
Extant?	Y	Y	Y
Population Type	Wild	Wild	Reintroduction
Population Trend *	D	D	D
Estimated Number of Sites	2	1	1
Estimated Number of Individual	three sporelings, seven premature and 43 mature individuals	37 sporelings, 58 premature and 54 individuals	three premature and 36 mature individuals

*I = increasing number of individuals; D = decreasing; C = constant (same individuals); S = stable population growth

SPECIES VIABILITY SUMMARY

Resiliency

For *Asplenium diellaciniatum* to maintain viability, the population must be resilient. The definition of resiliency is the ability of the population to withstand stochastic events, meaning they must have healthy, stable populations, good quality and quantity of habitat.

We measure resiliency for *A. diellaciniatum* by population trends over time and population size and structure. Populations which have abundant individuals in which all size classes can be found, have a more probable outcome for survival. Currently there are 40 sporelings, 65 premature and 97 mature individuals in two sub-populations.

Population sizes have decreased over time and habitat extent and quality have also declined. A reintroduction has been conducted on Kaua‘i and there is evidence that natural recruitment is occurring as a new individual was found in 2019. However, by considering the persistence of threats (ungulates and invasive plants) in the largest sub-population, there is reduced resiliency in the current condition. More than half of the existing population is not fenced and subjected to continued severe habitat degradation as well as predation by ungulates.

When evaluating the habitat quality of each population to determine the species’ resiliency, the following threats; habitat modification and destruction by feral pigs, deer, and goats and competition from nonnative plants combined with herbivory by nonnative ungulates, continue to degrade the suitable habitat for *Asplenium diellaciniatum*. Only one sub-population of the two

sub-populations is protected. The reintroduction at Po‘omau enclosure is within an ungulate-proof enclosure.

Overall, the resiliency of *Asplenium diellaciniatum* is low due to a low number of individuals (162, excluding sporelings), and only one sub-population is in an area in which some threats are being addressed (feral ungulates and invasive weeds). The population is in decline. The fence provides protection for less than half of the population which has slowed the threat of imminent extinction. Therefore, the resiliency for *A. diellaciniatum* on the species level is low in the current condition due to the low of number of individuals, as well as the decrease in the extent and quality of habitat, and the degree of threats remaining in the current population.

Redundancy

We evaluate redundancy for *Asplenium diellaciniatum* based on the metric of the number of populations and their distribution across the known range of the species. Historically, the species was known from Halemanu on Kaua‘i. Recent surveys have not relocated the species at Halemanu. Currently it is only known from one population (population unit A) consisting of two sub-populations at Kawai‘iki and Kaluahulu. The loss of populations and their low resiliency of the extant populations reduces the species range and increases the risk of further extirpation. For *A. diellaciniatum*, redundancy requires the presence of multiple, stable to increasing populations distributed across montane mesic forests on Kaua‘i. Therefore, redundancy in *A. diellaciniatum* is very low due to the loss of populations and species range constriction.

Representation

We define representation by the number of resilient populations expressing the full morphological variation of *Asplenium diellaciniatum*. The distribution of historic and current populations occurs at three sites in the montane mesic forests of northwest Kaua‘i; Kawai‘iki, Kaluahulu and Halemanu. Currently the species is only known from Kawai‘iki and Kaluahulu. Morphotypes *alexandri* and *erecta* are found at Kaluahulu and morphotype *kndusenii* is found at Kawai‘iki. As morphotypes are isolated in single subpopulations, the population has very low representation. The morphotypes, which are analogous to the breadth of genetic diversity within the species, are not adequately represented. The unique traits which exist in the remaining populations are at risk of being lost. Ideally, several resilient populations with a robust number of individuals would occur expressing each morphotype throughout the species range. Thus, representation for *A. diellaciniatum* is very low.

Species Viability Summary

The current condition of *Asplenium diellaciniatum* is described as having one population on Kaua‘i, with a new reintroduction possibly creating a new population if successful in the future. Overall, individuals in populations have generally been decreasing due to existing threats in suitable habitats. Some redundancy and representation is maintained in *ex situ* seed storage and greenhouses, and reintroduced individuals, and one example of recruitment (one individual) has been documented as a result of the reintroduction. As this species has low resiliency, very low redundancy, and very low representation in the current condition, the overall viability of this species is very low (

Table 2).

Table 2. Current viability of *Asplenium diellaciniatum*.

The 3Rs	Resiliency	Redundancy	Representation	Overall Viability
Current Condition	Low	Very Low	Very Low	Very Low

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