BEFORE THE UNITED STATES DEPARTMENT OF THE INTERIOR
AND THE UNITED STATES FISH AND WILDLIFE SERVICE

In the Matter of the Petition to Delist
the Coastal California gnatcatcher
(*Polioptila californica californica*)
from the List of Threatened Species
Under the Endangered Species Act

PETITION OF THE CENTER FOR ENVIRONMENTAL SCIENCE, ACCURACY AND RELIABILITY; COALITION OF LABOR, AGRICULTURE, AND BUSINESS; PROPERTY OWNERS ASSOCIATION OF RIVERSIDE COUNTY; NATIONAL ASSOCIATION OF HOME BUILDERS; AND THE CALIFORNIA BUILDING INDUSTRY ASSOCIATION TO REMOVE THE COASTAL CALIFORNIA GNATCATCHER FROM THE LIST OF THREATENED SPECIES UNDER THE ENDANGERED SPECIES ACT

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INTRODUCTION

Pursuant to 16 U.S.C. § 1533(b)(3) and 50 C.F.R. § 424.14(a), Petitioners the Center for Environmental Science, Accuracy and Reliability; Coalition of Labor, Agriculture, and Business; Property Owners Association of Riverside County; National Association of Home Builders; and the California Building Industry Association hereby petition the Secretary of the Department of Interior and the United States Fish & Wildlife Service (collectively "the Service") to delist the coastal California gnatcatcher (*Polioptila californica californica*) ("*P.c.c.*") from the list of threatened wildlife, 50 C.F.R. § 17.11(h), under the Endangered Species Act (ESA), 16 U.S.C. §§ 1531-1544. The requested delisting action is warranted because the best available scientific data show that the taxonomic classification of the *P.c.c.* as a subspecies is based on erroneous information. It is undisputed that the ***species Polioptila californica*** is a common bird and is not endangered or threatened. Because the subspecies delineation *P.c.c.* is invalid, there is no basis to continue to apply the ESA to gnatcatchers in any portion of the species' range.

This petition goes to the heart of the ESA because an objective, science-based listing process is central to the statute's integrity. The ESA's purpose is to protect genetically unique or evolutionarily distinct life forms. It does this by requiring that listing decisions be based on the "best scientific

. . . data available," 16 U.S.C. § 1533(b)(1)(A), and by requiring that a species or subspecies be threatened or endangered "throughout all or a significant portion of its range," *id.* § 1532(6) & (20). The failure to use the best scientific data available in listing decisions engenders cynicism that listing decisions are a product of ideological and regulatory motives rather than the best available scientific data. Failing to use the best data also diverts scarce private and public resources from more important conservation challenges.

The debate over the taxonomy of the coastal California gnatcatcher has raged since before the bird's listing as a threatened species under the ESA. We describe the history of this debate below in some detail because it is important to understanding the grounds for this petition.

First described as a separate species in 1881, the gnatcatcher was reclassified in the 1920s as a subspecies of the widespread and common black-tailed gnatcatcher. *See* 58 Fed. Reg. 16,742, 16,742 (Mar. 30, 1993). Throughout the twentieth century, various authorities posited different groupings of California gnatcatcher subspecies, the purported ranges of which occupied contiguous segments of the Baja California peninsula and Southern California. *See id.*; 60 Fed. Reg. 15,693, 15,698 (Mar. 27, 1995); 68 Fed. Reg. 20,228, 20,230 (Apr. 24, 2003). *See also* R.M. Zink, J.G. Groth, H. Vazquez-Miranda, and G.F. Barrowclough. 2013. Phylogeography of the California Gnatcatcher (*Polioptila californica*) Using Multilocus DNA

Sequences and Ecological Niche Modeling: Implications for Conservation. *Auk* 130:449-458 ("Zink et al. (2013)").

The Service's principal basis for the listing of the *P.c.c.* as a threatened subspecies has been the analysis of morphological data of gnatcatcher museum specimens collected by Dr. Jonathan Atwood (a petitioner for the listing) as part of his dissertation studies. In his petition to list the *P.c.c.*, Atwood took the position that there are three valid subspecies of *Polioptila californica* and that the range of *P.c.c.—the northernmost form*—extends from southern California to 30°N latitude in Baja California, Mexico. During the debate over the listing, Atwood acknowledged that the subspecies designation for the *P.c.c.* was central to the listing decision because "[n]o credible scientist would claim or has claimed that California gnatcatchers as a species are endangered or threatened throughout their entire range." (Testimony to California Fish and Game Commission, August 31, 1991.) This statement remains correct today.

As much as any ESA decision since the statute's 1973 passage, the listing of the *P.c.c.* underscores how ESA regulation has both profound real-world consequences for the conservation of biological resources, as well as significant impacts to society and the economy. The listing of the *P.c.c.* triggered an unprecedented twenty-year conservation planning process in

Southern California that continues today. This planning process has included the approval of numerous habitat conservation plans (HCPs) and natural community conservation plans (NCCPs) in Orange, Riverside, and San Diego Counties. Collectively, these plans regulate land-use on millions of acres. The plans have resulted in the establishment and management of regional conservation reserves of several hundred thousand acres of the coastal sage scrub habitat of the gnatcatcher and other species covered by the plans.

Since the listing of the *P.c.c.*, well over a dozen HCPs and NCCPs protecting the gnatcatcher and its habitat have been finalized and are now being implemented. These programs protect in perpetuity a significant amount of coastal sage scrub in reserve systems, establish important linkages that allow for natural dispersal and gene flow, implement and fund coordinated monitoring and adaptive management actions beneficial to the long-term conservation of the gnatcatcher and other special status species, and require adherence to specific measures that minimize impacts to the gnatcatcher (*e.g.*, avoidance of grading during breeding season).

Table 1 below provides a summary of the largest conservation plans.

TABLE 1

Large-Scale Regional Conservation Plans That Protect the Coastal California Gnatcatcher and Its Habitat and Contribute to the Species's Conservation

Conservation Plan	Year Finalized	Gnatcatcher Conservation Measures
1. County of Orange Central/Coastal Subregion NCCP/HCP	1996	• Creates a 37,378-acre reserve system, with over 18,000 acres of coastal sage scrub habitat in the reserve
2. San Diego MSHCP	1998	• Preserves over 73,000 acres of coastal sage scrub and integrated habitats in an interconnected network of preserves; requires adoption of sub-area plans by cities
3. San Diego Association of Governments MSHCP for Seven Incorporated Cities Northwestern San Diego County	2003	• Conservation of a minimum of 62% of known gnatcatcher sites • Conserves, enhances, and manages regionally critical stepping-stone linkage across the MSHCP plan area • Dependent on incorporation of sub-area plans by cities
4. Western Riverside MSHCP	2004	• Creates a 500,000-acre reserve system with approximately 82,000 acres of coastal sage scrub conserved in the plan area

5. County of Orange Southern Subregion HCP/MSAA	2006	• Conservation of 14,387 acres of coastal sage scrub with habitat linkages; preservation of 428 gnatcatcher locations

Several smaller-scale conservation plans also provide protections for the *P.c.c.* as well as important habitat linkages among larger preserve areas. These plans, located in San Diego, Orange, and Riverside Counties, include the San Diego Gas & Electric Subregional NCCP (2005), the City of Carlsbad/Fieldstone/La Costa Associates HCP, the Assessment District 161 MSHCP, the North Peak Development Project MSHCP, the Evergreen Nursery HCP, the Coyote Hills East HCP, the Temecula Ridge HCP, and the Shell Oil/Metropolitan Water District HCP.

Beyond these robust ongoing conservation planning efforts, a number of resource management and conservation programs have been established to ensure the further protection of coastal sage scrub and the conservation of the gnatcatcher. Among them are: Integrated Natural Resources Management Plans, prepared under the Sikes Act, 16 U.S.C. § 670, *et seq.*, at Marine Corps Base Camp Pendleton and Naval Air Station Miramar; National Wildlife Refuges; Bureau of Land Management lands; and privately held lands. In addition, the Service has concluded dozens of interagency consultations under Section 7 of the ESA that have resulted in the protection of tens of thousands of acres of coastal sage scrub habitat. For example, the Service's consultations with the Federal Highway Administration for the San Joaquin, Foothill, and

Eastern Transportation Corridors have resulted in an infusion of land and ongoing funding for restoration and management of the Orange County habitat reserve system.

This unprecedented planning process has imposed significant societal costs. The Service itself has estimated that land-use regulations triggered by the *P.c.c.*'s listing will cost nearly $1 billion through 2025. *See* U.S. Fish & Wildlife Serv., *Economic Analysis of Critical Habitat Designation for the California Gnatcatcher* 13 (Feb. 24, 2004).[1] The value of the public and private land committed to the conservation reserves easily exceeds this number.

The ESA recognizes that science is not static: scientific information essential to the listing process inevitably improves over time as new data are gathered and hypotheses are tested and falsified. Thus, the ESA provides the public with the right to petition the government to modify decisions to list a species or to change the listing status of a species, including delisting if warranted. *See* 50 C.F.R. § 424.14(a). Again, these decisions are all required to be made on the basis of the best scientific data available. In *Bennett v. Spear*, the United States Supreme Court held that Congress adopted that criterion to insure that the "ESA not be implemented haphazardly, on the basis

[1] *Available at* http://www.fws.gov/economics/Critical%20Habitat/Final%20Draft%20Reports/CA%20coastal%20gnatcatcher/CAGN_DEA_Feb2004.pdf (last visited May 28, 2014).

of speculation or surmise." 520 U.S. 154, 176 (1997). The Court explained that the best available science mandate, which "no doubt serves to advance the ESA's overall goal of species preservation," also serves "another objective (if not indeed the primary one)" of avoiding "needless economic dislocation produced by agency officials zealously but unintelligently pursuing their environmental objectives." *Id*. at 176-77.

This petition is based on a recent peer-reviewed study and published article in the respected ornithological journal *The Auk*, authored by Professor Robert Zink of the University of Minnesota and Dr. George Barrowclough of the American Museum of Natural History and their colleagues. *See* Zink et al. (2013), Exh. A. This 2013 study consists of an analysis of nuclear DNA obtained from gnatcatcher specimens throughout the range of the species (i.e., southern California south to the tip of Baja California, Mexico). As explained in greater detail below, the study concludes that there is no genetic basis for maintaining a subspecies classification for the *P.c.c.* Rather, members of this putative subspecies should be considered part of the same taxonomic grouping as the species *Polioptila californica*, which ranges from Ventura County in southern California to the southern tip of Baja California, Mexico. The study by Professor Zink and his colleagues is particularly important because, in the Service's most recent review of the gnatcatcher's taxonomy, the agency suggested that just such a nuclear DNA analysis would provide the best

available scientific data to disprove or confirm the gnatcatcher's subspecies classification. *See* 76 Fed. Reg. 66,255, 66,258 (Oct. 26, 2011).

Accordingly, for the reasons set forth below, Petitioners request that the Service delist the coastal California gnatcatcher (*P. c. californica*) from the ESA.

PETITIONERS

The Center for Environmental Science, Accuracy & Reliability is a California nonprofit corporation the primary purpose of which is to bring scientific rigor to regulatory decisions undertaken pursuant to environmental statutes, and to ensure consistent application of these statutes throughout all industries and sectors. The Center believes that these activities will generate additional support for environmental statutes, because the results of and bases for regulatory actions will be transparent and supported by good science. The Center believes that these goals will be furthered by delisting *P.c.c.* Delisting will demonstrate that ESA decision-making should not be based on politicized science. This goal is all the more important now, given the depressed California economy and the significant economic impact that the *P.c.c.*'s listing creates.

Petitioner Coalition of Labor, Agriculture, and Business unites the independent strengths of these sectors of the economy to protect and improve the natural and business environments of San Luis Obispo and Santa Barbara Counties, California. The Coalition engages in educational outreach, political

action, and issue advocacy. The Coalition supports the protection of private property rights, fiscal responsibility, and environmental legislation based on sound principals of science, as well as cost-effective solutions to issues associated with business and job creation. The Coalition's members are primarily comprised of farming and ranching families who have been stewards of the land for generations. The Coalition advocates for a balanced approach to environmental regulation, especially with respect to the administration of the ESA. To that end, in 2010 the Coalition, along with other parties, petitioned the Service to delist the *P.c.c.*

Petitioner Property Owners Association of Riverside County (Association), is a non-profit organization, the mission of which is to serve as an advocate for Riverside County property owners to ensure that landowners' rights are protected in the formation and implementation of public policies. The Association includes owners of real property in Riverside County whose interests are directly affected by government land-use regulations, including numerous land-use restrictions imposed by the ESA. In particular, the Association has two dozen members who are within the Western Riverside County Multiple Species Habitat Conservation Plan area, which includes *P.c.c.* habitat. In 2010, the Association joined the Coalition to petition the Service to delist the *P.c.c.*

Petitioner National Association of Home Builders ("NAHB") is a Washington, D.C.–based trade association founded in 1942. It is comprised of more than 800 state and local associations, with about one-third of NAHB's 235,000 members being home builders. The remaining members are associates working in closely-related fields within the housing industry. NAHB's goal is providing and expanding opportunities for all consumers to have safe, decent and affordable housing. The association represents the industry's interests on Capitol Hill and strives to ensure that housing remains a national priority when laws are made and policies are established. NAHB also works with federal agencies on regulations affecting the housing industry and the environment.

Petitioner California Building Industry Association represents approximately 3,500 members—including home builders, trade contractors, architects, engineers, designers, suppliers, and other industry professionals. CBIA members design and construct California's housing. CBIA's purpose is to advocate on behalf of the interests of its members, including, but not limited to, representation in regulatory matters and litigation affecting the ability of its members to provide housing, office, industrial, and commercial facilities for residents of California. Members of the CBIA have been actively involved in all regulatory and planning issues concerning the gnatcatcher since 1990 and have committed hundreds of millions of dollars and tens of thousands of acres of land to the conservation of the gnatcatcher in the various

habitat conservation plans and natural community conservation plans in Southern California.

BACKGROUND

A. The Convoluted Taxonomic History of the Coastal California Gnatcatcher

Polioptila californica (commonly referred to as the "California gnatcatcher") is a *species* of song bird that extends from the southern tip of the Baja California Peninsula, Mexico, north to Ventura County, California. It is common in central and southern Baja California but less common in northwestern Baja California and southern California. A closely related species, the black-tailed gnatcatcher (*Polioptila melanura*), ranges from southern Nevada into the Mexican States of Sonora and Chihuahua, and overlaps a small fraction of the California gnatcatcher's range in northeastern Baja California. Until 1988, the California gnatcatcher was considered to be a subspecies of the black-tailed gnatcatcher. Atwood (1988). The two species were split apart on the basis of the amount of white in their tail feathers and differences in vocalizations. These results were corroborated with mitochondrial DNA (mtDNA) sequence data. Zink & Blackwell (1998).

Up to five *subspecies* of California gnatcatcher have been described on the basis of the distributions of varying morphological characteristics (such as plumage color and tail feather length). All subspecies have varying degrees of overlap for each trait. The listing of the northernmost subspecies—the

P.c.c.—was based on early summaries of morphology by Miller et al. (1957), although no quantitative statistical analyses were done. Dr. Atwood included more specimens and applied modern quantitative analyses and concluded that the *P.c.c.* was a distinct subspecies, although his taxonomic boundaries were fluid. In 1988, Atwood described two subspecies of California gnatcatcher, with the southern boundary of the *P.c.c.* at approximately 25°N in Baja California. In 1990 and 1991, Atwood reported that there were actually three subspecies, a conclusion based on his reanalysis of data from his 1988 paper. Mellink & Rea (1994) further subdivided the *P.c.c.* into two subspecies (*P. c. californica and P. c. atwoodi*), with their boundary near the United States–Mexico border. This addition brought the total number of California gnatcatcher subspecies to five, although no more than three have ever been recognized by a single author.

Of interest is the geographic placement of subspecies boundaries. Miller et al. (1957) and Atwood (1991) suggested that the southern boundary of the *P.c.c.* was about 30°N, and Mellink & Rea (1994) placed it at about 32.5°N. No quantitative assessments of the differences in the southern boundary have been published, but obviously the different schemes result in different amounts of area being occupied by the *P.c.c.* That fact has implications for any management plan. The southernmost boundary used by the Service in the *P.c.c.* listing is 30°N, in the vicinity of El Rosario, Baja California. As explained in further detail below, setting the southernmost

boundary of the *P.c.c.* at 30°N was fundamental to the listing of the *P.c.c.* because gnatcatchers are a common bird in the Baja California Peninsula south of 30°N.

B. The 1993 Listing of the Coastal California Gnatcatcher: The Service Relies on Disputed Morphological Data To List a Subspecies

Because of Dr. Atwood's change in position on the number of gnatcatcher subspecies (from two subspecies to three subspecies) and on the range of the *P.c.c.*, many members of the public requested the Service and Atwood to provide an opportunity for public review of the morphological data that were the subject of his 1988 and 1991 papers. Despite repeated requests, Dr. Atwood declined to make the data available, claiming that they were "proprietary." For its part, the Service refused to demand that Atwood make the data available to the public during the listing process. The Service took the position that the Service could rely on Atwood's 1991 published paper and that the public had no right to review the data underlying Atwood's taxonomic conclusions.

In March, 1993, the Service listed the *P.c.c.* as a subspecies of *Polioptila californica* based largely on morphological data generated by Dr. Atwood. 58 Fed. Reg. 16,742 (Mar. 30, 1993). During the listing process, Dr. Barrowclough of the American Museum of Natural History and other scientists testified that the morphological data reported by Atwood did not

support a conclusion that the *P.c.c.* was a distinct subspecies. These scientists suggested that a genetic study should be conducted to resolve the serious questions that had been raised concerning the morphological data. The scientists testified that any morphological differences between gnatcatchers north and south of 30°N latitude could be explained by the aged condition of specimens (given that feather coloration fades over time, such that two groups of individuals sampled from the same place 50 years apart would appear to differ), technical problems with plumage color measuring devices, and environmental (not genetic) causes of color differences. In any event, the scientists explained that, because of recent scientific advance in analysis of genetic material, the Service's reliance on morphological measurements from museum specimens (some of which were 100 years old) did not constitute the best scientific data available.

These scientists presented scientific evidence that documented that the *P.c.c.* is not a separate subspecies because it is not taxonomically distinct from the estimated two million gnatcatchers in Baja California, south of El Rosario. Nevertheless, the Service dismissed the opinion of these nationally recognized scientists and elected instead to rely on the views of Dr. Atwood and others—despite the fact that Atwood had only a few years before reached diametrically contradictory conclusions regarding whether gnatcatchers in southern California and northern Baja California were a distinct subspecies.

C. Litigation Regarding the 1993 Listing and Court-Ordered Release of Dr. Atwood's Morphological Data; Deposition of Dr. Atwood

Shortly after the listing, public agencies and private parties filed a lawsuit challenging the Service's action. The lawsuit argued that the Administrative Procedure Act and the ESA required the Service to make Dr. Atwood's morphological data available for public review. In June, 1994, the United States District Court for the District of Columbia determined that the Service had violated the Administrative Procedure Act and the ESA in refusing to provide the public with an opportunity to review and comment on Atwood's morphological data. *Endangered Species Comm. v. Babbitt*, 852 F. Supp. 32 (D.D.C. 1994). The court noted that, in listing the *P.c.c.*, the Service had before it "a report by an author, who two years before had analyzed the same data and had come to an opposite conclusion." *Id*. at 37. Accordingly, "[w]here, as in this case, the underlying data from such a critical and disputed report is readily available to the [Service]," the agency must "make the data available to those interested parties from whom the Service sought comment." *Id*. By failing to make the data available, the Service deprived these interested parties of "important and material information from which they could make meaningful analysis in order to provide their views to the [Service]." *Id*. The court subsequently ordered the Service to obtain and release the morphological data and also ordered the deposition of Atwood.

The production of these materials opened yet another bizarre phase of this saga. The data released by Dr. Atwood raised new questions regarding the Service's reliance on his morphological data. The deposition of Atwood revealed, among other things, that he had not maintained the raw data on which his 1988 and 1991 studies had been based. *See* Deposition of Jonathan Lee Atwood, Nov. 14 - 16, 1994, vols. I at 33, 36, *Endangered Species Comm. v. Babbitt*, Doc. No. 92-2610 (SS) (D.D.C.), Exh. B ("Atwood Depo.") ("Q. With regard to those morphological measurements, when you measured museum specimens or specimens in the field, how did you manually record them? A. To the best of my remembrance, the majority were recorded on sheets of paper. . . . Q. And did you produce those sheets of paper today? A. They are no longer in existence.").

Dr. Atwood testified that he had transferred his raw data into tabulated summaries. Atwood also testified that his 1991 conclusions were based on the results of a statistical technique called a "UPGMA cluster analysis." Two independent sets of scientists were unable to replicate Atwood's cluster analysis using the Atwood data set. Atwood acknowledged that the UPGMA test would cluster sampling areas even in the absence of a step (*i.e.*, change) in morphological characteristics. Dr. Barrowclough testified that by itself "clustering should never be used to look for subspecies."

Dr. Atwood testified that the only morphological variables in his 1991 paper that exhibited any step at approximately 30°N were brightness of breast

feathers (BRSTB) and purity of back feathers (BACKP). When the data for these characteristics were plotted for all specimens in the data set, there was no "sharp step" at 30°N as claimed by Atwood in his listing petition.

Moreover, in a 1994 letter to the Service, Dr. Atwood confessed "serious doubts" about the accuracy of his own color measurements because of technical problems with the spectrophotometer (a device used by paint stores to classify paint colors) that he utilized to measure plumage color. Atwood Depo. v. II at 58. While the use of this device was common prior to the availability of genetic analysis, it is well known that the older machines such as used by Atwood were difficult to calibrate. One of the known problems is that the operator of the device obtains a different measurement when the bird specimen is placed in the portal because the feathers are not uniform and can shift. In Atwood's 1991 paper, data derived from the device accounted for six of the variables that showed the greatest "step" at 30°N. These data were used to determine the two key color characteristics—brightness of breast feathers and purity of back plumage—that Dr. Atwood cited as most important to distinguish the coastal California gnatcatcher.

Mellink and Rea (1994) criticized the use of the spectrophotometer because of the difficulty in getting consistent readings from the device. Atwood Depo. v. II at 57. Dr. Atwood stated that the data derived from spectrophotometer readings could not be relied upon because he was not able

to replicate the results underlying his 1991 paper. Atwood Depo. v. II at 59. Atwood also testified that with regard to purity of back color (dark plumage), another of the key characteristics relied upon to support his subspecies delineation, there "is apparently so much variation in the data associated with that value that it really does not seem to have any clear indication of any steps anywhere." Atwood Depo. v. III at 124. Dr. Barrowclough (1994) pointed out that Atwood (1991) displayed only the means and standard errors for his distinguishing morphological characters. This gave the appearance that the magnitude of differences was greater (breaks) than they actually were. When Barrowclough (1994) and McDonald et al. (1994) plotted all of the data points by latitude, they found no distinct breaks. Overlap in plumage characteristics was substantial and variation was clinal. Moreover, Atwood never considered the effect of age on feather color despite the fact that it is a well-documented phenomenon.

Throughout the lengthy dispute on the gnatcatcher's listing, the Service zealously defended Dr. Atwood's assertion that the *P.c.c.* was a valid subspecies and was genetically distinct from the two million gnatcatchers in central and southern Baja California. The Service first refused requests for public review and comment of the Atwood morphological data, claiming that the ESA and Administrative Procedure Act (APA) did not require public

access to the underlying taxonomic data. After the district court invalidated the listing and ordered the data to be made available, the Service continued to defend Atwood's subspecies claims. The Service summarily dismissed detailed analyses prepared by highly respected academic scientists (Drs. Barrowclough, Skalski, McDonald, and others) that indicated that analyses of the morphological data did not support Atwood's claim that the *P.c.c.* was a distinct subspecies. The Service rejected these criticisms partly because the Barrowclough, Skalski, and McDonald analyses at that time had not been published in a scientific journal. The Service also rejected requests that it conduct a genetic study to resolve the gnatcatcher taxonomic issues. Rather, the Service decided that it should rely on published studies of gnatcatcher morphology to conclude that the *P.c.c.* was a valid subspecies.

In 1995, notwithstanding the serious shortcomings with Dr. Atwood's work (including Atwood's own "serious doubts" about the accuracy of his key data), the Service reaffirmed the listing, finding that Atwood's work was still reliable. *See* 60 Fed. Reg. at 15,694-97. The Service also explained that the conclusion of Atwood's 1991 study—the *P.c.c.* constitutes its own subspecies with a southern range terminating at 30°N—was generally consistent with other gnatcatcher morphology studies from the twentieth century. *See id.* at 15,698. The Service acknowledged, however, that this taxonomic work was

not definitive, and suggested that additional research might support a different conclusion. *See id.* at 15,699.

D. The 2000 Zink, Barrowclough, Atwood, and Blackwell-Rago Analysis of Mitochondrial DNA Indicates That the Coastal California Gnatcatcher Is Not a Distinct Subspecies

Taking the cue from the Service's acknowledgment of the need for a genetic analysis, Dr. Zink spearheaded a new study that would focus not on gnatcatcher morphology but rather on the bird's mitochondrial DNA (mtDNA). R.M. Zink, G.F. Barrowclough, J.L. Atwood, and R.C. Blackwell-Rago. 2000. Genetics, Taxonomy, and Conservation of the Threatened California Gnatcatcher. *Conservation Biology* 14:1394-1405. [hereinafter Zink et al. (2000)] (Exh. C). This different approach was notable because mtDNA analysis leaves substantially less room for guesswork, judgment, and human error than morphological analysis standing alone. Skalski (2008). For example, measuring small body parts is prone to error, which if not accounted for statistically, seriously undermines morphological studies. Rojas-Soto (2003). In the past three decades, thousands of mtDNA studies have been published and applied to conservation questions. Avise (2000). In birds, it has been found that the average species has two genetically distinct mtDNA evolutionary units embedded within it, each of which could be considered independently in taxonomic, evolutionary, or conservation analyses. However, a species typically has more subspecies than mtDNA

groups. Zink (2004); Phillimore & Owens (2006). In fact, fewer than 10% of avian subspecies have been shown to be genetically distinct in their mtDNA. This has led most ornithologists to acknowledge that subspecies are not genetically or evolutionarily distinct, but instead are designations based on arbitrary or subjective divisions of gradual morphological gradients. Undue reliance on one or two morphological features while ignoring the remaining characters leads to further arbitrariness. Cronin (1997); Cronin (2006). In contrast, the mtDNA method is very sensitive, often discovers patterns of genetic diversity not apparent from subspecies classifications, and only rarely supports particular subspecies. Importantly, given the twofold increase in taxonomic diversity revealed by mtDNA analysis of *species*, the failure to discover mtDNA diversity within a species is a strong sign that no significant historical divisions exist that would otherwise support subspecies classifications. Avise (2000); Zink & Barrowclough (2008).

Perhaps unsurprisingly, Dr. Zink's 2000 study (in which Dr. Atwood was a co-author) found no abrupt change in gnatcatcher mtDNA characters at 25°N, 30°N, or any other latitude. Instead, the genetic change was gradual. Zink et al. (2000). Consequently, the study concluded that there is no mtDNA basis to support a subspecies classification for the California gnatcatcher.

Because gaps in sampling can mimic genetic gaps, Zink et al. (2000) drew on equally spaced sample populations throughout the range of the gnatcatcher and used direct sequencing of mtDNA to investigate genetic

distinctiveness. The study's authors concluded that haplotypes (*i.e.*, individually distinct mtDNA sequences) did not form exclusive clusters that conformed to recognized subspecies or to any other geographically restricted area. Based on the mtDNA, the authors found that "northern populations [of *Polioptila californica*] do not appear to constitute a unique component of gnatcatcher diversity." Zink et al. (2000). Therefore, Zink et al. (2000) concluded that no genetic distinction exists between the southern California populations of *Polioptila californica* and the flourishing *Polioptila californica* populations found south of the U.S.–Mexico border.

E. The 2010 Delisting Petition

In light of the Zink et al. (2000) study, the Service began to question the validity of the subspecies classifications of all prior authors, including Dr. Atwood. In 2003, perhaps in an effort to neutralize the study's impacts, the Service proposed to reclassify the *P.c.c.* as a distinct population segment of the California gnatcatcher species.[2] *See* 68 Fed. Reg. at 20,228. *Cf.* 16 U.S.C. § 1532(16) (defining "species" to include "any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when

[2] Remarkably, within *days* of the announcement of Zink et al. (2000), the Service stated in a press release that "the coastal California gnatcatcher would likely remain listed as a distinct population segment, even if scientific opinion eventually determines that its subspecies status is in question." Prior to the Service's press release, the Service had never suggested that the *P.c.c.* constitutes a distinct population segment.

mature"). As part of this proposed reclassification process, the Service, in 2004, convened a panel of gnatcatcher experts to review the bird's taxonomy. The panel concluded that, although Zink et al. (2000) cast doubt on prior gnatcatcher taxonomic work, the pre-existing morphological analyses (including Atwood's) were substantial enough that more genetic work had to be done before a change in taxonomy would be warranted. *See* 76 Fed. Reg. at 66,257-58 (discussing Eric Vander Werf, California Gnatcatcher Taxonomy Exercise (Dec. 1, 2004)).

A 2008 study, led by Professor John Skalski of the University of Washington, produced a rigorous statistical re-analysis of Atwood's 1988 and 1991 studies, and concluded that the published Atwood data do not support the existence of gnatcatcher subspecies. *See* John R. Skalski, R.L. Townsend, L.L. McDonald, J.W. Kern, and J.J. Millspaugh. 2008. Type I Errors Linked to Faulty Statistical Analyses of endangered subspecies classifications. *Journal of Agricultural, Biological, and Environmental Statistics* 13:199-220. Skalski et al. (2008) suggested that Atwood's analyses, based on what were then standard off-the-shelf statistical packages, produced a high rate of false positives, a conclusion that, applied to the *P.c.c.*, would undercut its subspecies listing. The study explained that Atwood's data, properly analyzed, revealed a geographic cline (*i.e.*, a gradual change over geography), not distinct breaks in morphological characters that would support a subspecies classification. Along with other critics, the study observed that the Atwood

raw data were very probably confounded by the age of specimens analyzed. *See id.* at 206.

Relying on Zink et al. (2000) and Skalski et al. (2008), a coalition of property owners and other groups concerned about the negative economic impacts of defective ESA listings (including two of the Petitioners here) petitioned the Service in 2010 to delist the *P.c.c.*

F. The Service Rejects the 2010 Delisting Petition and Continues To Rely on Contested Morphological Data

On October 26, 2011, the Service denied the petition to delist the *P.c.c.*[3] 76 Fed. Reg. 66,255. Relying on its 2004 taxonomy review and a 2010 status review, the Service determined that the Zink analysis, although probative, was not decisive. *See id.* at 66,258. The Service strongly suggested that mtDNA analysis, standing alone, is insufficient to overturn the gnatcatcher's subspecies classification, and that a nuclear DNA analysis should be done. *Id.* The Service explained, based on Edwards et al. (2005), that "nuclear genes, not mtDNA, should have priority in determining avian species delimitation." *Id*. Relying on other studies, the Service suggested that "the best approach for subspecies recognition is to include multiple characters (mtDNA, nuclear DNA, morphology) and that reliance on a single locus with unique properties,

[3] In addition to affirming the subspecies classification, the Service withdrew its 2003 proposal to designate the *P.c.c.* as a distinct population segment. *See* 76 Fed. Reg. at 66,260.

such as mtDNA, may not accurately reflect the genetic differences among populations due to random genetic effects." *Id.*

The Service overlooked the fact that Edwards et al. (2005) was concerned with delimiting species—not subspecies—and thereby the agency failed to acknowledge a huge wealth of evidence in which mtDNA provided useful tests for subspecies designation. In fact, very few mtDNA studies have failed to detect divisions (*e.g.*, subspecies) within species where a nuclear DNA analysis would. Zink & Barrowclough (2008).[4]

With respect to statistics, the Service acknowledged that the Skalski study had revealed statistical shortcomings with Dr. Atwood's work, but the agency found the study inadequate because it considered only one of Atwood's morphological characters. 76 Fed. Reg. at 66,259.

Finally, although the Service acknowledged the possibility that Dr. Atwood's data and his analysis of the data set suffered from some of the problems identified above, the agency pointed to subsequent morphological re-analyses that, taking account of the risk of changes in the color of museums due to the age of the specimen, seemed to confirm Atwood's subspecies conclusion. *Id.*

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[4] Presumably, the Service dismissed the significance of Zink et al. (2000) and similar mtDNA studies because they provide "negative" evidence, *i.e.*, they can demonstrate the absence of a genetic structure that would otherwise be expected in a species containing historical divisions indicative of subspecies classification.

In summary, the Service's 2010 delisting denial affirmed the *P.c.c.*'s subspecies status based on measurement of morphological characteristics collected from museum specimens (some of which were 100-years old), despite (1) the availability of mtDNA concluding that there were no distinct subspecies of *Polioptila californica*, and (2) Dr. Atwood's acknowledgment that he had "serious doubts" about the accuracy of several of the measurements that were key to the delineation of the *P.c.c.* as a subspecies with a southern range limit at 30°N. That the Service would not acknowledge mtDNA as the best scientific data is particularly noteworthy given the Service's and the National Marine Fisheries Service's reliance on mtDNA in other regulatory decisions under the ESA. Indeed, on more than 80 occasions, the Services have relied on mtDNA evidence to make listing determinations under the ESA. *See* Exh. D.

NEW GENETIC ANALYSIS CONFIRMS THAT THE COASTAL CALIFORNIA GNATCATCHER IS A NOT A VALID SUBSPECIES

A. The Service Is Required To Delist a Species Where the Best Data Available Show That the Original Listing Was in Error

The Service's regulations provide that a listed entity must be delisted if the best scientific and commercial data available show that the original listing was in error. 50 C.F.R. § 424.11(d)(3). The Service's regulations also

provide that taxonomic determinations must be based on "standard taxonomic distinctions and the biological expertise of the Department [of Interior] and the scientific community." *Id.* § 424.11(a). As noted above, the Service has suggested that "the best approach for subspecies recognition is to include multiple characters (mtDNA, nuclear DNA, morphology)." 76 Fed. Reg. at 66,258. Under the Service's own approach, the best available data dictate that the *P.c.c.* should be delisted.

B. Zink et al. (2000) and Zink et al. (2013) Constitute the Best Available Scientific Data

In 1993, the Service listed the gnatcatcher as a threatened subspecies, relying largely on the morphological and statistical research of Dr. Atwood. Atwood's work has for long been the subject of intense scientific debate, as the Service itself has admitted. *See* 76 Fed. Reg. at 66,258 ("We acknowledge that the taxonomic classification of the coastal California gnatcatcher has been the subject of considerable scientific debate."). Zink et al. (2000), in which Atwood himself was a co-author, concluded that, "based on mtDNA data, northern populations [of gnatcatcher] do not appear to constitute a unique component of gnatcatcher diversity." Zink et al. (2000). That study also noted that, based on the mtDNA analysis, "there probably is no general pattern of variation in morphological characteristics consistent with historical isolation and independent evolution of populations." The latest study of Professor Zink

and his colleagues, which focuses on nuclear DNA, provides the called-for genetic data. The study responds directly to the Service's position that analyses of nuclear genes is required to corroborate the mtDNA results.

Zink et al. (2013) conducted a genetic analysis using eight different markers (nuclear loci) and a somewhat reduced data set from Zink et al. (2000). *See* Zink et al. (2013). "Analysis of [the] nuclear loci . . . identified no geographic groupings that corresponded with any previously suggested subspecies, nor any other significant evolutionary divisions." Rather, the nuclear DNA analysis was consistent with the conclusion that the gnatcatcher has relatively recently expanded from a southern home base. After discussing the results of Zink et al. (2000) and Skalski et al. (2008), the study concluded that "the California Gnatcatcher is not divisible into discrete, listable units." *Id.* at 456.

Zink et al. (2013) also presents the results of an ecological niche analysis. As the study explains, "quantitative tests of niche divergence can show whether a population is ecologically distinct" by distinguishing between populations that "harbor evolved ecological adaptations" and those "that simply reflect a generalist ecological strategy." In other words, "niche modeling provides a basis for making quantitative assessments of ecological differentiation in a hypothesis-testing framework." Such assessments can help determine whether, regardless of genetics or morphology, a given population exhibits "significantly different niche characteristics" such that listing as a

distinct population segment might be warranted. Hence, niche analysis "provides a more complete perspective on threatened and endangered species in the context of their preservation."

Zink et al. (2013) concludes that, "[a]lthough the [gnatcatcher] species . . . in the north occupies the distinctive [coastal sage scrub] habitat, . . . the two groups [*i.e.*, northern and southern gnatcatchers] do not exhibit significant niche divergence if the backgrounds of each environment are taken into account." To be sure, the study acknowledges that "the methods for testing niche divergence are in a relatively early stage and that the test is only as good as the models and input data." Nevertheless, the study concludes that ecological distinction does not provide a basis for any taxonomic subdivision of the California gnatcatcher species.

Zink et al. (2013) presents an important test of the ESA command that the Service use the best available scientific data in listing determinations. In rejecting the 2010 petition and the Zink et al. (2000) mtDNA study on which the petition was mainly based, the Service suggested that the mtDNA evidence reported in Zink et al. (2000) needed to be supplemented with an analysis of nuclear genes. Zink et al. (2013) provides precisely the data set that the Service acknowledged "should have priority" in avian taxonomy.

In rejecting Zink et al. (2000), the Service chose instead to rely on taxonomic classifications that were all based on morphological data. None of

these prior morphological classifications (some of which dated to 1922) used modern genetic analysis. Morphological characteristics are, at best, an indirect measure of underlying genetic variation among populations. Using morphology to identify subspecies of gnatcatchers fundamentally rests on numerous assumptions, such as: (1) the measurements of the plumage color of dated museum specimens is representative of birds in the wild; (2) the device used to measure the differences in plumage color can produce consistent, repeatable results; (3) the variations in morphology do not reflect environmental influences such as food sources or climate; (4) the variations are sufficiently substantial that they indicate a sharp "break" or "step"; and (5) the morphological variations are the product of genetic differentiation among populations of gnatcatchers. In the case of the morphological data purportedly supporting the validity of the *P.c.c.* listing, there is continuing disagreement regarding all of the above issues. It is noteworthy that no reanalysis of Dr. Atwood's morphological data has supported his original subspecies boundaries.

The extensive scientific controversy over the use of gnatcatcher morphology to list the *P.c.c.* as a threatened subspecies vividly illustrates the problems associated with the Service's continued reliance on analysis of gnatcatcher morphology. This is particularly the case where a robust analysis of both mtDNA and nuclear DNA exists to evaluate directly genetic differences among gnatcatcher populations. In fact, the reanalysis of

morphological data, mtDNA data, nuclear gene data, and ecological niche modeling (Zink et al. (2013)) are remarkably consistent in their unified support of the lack of subspecies in the California gnatcatcher. Given the dramatic advances in genetic analysis in the last two decades, it is no longer legally or scientifically defensible for the Service to continue to rely on measurements of such characteristics as brightness of breast feathers and purity of back feathers from differently aged museum specimens to determine whether the *P.c.c.* is a valid subspecies. The best available data agree that the California gnatcatcher is not divisible into discrete, listable units, but instead is a single historical entity throughout its geographic range. Therefore, there is no scientific basis for listing any part of the range under the ESA.

CONCLUSION

As the foregoing makes clear, the listing of the *P.c.c.* was and is in error because the best available data actually did not, and currently do not, support a subspecies classification that could serve as a basis for listing under the ESA. The Service has persisted in its subspecies classification based on the morphological work of Dr. Atwood. Yet not only have published studies cast serious doubt on the value of that morphological work (*see, e.g.*, Skalski et al. (2008)), two genetic studies analyzing both mtDNA and nuclear DNA have concluded that subspecies classification is not justified.

The Service rejected the 2010 delisting petition largely based on its claim that mtDNA results reported in Zink et al. (2000) needed to be

supplemented with an analysis of nuclear DNA. Zink et al. (2013) provides the analysis of nuclear DNA suggested by the Service and also includes an analysis of niche divergence. It is now time for the Service to act. Based on the best available scientific data, the *P.c.c.* should be delisted.

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Respectfully submitted,

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EXHIBIT A

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PHYLOGEOGRAPHY OF THE CALIFORNIA GNATCATCHER (*POLIOPTILA CALIFORNICA*) USING MULTILOCUS DNA SEQUENCES AND ECOLOGICAL NICHE MODELING: IMPLICATIONS FOR CONSERVATION

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Abstract.—An important step in conservation is to identify whether threatened populations are evolutionarily discrete and significant to the species. A prior mitochondrial DNA (mtDNA) phylogeographic study of the California Gnatcatcher (*Polioptila californica*) revealed no geographic structure and, thus, did not support the subspecies validity of the threatened coastal California Gnatcatcher (*P. c. californica*). The U.S. Fish and Wildlife Service concluded that mtDNA data alone were insufficient to test subspecies taxonomy. We sequenced eight nuclear loci to search for historically discrete groupings that might have been missed by the mtDNA study (which we confirmed with new ND2 sequences). Phylogenetic analyses of the nuclear loci revealed no historically significant groupings and a low level of divergence (G_{ST} = 0.013). Sequence data suggested an older population increase in southern populations, consistent with niche modeling that suggested a northward range expansion following the Last Glacial Maximum (LGM). The signal of population increase was most evident in the mtDNA data, revealing the importance of including loci with short coalescence times. The threatened subspecies inhabits the distinctive Coastal Sage Scrub ecosystem, which might indicate ecological differentiation, but a test of niche divergence was insignificant. The best available genetic, morphological, and ecological data indicate a southward population displacement during the LGM followed by northward range expansion, without the occurrence of significant isolating barriers having led to the existence of evolutionarily discrete subspecies or distinct population segments that would qualify as listable units under the Endangered Species Act. *Received 19 December 2012, accepted 19 April 2013.*

Key words: California Gnatcatcher, ecological niche modeling, Endangered Species Act, mitochondrial DNA, nuclear DNA, phylogeography, *Polioptila californica*, subspecies.

Filogeografía de *Polioptila californica* basada en Secuencias de ADN Multilocus y Modelamiento de Nicho Ecológico: Implicaciones para su Conservación

Resumen.—Un paso importante en la conservación es identificar si las poblaciones amenazadas son discretas evolutivamente y significativas para la especie. Un estudio filogeográfico previo con ADN mitocondrial (ADNmt) de *Polioptila californica* reveló la ausencia de estructura geográfica, por lo cual no sustentó la validez como subespecie de la población costera amenazada (*P. c. californica*). El Servicio de Pesca y Vida Silvestre de los Estados Unios concluyó que los datos de ADNmt por sí solos no eran suficientes para evaluar la taxonomía a nivel de subespecies. Secuenciamos ocho loci nucleares para buscar agrupamientos históricos discretos que podrían haber sido pasados por alto en el estudio con ADNmt, el cual confirmamos con nuevas secuencias del gen ND2. Los análisis filogenéticos de los loci nucleares indicaron que no existen agrupamientos históricos significativos y que hay un bajo nivel de divergencia (G_{ST} = 0.013). Los datos de secuencias sugieren un incremento antiguo en el tamaño de las poblaciones del sur, lo que concuerda con los modelos de nicho que sugieren una expansión de la distribución hacia el norte después del último máximo glacial La señal de incremento poblacional fue más evidente en los datos de ADNmt, lo que demuestra la importancia de considerar loci con tiempos de coalescencia cortos. La subespecie amenazada habita el ecosistema de matorral costero, lo que puede indicar diferenciación ecológica, pero una prueba de divergencia de nicho no fue significativa. Los mejores datos genéticos, morfológicos y ecológicos disponibles indican un desplazamiento poblacional hacia el sur durante el último máximo glacial, seguido por una expansión hacia el norte, sin la aparición de barreras significativas que pudieran llevar a la existencia de subespecies discretas o de segmentos de poblaciones distintos que pudieran ser aprobados como unidades incluidas en el Acta de Especies Amenazadas.

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Plans to list populations and species under the U.S. Endangered Species Act (ESA) or under similar legislation in other countries can be informed by several types of information. Morphological studies, especially those focused on subspecies taxonomy, suggest hypotheses for evaluating whether different parts of a species' range are evolutionarily independent. Modern genetic studies can reveal whether populations or subspecies are evolutionarily discrete or significant in relation to the species as a whole and document levels and pathways of historical or ongoing gene flow. Furthermore, genetic data can show whether particular populations are lacking in genetic variability owing to inbreeding or bottlenecks and whether populations have undergone recent range and population increases. Various genetic markers exist, each suited to answering different questions about threatened species (Brito and Edwards 2009). For example, recent restrictions to gene flow might best be revealed by analyzing microsatellite allele frequencies (Barr et al. 2008). Major evolutionary divisions are perhaps best identified by analysis of mitochondrial DNA (mtDNA) or nuclear DNA sequences (Zink and Barrowclough 2008).

Although it has received less attention than morphological and genetic data, quantitative tests of niche divergence can show whether a population is ecologically distinct. Threatened populations that occur in different habitats present a concern for conservation biologists because it must be determined whether such populations harbor evolved ecological adaptations or simply reflect a generalist ecological strategy. Short of long-term, costly, and logistically difficult cross-fostering or common garden experiments, ecological niche modeling provides a basis for making quantitative assessments of ecological differentiation in a hypothesis-testing framework (Warren et al. 2010). If populations from different areas have significantly different niche characteristics, one might conclude that the populations qualify as a distinct population segment under the ESA (U.S. Fish and Wildlife Service [USFWS] and National Marine Fisheries Service 1996), irrespective of whether they were also genetically or morphologically discrete. Information from multiple sources provides a more complete perspective on threatened and endangered species in the context of their preservation (May et al. 2011).

We assessed the genetic and ecological distinctiveness of the subspecies of the California Gnatcatcher (*Polioptila californica*). The California Gnatcatcher is a small nonmigratory songbird that ranges from Los Angeles, California, to the southern tip of the Baja California peninsula (Atwood and Bontrager 2001). The species occupies coastal sage scrub (CSS) in the northern portion of its range; to the south of the CSS, the habitat occupied is typical of the drier Sonoran Desert and population density is much higher. Three independent taxonomic studies based on morphology supported a subspecies in the CSS, although the proposals differed in the geographic locations of taxonomic boundaries (Miller et al. 1957, Atwood 1991, Mellink and Rea 1994). The northern, coastal subspecies (*P. c. californica*) is listed as threatened under the ESA (USFWS 1993) because of habitat loss and fragmentation and concomitant population declines. The California Gnatcatcher has served as a flagship species for preservation of the highly fragmented CSS ecosystem (Atwood 1993). The enormous economic value of real estate in this area of already high and increasing human population density has led to scrutiny of the subspecies status of the coastal California Gnatcatcher and whether it should be listed under the ESA (Cronin 1997). Cronin (1997:663) stated that "I believe that subspecies designations of *P. californica* should be ignored until thorough phylogenetic analyses of genetically-based characters are done." The USFWS (2011:66255) concluded "that the coastal California Gnatcatcher constitutes a valid subspecies…."

Comparison of mtDNA sequences obtained throughout the range of the California Gnatcatcher revealed lower genetic diversity in the CSS but did "not support any subspecies scheme, either previously described [Fig. 1] or unforeseen" (Zink et al. 2000:1398). In the present study, we integrate new nuclear gene-sequence data with past studies of mtDNA (Zink et al. 2000) and morphology (Atwood 1991). In addition, we test for significant niche divergence (McCormack et al. 2010) as a proxy for ecological distinctiveness. Our goal is to obtain a more complete understanding of the species' recent history and to provide a perspective on its conservation from multiple sources of data.

Methods

Samples.—Samples (Table 1) were those used in Zink et al. (2000), although degradation of the DNA reduced the sample size. Some analyses were conducted on divisions of the sample localities into northern and southern, based on the pattern of nucleotide diversity found in Zink et al. (2000), with the dividing point at 28°N, between El Rosarito and San Ignacio (see Fig. 2). Exact sample sizes per locus per locality can be obtained from the GenBank accessions (KC863990–864745).

Genetic analyses.—We sequenced seven introns and one exon (Table 2) using primers from Backström et al. (2008) and Kimball et al. (2009). In addition, as a check on the mtDNA control-region sequences, and for calibrating population size changes, we sequenced 43 individuals for the mitochondrial gene ND2 (23 individuals from the north, 20 from the south, with one sequence of the sister taxon, *P. melanura*, as an outgroup); these data were analyzed with ARLEQUIN (Excoffier et al. 2005). From initial sequencing runs, we determined whether each nuclear sequence included two or more heterozygous sites; all such sequences were phased into component alleles using specially constructed polymerase-chain-reaction primers that would anneal with only one of the two alternate bases at the most 5′ heterozygous site. The resulting sequence allowed us to determine the sequence of one of the two alleles, and the other allele was inferred by subtracting this allele from the ambiguous sites. For each of the seven introns and one exon, we computed the number of gene copies sequenced, the number of sequenced base pairs, the number of alleles observed, nucleotide diversity for each population sample, and the G_{ST} statistic of Holsinger and Mason-Gamer (1996). We also computed the mean sample size and nucleotide diversity, with bootstrap confidence intervals, over loci for each population. We computed G_{ST} across loci among all populations and among populations with sample sizes >4, along with bootstrap confidence intervals. These statistics were computed using software written by G.F.B.

For each locus, we used a four-gamete test to determine the longest fragment of DNA consistent with no recombination (Hudson and Kaplan 1985), using PAUP*, version 4.0b10 (Swofford 1998). We constructed minimum spanning networks of alleles for those fragments using PAUP* and computed the frequency of

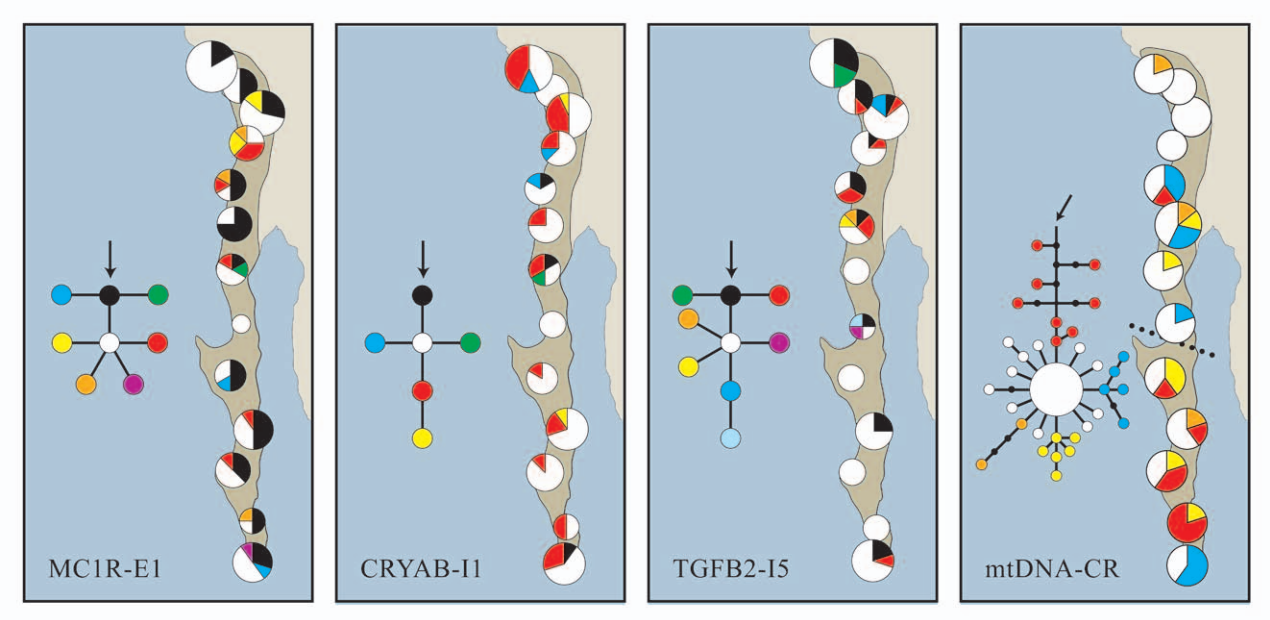

FIG. 1. Examples of patterns of geographic variation at three nuclear loci and the mitochondrial DNA control region of California Gnatcatchers (from Zink et al. 2000). Arrows indicate the root of the network derived from phylogenetic analysis using *P. melanura* as the outgroup. Darker shading indicates the range of the California Gnatcatcher. Names of sample locations are given in Figure 2 and Zink et al. (2000). Numbers of individuals per sample are given in Table 1 and in GenBank entries. The dotted line (far right) indicates the division between northern and southern localities based on the pattern of nucleotide diversity discovered in mtDNA.

each of the alleles at each sampling locality to show the geographic deployment of genetic variation. We used the Hudson-Kreitman-Aguade (HKA) test (Hudson et al. 1987) implemented in the HKA program (see Acknowledgments; Hey 2004) to test for departures from neutrality in the ND2 and all nuclear loci and alleles simultaneously (using *P. melanura* as an outgroup) by employing 10,000 coalescent simulations to assess statistical significance.

We used the program STRUCTURE (Pritchard et al. 2000) to determine the number of groups based on the nuclear DNA alleles, as determined above. In brief, this program considers variation among loci simultaneously and attempts to determine whether there is more than one genetically distinct group represented in the sample. The seven variable nuclear loci were formatted as single-nucleotide polymorphisms (SNP; see Manthey et al. 2011). The STRUCTURE analyses assumed an admixture model, correlated allele frequencies, and a fixed lambda value (which was inferred by setting $K = 1$ and allowing lambda to be estimated in an initial analysis). We analyzed the data for $K = 1$ to 6 with five replicates for each value of K. Each run contained 100,000 steps as burn-in, followed by 500,000 steps. The ΔK was calculated ad hoc (Evanno et al. 2005) and used to identify the best estimate of K.

We used the extended Bayesian skyline plot (EBSP) method (Heled and Drummond 2008) implemented in BEAST, version 1.7.4 (Drummond et al. 2012), to estimate changes in population size through time with multiple loci in a coalescence-based framework (Heled and Drummond 2008). We applied the EBSP analysis to the nuclear plus mitochondrial ND2 sequences for individuals

TABLE 1. Nucleotide diversity (π) in California Gnatcatchers over seven nuclear loci. Localities are shown in Figures 1 and 2.

	Los Angeles County	Riverside County	Orange County	San Diego County	Punta Banda	San Telmo	Misión San Fernando
$n_{maximum}$	18	8	14	8	8	8	8
$n_{average}$	16.3	7.3	12.9	7.7	6.6	8.0	6.0
π	0.0022	0.0013	0.0027	0.0029	0.0034	0.0031	0.0026
95% CI	0.0012–0.0045	0.0008–0.0018	0.0012–0.0057	0.0015–0.0062	0.0017–0.0068	0.0014–0.0063	0.0012–0.0049
	El Rosarito	San Ignacio	Mulegé	Villa Insurgentes	La Paz	Cabo San Lucas	
$n_{maximum}$	4	8	10	8	6	10	
$n_{average}$	3.1	5.7	9.1	7.4	4.3	10.0	
π	0.0034	0.0028	0.0025	0.0027	0.0016	0.0023	
95% CI	0.0004–0.0090	0.0010–0.0061	0.0013–0.0054	0.0009–0.0057	0.0011–0.0020	0.0012–0.0044	

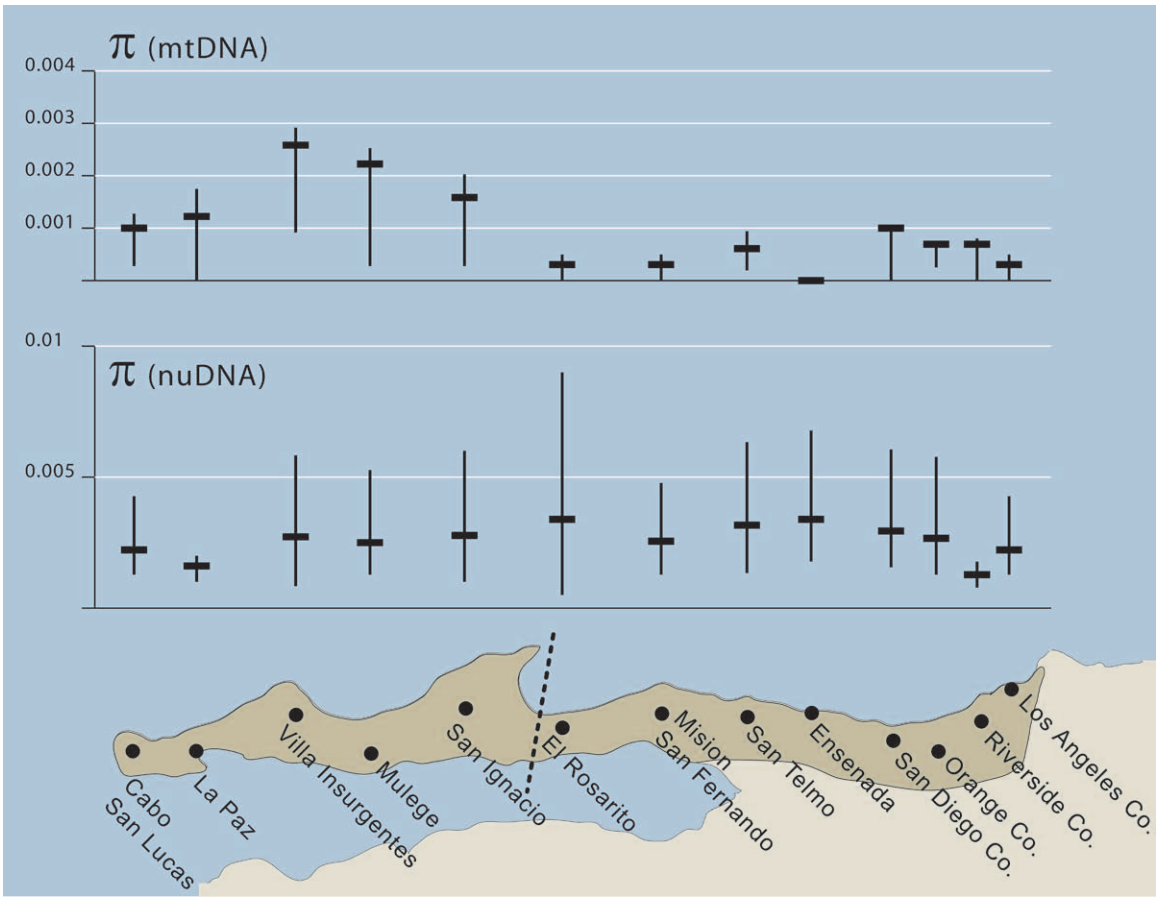

Fig. 2. Geographic distribution of nucleotide diversity (π) in mtDNA and seven pooled nuclear loci (nuDNA) in California Gnatcatchers. MtDNA data (top panel) suggest that populations north of 28°N were genetically less variable than populations to the south of the line, where mtDNA nucleotide diversity shifted from high (south) to low. The mtDNA pattern was not observed among the nuclear loci.

identified as northern or southern by Zink et al. (2000). The ND2 gene was set as the reference locus to calibrate time and population size with a substitution rate of 0.0125 site^{-1} lineage^{-1} Ma^{-1} (Smith and Klicka 2010) using a strict clock prior. By definition, strict clock rates do not incorporate error rates or confidence intervals (adding deviation estimates would convert such a rate into a relaxed clock, even for small ranges) and are the appropriate rates for the estimation of divergences in data sets with low variation (Brown and Yang 2011). The generation time was set at 1 year for small passerine birds. Although we acknowledge that 1 year is an estimate for this species in the absence of a detailed life table, juvenile males and females could start breeding in their first year (Atwood and Bontrager 2001). The EBSP analyses included a Markov chain Monte Carlo (MCMC) run of 100 million steps, sampled each 1,000 steps with a strict clock prior for the mitochondrial locus and relaxed exponential priors for the nuclear loci

Table 2. Gene locus characteristics in a population survey of California Gnatcatchers. [a] Note that MC1R is an exon, whereas all other loci are introns.

	ACON1-I15	CEPUS-I1	CRYAB-I1	bFIB-I7	MC1R	RHO-I1	TGFB2-I5	TROP-I5	CR/ND6
Chromosome [b]	Z	24	24	4	11	12	3	10	mtDNA
Number of base pairs	529	249	408	278	506	749	376	271	1399
Sample size [c]	82	82	82	76	86	92	74	84	47
Number of alleles [d]	7	8	6	6	8	8	9	1	16
Mean π	0.0011	0.0032	0.0016	0.0131	0.0016	0.0017	0.0015	0.0000	0.0010
G_{ST}	0.103	0.027	0.021	–0.076	–0.089	–0.073	0.244	n/a	0.018

[a] For 9 populations with mean $n > 4$.
[b] Based on *Gallus* and *Taeniopygia* genomes.
[c] Copies sequenced; 2N for autosomes, $2N_m + N_f$ for Z, N for mtDNA.
[d] Number of haplotypes for mtDNA.

to account for rate variation among different loci, and a linear demographic model. Trace plots were checked using TRACER, version 1.5 (Rambaut and Drummond 2007), to assess convergence in MCMC analyses. If the 95% high posterior density (HPD) of the estimate of the number of size-change steps (the parameter "demographic.populationSizeChanges") excluded zero, we concluded that a significant change in population size occurred (Lim and Sheldon 2011). To evaluate the contribution of the mtDNA data to the overall results, we repeated the EBSP analysis using only nuclear loci, to check for population increases at different relative times (e.g., excluding a temporal calibration).

Ecological analyses.—The occurrence of coastal California Gnatcatchers in the mesic CSS could indicate ecological differentiation sufficient for recognition as a distinct population segment. We constructed correlative ecological niche models (ENM; Peterson et al. 1999, Elith et al. 2011) using breeding records from the Breeding Bird Survey and the Global Biodiversity Information Facility (see Acknowledgments), which were input into MAXENT, version 3.2.2 (Phillips et al. 2006). We divided locality points into those representing CSS (n = 144) and southern populations (n = 48). Climatic data (19 layers) were obtained from the Worldclim bioclimatic database (Hijmans et al. 2005). Each ENM was based on the average of five MAXENT runs and plotted using DIVA-GIS, version 7.1.7.2 (Hijmans et al. 2004). Distribution maps were coded so that predicted presence or absence was based on the logistic threshold for equal training and testing specificity produced by MAXENT. We used the niche identity test and the background test for niche divergence in ENM Tools (Warren et al. 2010); random localities used in the test (Warren et al. 2008) were obtained from Hawth's Tools (Beyer 2004) in ARCGIS, version 9. We show results based on Schoener's D (Schoener 1968), which ranges from 0 to 1 (identical niches). We also estimated the distribution of suitable habitat for the California Gnatcatcher at the Last Glacial Maximum (LGM; 21,000 years ago) using all 192 distribution points.

RESULTS

Seven of the eight loci surveyed were variable, although each locus lacked a structured geographic pattern (Fig. 1 and Table 1), as did the ND2 sequences (F_{ST} = 0.021, P = 0.37). The locus MC1R has been associated with darker-colored phenotypes in some organisms (Baião et al. 2007); this locus also lacked geographic structure in California Gnatcatchers, despite the CSS populations being characterized as having somewhat darker plumage (Atwood 1991). Nucleotide diversity (π) did not differ across localities, unlike the result of the mtDNA study (Fig. 2) and for the new ND2 sequences (π = 0.0013 for north, 0.0030 for south). The pie charts in Figure 1 suggest a greater diversity of rare alleles in the north; however, the average sample size across loci was 67.9 in the north and 36.5 in the south (Table 1), which suggests a sample-size explanation for the apparent higher number of rare alleles in the north. The G_{ST} values across loci ranged from −0.089 to 0.244 (Table 1), and the overall G_{ST} was 0.013 (95% bootstrap confidence interval: −0.058 to 0.104), indicating a negligible level of genetic divergence across localities. A similar result was obtained for mtDNA (control region) data (Zink et al. 2000). Plots of genetic distance versus geographic distance suggested a lack of isolation by distance for mtDNA and autosomal loci (Fig. 3), further supporting an inference of no geographic structure. The HKA test was insignificant (χ^2 = 8.14, df = 7, P = 0.32), indicating that selection did not influence the pattern of genetic variation.

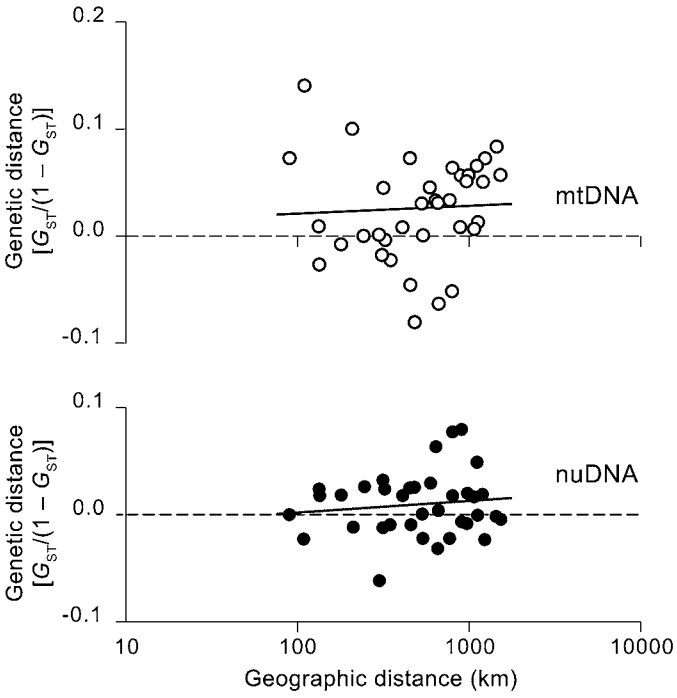

FIG. 3. Relationship in California Gnatcatchers between genetic and (log) geographic distance, showing modest isolation by distance (P = 0.37 for nuclear DNA; P = 0.08 for mtDNA; Spearman's rank correlation). This plot also reveals a lack of geographic breaks, which would appear as displaced groups of points.

Analyses of nuclear loci combined from STRUCTURE (Fig. 4) identified no geographic groupings that corresponded with any previously suggested subspecies, nor any other significant evolutionary divisions. The ΔK statistic was nonsensical because the most likely value of K was 1. Thus, the nuclear gene-sequence data do not support individual population assignment or clustering to named subspecies or any other geographic groupings.

The EBSP analyses based on all loci (Fig. 5) suggested that the southern population underwent an increase that began ~3,500 years ago, whereas the northern population increased to a lesser degree, and more recently. Because the timing of the increases depended on the calibration used, we do not interpret the dates precisely but find only that there was at least one expansion in the south and, potentially, a more recent one in the north. Using only nuclear loci (not shown), the results for both northern and southern populations had HPD confidence intervals for the demographic-size-changes parameter that overlapped zero, and we therefore could not infer a population increase, although the most frequent size-change factor found by the Markov chain in the south was 1.

Niche models (Fig. 6) for the two groups of populations did not cross-predict the majority of each other's ranges, although each predicted co-occurrence around 28–30°N, where the more mesic CSS meets the drier southern vegetation. The observed niche

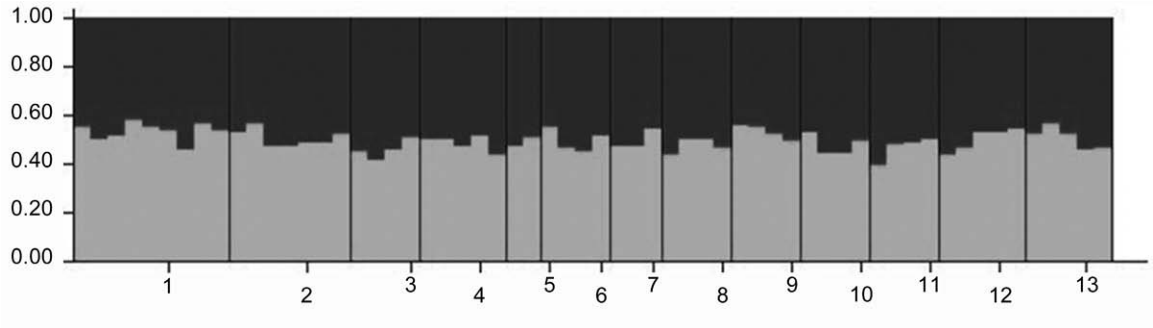

Fig. 4. Results of STRUCTURE analysis for seven variable nuclear loci showing a lack of geographic structure and assuming that $K = 2$ (i.e., two groups of California Gnatcatchers). The same result was obtained when $K = 3$. The highest probability of the data occurred when $K = 1$. Numbers across the bottom correspond to the samples from north (left) to south in Figure 2. The scale on the *y*-axis is percentage.

identity (0.343) was less than the distribution of randomized values (Fig. 7), which suggests that the niches differ more than expected by chance. Background tests suggest that the niches are not significantly divergent (Fig. 8). The estimated distribution of California Gnatcatchers in the LGM (Fig. 9) corresponded to the southern half of the present-day distribution and involved considerable area to the west of the current coastline that was above water at that time.

Discussion

Genetic results and comments on molecular markers.—Some authors have suggested that natural selection biases the pattern of variation in mtDNA genomes (Ballard and Whitlock 2004, Galtier et al. 2009:4546, Balloux 2010), but the question is whether selection is sufficiently strong to obscure evolutionary patterns and processes, which several studies suggest is not the case for birds (Zink 2005, Zink et al. 2006, Hung et al. 2012). Our HKA test revealed no evidence of strong selection that would bias mitochondrial or nuclear loci. We therefore believe that the loci we analyzed provide a basis for understanding the recent demographic and evolutionary history of the California Gnatcatcher.

Our assessment of genetic population structure of the California Gnatcatcher was the same for mtDNA and nuclear loci, both of which suggest that no evolutionarily significant divisions exist within the species. Thus, the mtDNA data provided a proper assessment of genetic structure. In general, relatively few avian subspecies qualify as valid evolutionary entities (Zink 2004, Phillimore and Owens 2006). We recommend that an mtDNA survey should be part of attempts to determine whether a species includes multiple lineages, owing to the rapid coalescence time for mtDNA gene trees. However, any single gene tree could misrepresent the lineage tree (Toews and Brelsford 2012), and, hence, both phylogeography and conservation genetics have become multilocus efforts. The question is what type of nuclear gene information should be used in concert with mtDNA (recognizing that all nuclear loci, including microsatellites, have longer coalescence times than mtDNA). We advocate the use of sequences from nuclear loci because we think that the potential to compare coalescence analyses based on both nuclear and

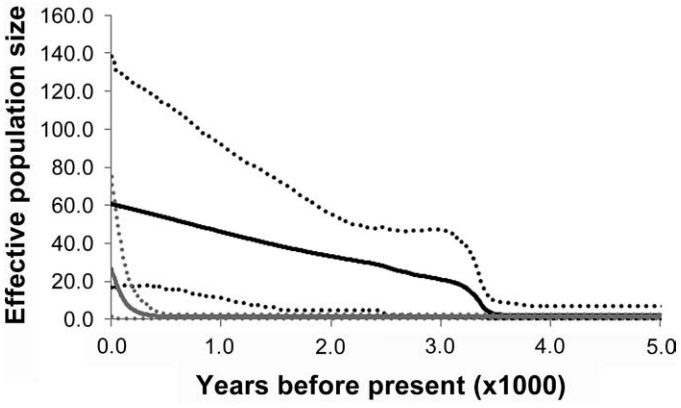

Fig. 5. Bayesian skyline plots for northern (gray lines) and southern (black lines) populations of California Gnatcatchers (dashed lines indicate 95% HPD intervals). The southern population underwent an expansion beginning ~3,500 years ago (or earlier, depending on the calibration used), whereas the northern population increased to a lesser degree and more recently. The closeness of the 95% HPD to zero for the northern sample suggests caution in inferring a population increase.

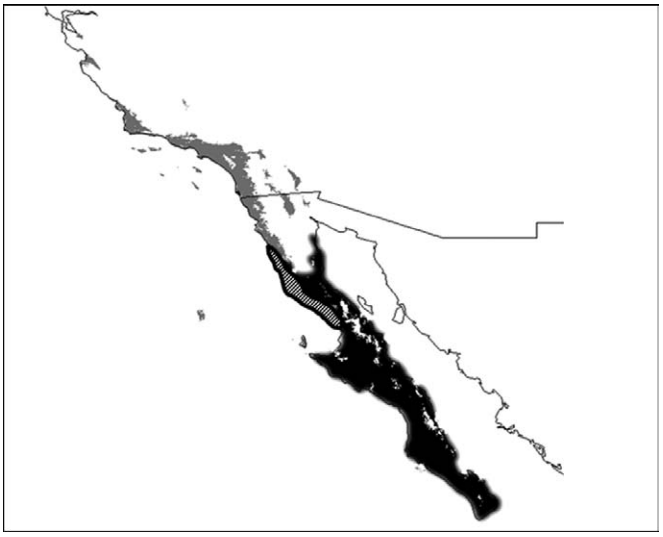

Fig. 6. Niche models showing predicted occurrence of California Gnatcatchers in coastal sage scrub (gray) and southern populations (black); hatched area indicates predicted overlap.

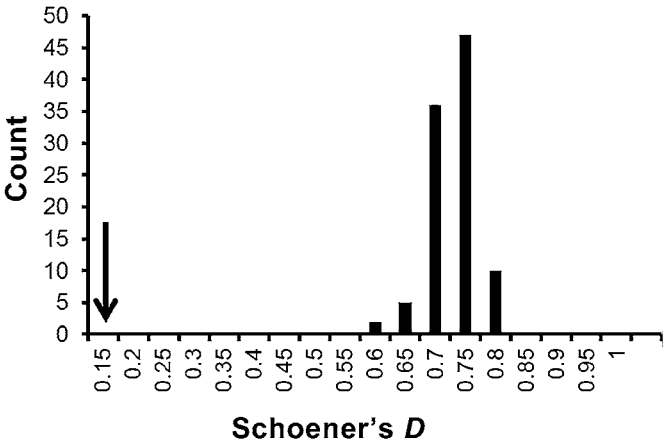

FIG. 7. Distribution of 100 random values of Schoener's *D* for the samples from the coastal sage scrub and the south. Arrow shows observed Schoener's *D* value, which indicates that the two groups of California Gnatcatchers do not have identical niches.

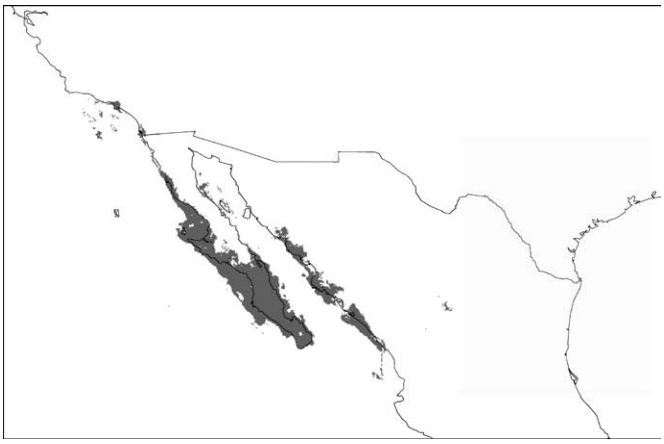

FIG. 9. Predicted distribution of California Gnatcatchers at Last Glacial Maximum based on locality points from coastal sage scrub and southern parts of the current range. Considerable habitat existed offshore of western Baja California Sur, which is now under water. Although the analysis shows the occurrence of suitable niche space on the west coast of Mexico, the species currently does not occur there.

mtDNA sequences provides a sounder basis for making evolutionary inferences than comparison of mtDNA sequences with microsatellite allele frequencies (Brito and Edwards 2009, Zink 2010).

Populations north of 28°N harbored less mtDNA variability than those to the south (Zink et al. 2000). Furthermore, several southern mtDNA haplotypes rooted basally on an otherwise unstructured haplotype tree, also suggesting a southern refugium. The geographic break in level of variability coincides with a biogeographic split in many species, potentially owing to a mid-peninsular seaway (Lindell et al. 2006, Leaché et al. 2007). Zink et al. (2000) suggested that California Gnatcatchers recently invaded the northern part of their current range, which could explain the lower nucleotide diversity in the north, owing to leading edge expansion (Hewitt 2000). This is consistent with the estimated distribution of California Gnatcatchers at the LGM (Fig. 9), which suggests that they were limited to the southern half of the current range. In contrast to the mtDNA data, we found no difference in nucleotide diversity at nuclear loci (Fig. 2) between southern and northern parts of the range. We suggest that because of the larger effective population size of nuclear loci, the signature of a northward expansion might be "erased" more quickly at nuclear loci than for mtDNA, owing to the fact that dispersing individuals carry two copies of nuclear genes, whereas only females carry a haploid mitochondrial genome.

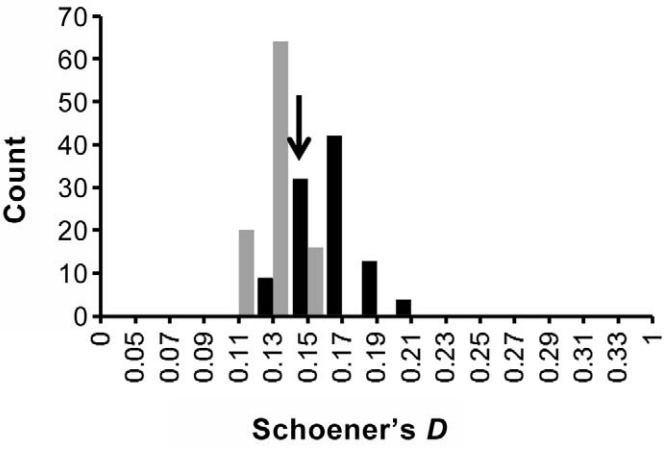

FIG. 8. Results of test for niche divergence between coastal sage scrub and southern populations of California Gnatcatchers. Gray bars indicate the distribution of random samples from the coastal sage scrub population, and black bars indicate those for the southern population. Arrow indicates observed Schoener's *D* value for the two populations, indicating neither niche divergence nor conservatism.

Mismatch distributions based on mtDNA control-region data (Zink et al. 2000) and ND2 (not shown) suggested that populations increased at different times, with a southern expansion preceding a northern one. Our EBSP plots (Fig. 5) based on all loci are consistent with this finding and suggest that the population expansion began not immediately at the end of the LGM but perhaps as recently as 3,000 years ago. We did not find a similar signal in EBSP analyses based on nuclear data alone, especially for the northern population. This suggests that it is important to include mtDNA in such analyses because it likely provides more mutations in the relevant time frame of population expansion (Keinan and Clark 2012).

Morphological support for subspecies.—It is possible that neither mtDNA nor nuclear loci coalesce rapidly enough to capture recent geographic isolation, owing to the lag time inherent in all molecular markers (Zink and Barrowclough 2008). If polygenic morphological characters were under selection and they possessed more additive genetic variance than a single locus (such as mtDNA), they might provide support for subspecies that originated in less time than required for reciprocal monophyly at mtDNA (i.e., $2Ne_f$ generations). Evidence for evolutionarily significant morphological units would include concordant character step-clines, consistent with a morphology-wide response to historical isolating events (Barrowclough 1982). The molecular

results suggest scrutiny of the morphological basis of the subspecies upon which the USFWS based its opinion.

Reanalysis of the morphological data (Atwood 1991) on California Gnatcatchers led Skalski et al. (2008) to conclude that the coastal California Gnatcatcher was "incorrectly listed under the ESA due to misinterpretation of morphological data." Instead of a concordant pattern of discrete character gaps across common geographic localities, these authors found a pattern of gradual morphological change across the range, with inconsistent patterns among characters. This geographic pattern of morphological variation is consistent with genetic data, which suggests that the subspecies were arbitrary divisions of idiosyncratic morphological gradients, and not equivalent to discrete evolutionary (listable) entities.

Ecological distinctiveness.—If the sole criterion for ecological distinctiveness is successful persistence in two or more environments, then the gnatcatchers could qualify as two DPSs (Fig. 6). However, we suggest that the intent of this criterion is to protect populations that have differentially adapted to novel environments. Populations that occupy different environments across a species' range could simply represent a species with broad ecological tolerance, and not indicate that each population possesses evolved ecological adaptations to differing habitats. For example, McCormack et al. (2010:1231) discovered that although allopatric populations of jays in the genus *Aphelocoma* occupied different habitats, niches were not significantly different because "the allopatric environments they occupy are not significantly more divergent than expected under a null model." We found a similar result in California Gnatcatchers (Fig. 8). Although the species occupies the distinctive CSS habitat in the north (Fig. 7), the two groups do not exhibit significant niche divergence if the backgrounds of each environment are taken into account. In other words, the species appears to be a habitat generalist. To our knowledge, this is the first attempt to quantify the ecological distinctiveness criterion of the ESA using the niche background test.

As a caveat, we add that the methods for testing niche divergence are in a relatively early stage and that the test is only as good as the models and input data. For example, we tested for differences in what has been termed "Grinnellian" niche dimensions (Soberón 2007), and inclusion of other types of variables could yield a different result.

Conservation implications.—The U.S. Congress directed the USFWS to list taxa under the ESA on the basis of the best available scientific and commercial data. In denying a petition to delist the California Gnatcatcher, the USFWS (2011:66258) relied on statements such as these:

> [T]he argument that the California Gnatcatcher is not distinct from other populations is based on a single genetic character, mtDNA, and this is a far too narrow and limited technique for making determinations of taxonomic validity.... [The mtDNA data set alone] does not present substantial information that the current subspecies taxonomic classification of the coastal California Gnatcatcher may be in error.

These arguments could be construed to mean that mtDNA does not qualify as the best available evidence if used alone. One of the litmus tests for assessing the scientific value of a particular data set is its degree of congruence with other data sets. We show that results from morphology, mtDNA, nuclear DNA, and ecological niche modeling agree that the California Gnatcatcher is not divisible into discrete, listable units. For listing, we believe that at least one data set should provide clear evidence of distinctiveness and significance. Furthermore, the U.S. Congress advised that the ESA should be applied "sparingly" when listing DPSs (Bernhardt 2008). Given that the coastal California Gnatcatcher lacks morphological, genetic, and ecological significance, it becomes difficult to justify its listing. Although it is difficult to prove a hypothesis of no divergence, the congruence of evidence suggests that this is the most strongly supported hypothesis given the best available data. This outcome is unfortunate, in that preservation of CSS has benefited from listing of this subspecies. Our analysis refocuses attention on the importance of ecosystem-wide preservation.

Acknowledgments

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Literature Cited

Atwood, J. L. 1991. Species limits and geographic patterns of morphological variation in California Gnatcatchers (*Polioptila californica*). Bulletin of the Southern California Academy of Sciences 90:118–133.

Atwood, J. L. 1993. California Gnatcatchers and coastal sage scrub: The biological basis for endangered species listing. Pages 149–169 *in* Interface between Ecology and Land Development in California (J. E. Keeley, Ed.). Southern California Academy of Sciences, Los Angeles.

Atwood, J. L., and D. R. Bontrager. 2001. California Gnatcatcher (*Polioptila californica*). *In* Birds of North America Online (A. Poole, Ed.). Cornell Lab of Ornithology, Ithaca, New York. Available at bna.birds.cornell.edu.ezp1.lib.umn.edu/bna/species/574.

Backström, N., S. Fagerberg, and H. Ellegren. 2008. Genomics of natural bird populations: A gene-based set of reference markers evenly spread across the avian genome. Molecular Ecology 17:964–980.

Baião, P. C., E. A. Schreiber, and P. G. Parker. 2007. The genetic basis of the plumage polymorphism in Red-footed Boobies (*Sula sula*): A melanocortin-1 receptor (MC1R) analysis. Journal of Heredity 98:287–292.

Ballard, J. W. O., and M. C. Whitlock. 2004. The incomplete natural history of mitochondria. Molecular Ecology 13:729–744.

Balloux, F. 2010. The worm in the fruit of the mitochondrial DNA tree. Heredity 104:419–420.

Barr, K. R., D. L. Lindsay, G. Athrey, R. F. Lance, T. J. Hayden, S. A. Tweddale, and P. L. Leberg. 2008. Population structure in an endangered songbird: Maintenance of genetic differentiation despite high vagility and significant population recovery. Molecular Ecology 17:3628–3639.

Barrowclough, G. F. 1982. Geographic variation, predictiveness, and subspecies. Auk 99:601–603.

Bernhardt, D. L. 2008. U.S. Fish and Wildlife Service Authority under Section 4(c)(l) of the Endangered Species Act to Revise Lists of Endangered and Threatened Species to "Reflect Recent Determinations," Memorandum M-37018 to Director, U.S. Fish and Wildlife Service, 12 December 2008.

Beyer, H. L. 2004. Hawth's analysis tools for ArcGIS. [Online.] Available at www.spatialecology.com/htools.

Brito, P. H., and S. V. Edwards. 2009. Multilocus phylogeography and phylogenetics using sequence-based markers. Genetica 135:439–455.

Brown, R., and Z. Yang. 2011. Rate variation and estimation of divergence times using strict and relaxed clocks. BMC Evolutionary Biology 11:271.

Cronin, M. A. 1997. Systematics, taxonomy, and the endangered species act: The example of the California Gnatcatcher (*Polioptila californica*). Wildlife Society Bulletin 25:661–666.

Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29:1969–1973.

Elith, J., S. J. Phillips, T. Hastie, M. Dudík, Y. E. Chee, and C. J. Yates. 2011. A statistical explanation of MaxEnt for ecologists. Diversity and Distributions 17:43–57.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14:2611–2620.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50.

Galtier, N., B. Nabholz, S. Glémin, and G. D. D. Hurst. 2009. Mitochondrial DNA as a marker of molecular diversity: A reappraisal. Molecular Ecology 18:4541–4550.

Heled, J., and A. J. Drummond. 2008. Bayesian inference of population size history from multiple loci. BMC Evolutionary Biology 8:289.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913.

Hey, J. 2004. HKA—a computer program for tests of natural selection. [Online.] Available at genfaculty.rutgers.edu/hey/software#HKA.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965–1978.

Hijmans, R. J., L. Guarino, C. Bussink, P. Mathur, M. Cruz, I. Barrantes, and E. Rojas. 2004. DIVA-GIS, version 4. A geographic information system for the analysis of biodiversity data. [Online.] Available at www.diva-gis.org.

Holsinger, K. E., and R. J. Mason-Gamer. 1996. Hierarchical analysis of nucleotide diversity in geographically structured populations. Genetics 142:629–639.

Hudson, R. R., and N. L. Kaplan. 1985. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. Genetics 111:147–164.

Hudson, R. R., M. Kreitman, and M. Aguadé. 1987. A test of neutral molecular evolution based on nucleotide data. Genetics 116:153–159.

Hung, C.-M., S. V. Drovetski, and R. M. Zink. 2012. Multilocus coalescence analyses support a mtDNA-based phylogeographic history for a widespread Palearctic passerine bird, *Sitta europaea*. Evolution 66:2850–2864.

Keinan, A., and A. G. Clark. 2012. Recent explosive human population growth has resulted in an excess of rare genetic variants. Science 336:740–743.

Kimball, R. T., E. L. Braun, F. K. Barker, R. C. K. Bowie, M. J. Braun, J. L. Chojnowski, S. J. Hackett, K.-L. Han, J. Harshman, V. Heimer-Torres, and others. 2009. A well-tested set of primers to amplify regions spread across the avian genome. Molecular Phylogenetics and Evolution 50:654–660.

Leaché, A. D., S. C. Crews, and M. J. Hickerson. 2007. Two waves of diversification in mammals and reptiles of Baja California revealed by hierarchical Bayesian analysis. Biology Letters 3:646–650.

Lim, H. C., and F. H. Sheldon. 2011. Multilocus analysis of the evolutionary dynamics of rainforest bird populations in Southeast Asia. Molecular Ecology 20:3414–3438.

Lindell, J., A. Ngo, and R. W. Murphy. 2006. Deep genealogies and the mid-peninsular seaway of Baja California. Journal of Biogeography 33:1327–1331.

Manthey, J. D., J. Klicka, and G. M. Spellman. 2011. Isolation-driven divergence: Speciation in a widespread North American songbird (Aves: Certhiidae). Molecular Ecology 20:4371–4384.

May, S. E., K. A. Medley, S. A. Johnson, and E. A. Hoffman. 2011. Combining genetic structure and ecological niche modeling to establish units of conservation: A case study of an imperiled salamander. Biological Conservation 144:1441–1450.

McCormack, J. E., A. J. Zellmer, and L. L. Knowles. 2010. Does niche divergence accompany allopatric divergence in *Aphelocoma* jays as predicted under ecological speciation?: Insights from tests with niche models. Evolution 64:1231–1244.

Mellink, E., and A. M. Rea. 1994. Taxonomic status of the California Gnatcatchers of northwestern Baja California. Western Birds 25:50–62.

Miller, A. H., H. Friedmann, L. Griscom, and R. T. Moore. 1957. Distributional check-list of the birds of Mexico, part 2. Pacific Coast Avifauna, no. 33.

Peterson, A. T., J. Sobersón, and V. Sánchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. Science 285:1265–1267.

Phillimore, A. B., and I. P. F. Owens. 2006. Are subspecies useful in evolutionary and conservation biology? Proceedings of the Royal Society of London, Series B 273:1049–1053.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190:231–259.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Rambaut, A., and A. J. Drummond. 2007. TRACER, version 1.5. [Online.] Available from beast.bio.ed.ac.uk/Tracer.

Schoener, T. W. 1968. The *Anolis* lizards of Bimini: Resource partitioning in a complex fauna. Ecology 49:704–726.

Skalski, J. R., R. L. Townsend, L. L. McDonald, J. W. Kern, and J. J. Millspaugh. 2008. Type I errors linked to faulty statistical analyses of endangered subspecies classifications. Journal of Agricultural, Biological, and Environmental Statistics 13: 199–220.

Smith, B. T., and J. Klicka. 2010. The profound influence of the Late Pliocene Panamanian uplift on the exchange, diversification, and distribution of New World birds. Ecography 33:333–342.

Soberón, J. 2007. Grinnellian and Eltonian niches and geographic distributions of species. Ecology Letters 10:1115–1123.

Swofford, D. L. 1998. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, Massachusetts.

Toews, D. P. L., and A. Brelsford. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Molecular Ecology 21:3907–3930.

U.S. Fish and Wildlife Service. 1993. Endangered and threatened wildlife and plants: Special rule concerning take of the threatened coastal California Gnatcatcher. Final Rule. Federal Register 58:65088–65096.

U.S. Fish and Wildlife Service. 2011. Endangered and threatened wildlife and plants; 90-day finding on a petition to delist the coastal California Gnatcatcher as threatened. Federal Register 76:66255–66260.

U.S. Fish and Wildlife Service and National Marine Fisheries Service. 1996. Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. Federal Register 61:4722–4725.

Warren, D. L., R. E. Glor, and M. Turelli. 2008. Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. Evolution 62:2868–2883.

Warren, D. L., R. E. Glor, and M. Turelli. 2010. ENMTools: A toolbox for comparative studies of environmental niche models. Ecography 33:607–611.

Zink, R. M. 2004. The role of subspecies in obscuring biological diversity and misleading conservation policy. Proceedings of the Royal Society of London, Series B 271:561–564.

Zink, R. M. 2005. Natural selection on mitochondrial DNA in *Parus* and its relevance for phylogeographic inference. Proceedings of the Royal Society of London, Series B 272:71–78.

Zink, R. M. 2010. Drawbacks with the use of microsatellites in phylogeography: The Song Sparrow (*Melospiza melodia*) as a case study. Journal of Avian Biology 41:1–7.

Zink, R. M., and G. F. Barrowclough. 2008. Mitochondrial DNA under siege in avian phylogeography. Molecular Ecology 17:2107–2121.

Zink, R. M., G. F. Barrowclough, J. L. Atwood, and R. C. Blackwell-Rago. 2000. Genetics, taxonomy, and conservation of the threatened California Gnatcatcher. Conservation Biology 14:1394–1405.

Zink, R. M., S. V. Drovetski, and S. Rohwer. 2006. Selective neutrality of mitochondrial ND2 sequences, phylogeography and species limits in *Sitta europaea*. Molecular Phylogenetics and Evolution 40:679–686.

Associate Editor: L. Joseph

EXHIBIT B

ENDANGERED SPECIES COMMITTEE

VS.

BRUCE BABBITT, et al

Miniscript depositions

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MS. BANNING: Objection.
Q. With regard to those morphological measurements, when you measured museum specimens or specimens in the field, how did you manually record them?
A. To the best of my remembrance, the majority were recorded on sheets of paper. There may be a few that were added, entered directly into a computer.
Q. Were they recorded in any other way?
A. No.
Q. Those are the only two ways you recorded morphological specimens?
A. Yes.
Q. And did you produce those sheets of paper today?
A. They no longer are in existence.
Q. Would you explain, please, why they are no longer in existence?
A. After they had been transcribed into a computer data file, they ceased of be of real value to me. And in 1985 or 1986 when I was preparing to move to Massachusetts, I decided that it was

pointless to keep those pieces of paper, primarily because I had exact copies of that information in computer files.
Q. Is it possible for you to be more specific about the period during which you destroyed those sheets?
MS. BANNING: Objection.
A. I didn't destroy those sheets.
Q. I don't want to put words in your mouth. What did you do with those sheets?
A. I probably recycled them.
Q. But am I correct they are no longer in existence?
A. Yes.
Q. Did you file those sheets with U C L A?
A. No.
Q. Did you give copies of those sheets to anybody?
A. No.
Q. Is it your testimony this morning that no copy of those sheets is in existence anywhere?
MS. BANNING: To your knowledge.
A. Not that I remember.

Q. Do you recall specifically how you disposed of the sheets?
A. No, I don't.
Q. Can you be more precise as to the time period during which you destroyed or disposed of these data sheets?
A. I began work at Manomet Observatory in April of 1986. I left California I believe in March of 1986, mid-March 1986, and sometime during February to mid-March of 1986, when I knew that I was moving to Massachusetts and was going through a major housecleaning, it was probably during at that time period.
Q. When were you actually awarded your Ph.D. from U C L A?
A. In June of 1986.
Q. Is it correct, then, that you disposed of your original data sheets before you were awarded your Ph.D.?
A. I disposed of the data forms themselves.
Q. That is all --
A. The raw data of course were still on my computer.

Q. I don't want to mislead you. All I'm referring to now is the data sheets on which you originally entered the data.
A. That's correct.
Q. And those data sheets were discarded or destroyed before you were awarded your Ph.D.?
A. Correct.
Q. And they were destroyed before the publication of Exhibit 3?
A. Correct.
Q. Now, did you undertake any steps to insure that the data entries into your computer files were the same as those on the physical data sheets?
A. Yes.
Q. What steps did you take?
A. Proof checks made on the data files while they were displayed on the screen as well as printouts of those data files and cross checks against the raw data sheets.
Q. Would you explain to me what you mean by cross checks against the original data sheets?
A. Simply making sure that the value that was entered for a particular data field coincided with

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measurements taken along each graph line that was produced and then an equation that followed a publication by I believe someone named Judd to produce 3 different types of measures of color.

Q. Are the measurements that were produced on the drums by the spectrophotometer part of the documents that you produced today?

A. No.

Q. Were those documents, was that output part of the material that you discarded in 1986?

A. That's correct.

Q. Is it correct to say that all of those coloration characteristics would be included in Exhibit 4?

A. Yes.

Q. Now, if you would refer please to Page 5 of Exhibit 4, Paragraph 3 beginning M E L L I N K and R E A. You have that paragraph in front of you?

A. Yes.

Q. If I am reading this correctly, it criticizes the use of spectrophotometer, is that correct?

MS. BANNING: Objection.

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A. That was my understanding.

Q. To your knowledge, apart from the Mellink and Rea report which is referred to here, has there been any other criticism of the use of the spectrophotometer to measure color characteristics?

A. I don't know.

Q. Are you aware of any other studies?

A. No, I'm not.

Q. I would like to refer you to the last paragraph. Sorry. The last sentence, in that long paragraph that says, because plumage coloration appears to be one of the primary characters, that sentence?

A. Yes.

Q. When you wrote this letter, what did you mean by serious doubts about the advisability of attempting to use the spectrophotometer data?

A. I think that Mellink and Rea's paper indicates that at the very least there needs to be a scientific question raised by whether or not spectrophotometer data are useful way to quantify color measurements.

Q. Does Exhibit 3 raise that question anyway?

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MS. BANNING: Objection.

Q. Can you answer the question.

A. No, to my remembrance.

Q. Now, in light of that statement on Paragraph 5 of your letter, if you were doing Exhibit 3 for the first time, would you use the spectrophotometer to measure coloration characteristics?

MS. BANNING: Objection.

A. I would certainly have used a more strict quality control procedure so that I would be able to talk with you folks about it.

Q. What leads you to conclude that if you were to redo the study you would use a more strict quality control procedure?

A. Because Mellink and Rea's results suggested that values obtained from a single specimen on successive runs were not entirely consistent with each other.

Q. Have you discussed the issue of using the spectrophotometer to measure coloration characteristics with either Mellink and Rea?

A. No.

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Q. Have you discussed it with any employee of the Fish and Wildlife Service?

A. No.

Q. Have you discussed it with any representative from the Natural Resources Defense Council?

A. No.

Q. Let me refer again back to Exhibit 3. Let me ask one other question. Have you discussed the propriety of using the spectrophotometer to measure coloration characteristics with any employee of the United States government?

A. No.

Q. In any department?

A. No.

Q. Have you discussed--

A. Except as contained in that letter.

Q. Have you discussed it with anybody?

A. No.

Q. Referring back to Table 1 in Exhibit 3 on Page 5, if I'm reading this correctly, you measured and reported on 196 linear measurements for May specimens of Polioptila Californica, is that

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result section identified a variety of variables belonging to what I referred to here as cluster 2. Those variables include breast brightness, back brightness, the length of the tail spot on rectrices 5 and 6, the extent of tail spot white expressed as a percent for rectrices 5 and 6, relative lengths of tail feathers 5 and 6 and the amount of white on rectrix 5 and 6. The sentence reads that the 10 components of this cluster had little variation, variably distinct break located between 31 degrees 30 minutes and 30 degrees north latitude and then cites figure 3 A and B as examples of that. So to answer your question, if there was additional supportive evidence for that, I would interpret that sentence to mean that the other components of cluster 2 showed similar patterns but are not presented here in the manuscript.

Q. Directing your attention to page 121, continuing down a couple of sentences, the sentence that begins, "In specimens obtained from sample area B G 27," do you see that sentence?

A. Yes.

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Q. And do I understand that your reference to figure 3 C is the evidence to support that statement?

MS. BANNING: Objection.

A. That is provided as an example of the types of patterns that apparently were found in cluster 3 which in addition to back purity as shown in figure 3 C also included going back to the first paragraph of the result section, breast wavelength, and back wavelength.

Q. Did you run Gabriel's technique with regard to breast wavelength and back wavelength?

A. I don't recall. I would assume so based on this statement.

Q. Have you retained a record of the results of Gabriel's technique for those characteristics?

A. No.

Q. Does Gabriel's technique with regard to back P as shown on figure 3 C provide evidence in support of the statement of a sharp step at 30 degrees?

MS. BANNING: Is that the end of the question?

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MR. THORNTON: Yes.

MS. BANNING: I object.

A. Could you direct me to the statement about the sharp step at 30 degrees again, please?

Q. Let me rephrase the question: On Page 121 appears to be the beginning of the third line of the text on that page that begins, "The three variables included in cluster 3 show little variation. " Do you see that sentence?

A. Yes.

Q. Do you see the reference to a sharp step?

A. Yes.

Q. My question is: Does the result of Gabriel's technique with regard to variable back purity suggest support for that statement?

A. That is the reference given there in that sentence, a sharp step occurred between 31 degrees 30 minutes and 30 degrees latitude (figure 3 C.

Q. I see what the sentence says. My question to you though is whether you interpreted Gabriel's technique applicable to figure 3 C to support that statement.

MS. BANNING: Objection.

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A. All I can say is what the sentence says.

Q. Well, looking at the results of Gabriel's technique shown to the right of figure 3 C, do you now believe it supports that statement?

A. This was one of three characters referred to in cluster 3. It was provided as an example. I have no recollection of the pattern of variation given in the other two characters nor a recollection of the results of Gabriel's test for the other two characters. Based on back purity alone, I would say that there is suggestion of something that is happening in the vicinity of latitude 30, but there is apparently so much variation in the data associated with that value that it really does not seem to have any clear indication of any steps anywhere. Despite the fact that on the figure itself the means, the plot of means was suggested, there is something that is transpiring on a north/south gradient.

Q. You made reference to the figure indicating variation. Were you referring to the vertical line representing the results of Gabriel's technique?

A. That analysis, if I understand it

EXHIBIT C

Genetics, Taxonomy, and Conservation of the Threatened California Gnatcatcher

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Abstract: *The California Gnatcatcher (*Polioptila californica*) has become a flagship species in the dispute over development of southern California's unique coastal sage scrub habitat, a fragile, geographically restricted ecosystem with high endemism. One aspect of the controversy concerns the status of the subspecies of this bird in southern California coastal sage scrub that is currently listed as threatened under the U.S. Endangered Species Act. To investigate the recent population history of this species and the genetic distinctiveness of subspecies and to inform conservation planning, we used direct sequencing of mitochondrial DNA (mtDNA) for 64 individuals from 13 samples taken throughout the species' range. We found that coastal sage scrub populations of California Gnatcatchers are not genetically distinct from populations in Baja California, which are dense and continuously distributed throughout the peninsula. Rather, mtDNA sequences from this species contain the signatures of population growth and support a hypothesis of recent expansion of populations from a southern Baja California refugium northward into the southern coastal regions of California. During this expansion, stochastic events led to a reduction in genetic variation in the newly occupied range. Thus, preservation of coastal sage scrub cannot be linked to maintaining the genetic diversity of northern gnatcatcher populations, despite previous recognition of subspecies. Our study suggests that not all currently recognized subspecies are equivalent to evolutionarily significant units and illustrates the danger of focusing conservation efforts for threatened habitats on a single species.*

Genética, Taxonomía, y Conservación de la Perlita de California Amenazado de Extinción

Resumen: *La perlita de California (*Polioptila californica*) se ha convertido en una especie insignia en la disputa sobre el desarrollo del exclusivo hábitat de chaparral de salvia costero (CSS) del sur de California, un ecosistema frágil y geográficamente restringido con un endemismo elevado. Un aspecto de la controversia tiene que ver con la situación de la subespecie de esta ave en el CSS del sur de California y que se encuentra actualmente enlistada como amenazada bajo el Acta de Especies Amenazadas de los Estados Unidos. Utilizamos un secuenciado directo de ADN mitocondrial (mtDNA) de 64 individuos de 13 muestras tomadas a lo largo del rango de distribución de la especie para investigar la historia poblacional reciente de la especie y la diferenciación de subespecies, y para documentar planes de conservación. Encontramos que las poblaciones de la perlita de California de CSS no son genéticamente distintas de las poblaciones de Baja California, las cuales son densas y tienen una distribución continua a lo largo de la península. Más bien, las secuencias de mtDNA de esta especie contienen la firma de un crecimiento poblacional y apoya una hipótesis de expansión reciente de poblaciones de un refugio sureño de Baja California hacia el norte y hacia adentro de las regiones sureñas costeras de California. Durante esta expansión, los eventos estocásticos conducen a una reducción en la variación genética en el rango recientemente ocupado. Por lo tanto, la conservación del CSS no puede ser vinculada con el mantenimiento de la diversidad genética de poblaciones norteñas de perlitas, a pesar de su previo reconocimiento como subespecie. Nuestro estudio sugiere que no todas las subespecies actualmente reconocidas son equivalentes a las unidades evolutivamente significativas e ilustra el peligro de enfocar los esfuerzos de conservación de hábitats amenazados en una sola especie.*

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Introduction

Since 1940, the human population of southern California has increased at a rate twice that of many developing countries (Mann & Plummer 1995). Not surprisingly this growth has had a negative effect on the native flora and fauna. One particularly hard-hit community is coastal sage scrub, where approximately 100 endemic species and subspecies of plants and animals are potentially endangered (Atwood 1993). A characteristic inhabitant of coastal sage scrub, a small, nonmigratory songbird known as the California Gnatcatcher (*Polioptila californica*), has been at the center of efforts to preserve this habitat and its unique flora and fauna. Populations of gnatcatchers in coastal sage scrub are considered threatened under the U.S. Endangered Species Act (ESA; U.S. Fish and Wildlife Service 1993, 1995) as a result of loss of 70–90% of the original habitat (Atwood 1993). The remaining highly fragmented tracts of coastal sage scrub are of high economic value because of their proximity to the Pacific Ocean and major urban, retirement, and commercial areas in Los Angeles, Riverside, Orange, and San Diego counties. Some patches of coastal sage scrub are valued at $3 million per acre (0.40 ha; Mann & Plummer 1995). Because a pair of California Gnatcatchers may occupy a year-round home range in excess of 10 acres (4 ha; Atwood 1993) the value of real estate required to support a population of, for example, 50 pairs of these birds might exceed $1 billion. Few other species better typify the conflicts and tradeoffs among legal, environmental, and economic priorities.

The abundance of gnatcatchers throughout their range reveals a potential conflict between legal and biological concerns. California Gnatcatchers occur from Los Angeles, California, to the southern tip of the Baja peninsula (Fig. 1). Northern populations are least dense, especially from El Rosario (Baja California, lat 30°N) north to Los Angeles. These threatened populations comprise many small groups of individuals, each often isolated by urban sprawl, which potentially promotes local inbreeding. In contrast, populations in central and southern Baja California and throughout Baja California Sur are large and continuous (Atwood 1993). Thus, the ESA mandates protection of populations of a species that are historically restricted (and threatened) in the United States, whereas populations elsewhere in the contiguous range are "healthy." Therefore, the species as a whole is not threatened; rather, the issue involves preservation of populations within a relatively small part of the range that transcends an international boundary (Hunter & Hutchinson 1994).

Conservation of the species has been complicated by past taxonomic studies. Before 1989, the California Gnatcatcher was classified as a subspecies of the Black-tailed Gnatcatcher (*Polioptila melanura*). Studies by Atwood (1988), however, revealed that subspecies along the coast in California and those south of 28°N latitude throughout the Baja California peninsula were distinct

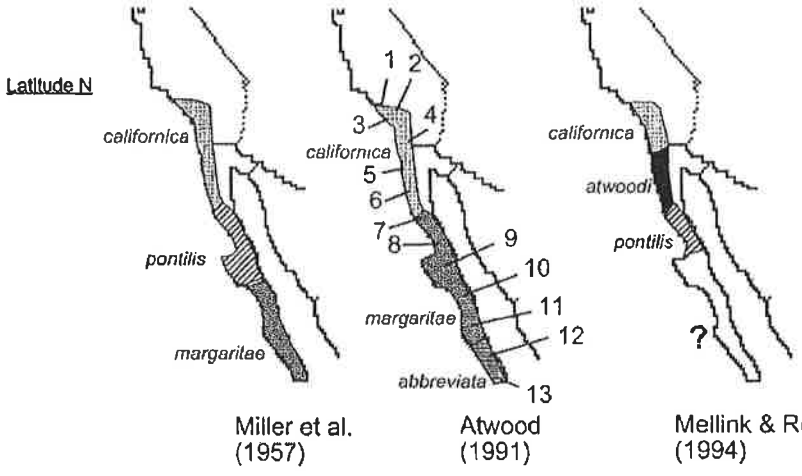

Figure 1. Three subspecies schemes proposed by Miller et al. (1957), Atwood (1991), and Mellink and Rea (1994) for the California Gnatcatcher, based on morphological characteristics of the external phenotype. Mellink and Rea (1994) did not explicitly state their recommendation for subspecific taxonomy south of 27°N latitude; they recognized P. c. margaritae, *however, for a total of at least four subspecies. Combining the treatments suggests a total of five subspecies. Sample sites for mtDNA study shown with numbers on the subspecies scheme of Atwood: 1, Los Angeles County; 2, Riverside County; 3, Orange County; 4, San Diego County; 5, Ensenada; 6, San Telmo; 7, Mision San Fernando; 8, El Rosarito; 9, San Ignacio; 10, Mulege; 11, Villa Insurgentes; 12, La Paz; and 13, Cabo San Lucas. The boundary between the states of Baja California and Baja California Sur is at 28°N latitude.*

from populations of *P. melanura* to the east. Analysis of the amount of white in the tail feathers and especially of vocalizations provided key evidence for the species-level distinctiveness of these subspecies and led to their formal recognition (American Ornithologist's Union 1989) as the California Gnatcatcher (*P. californica*). Analyses of mitochondrial DNA (mtDNA) sequences subsequently corroborated Atwood's recognition of the California Gnatcatcher (Zink & Blackwell 1998). The subspecific taxonomy of the California Gnatcatcher, however, has been controversial. Based on differing interpretations of geographic patterns of coloration, size, and shape, three recent subspecies schemes have been proposed (Fig. 1). Although these subspecies classifications differ, all suggest that the northern part of the range, including the coastal sage scrub populations, includes one or two subspecific units. The controversy over subspecies taxonomy suggests that new data are required to clarify the significance of geographic variation relative to conservation of both the species itself and of the coastal sage scrub.

From the viewpoint of conservation genetics, the issue involves the distribution of genetic diversity within the species: is the species uniform throughout its range or is it subdivided into smaller units, termed evolutionarily significant units (ESU; Ryder 1986; Barrowclough & Flesness 1996)? To qualify as an ESU (Moritz 1994; Waples 1995), phylogenetic analysis of mtDNA haplotypes must show that haplotypes from a given region are more closely related to each other than they are to haplotypes from other regions (termed reciprocal monophyly). If haplotypes from a given region do not form such an exclusive group, then either gene flow is ongoing or it has ceased recently. In either of the latter two cases, there are no geographic units that have had significant periods of isolation and independent evolution. Thus ESUs are diagnosed by the pattern of haplotype variation, not the level of sequence divergence. That is, reciprocally monophyletic groups can differ by 1% (a typical lower value for birds) to over 8% (Avise & Walker 1998). Evolutionarily significant units defined by genetic criteria constitute significant elements of biodiversity "below" the species level and are often considered units of conservation (U.S. Departments of the Interior and U.S. Department of Commerce 1996).

In cases in which direct genetic information is lacking, the taxonomic category of subspecies serves as a proxy for the ESU, as in the case of the California Gnatcatcher. The relevant conservation question here is whether putative subspecies of the California Gnatcatcher (Fig. 1) reflect ESUs or whether the genetic composition of the species is more homogeneous than previous subspecies schemes imply (Cronin 1997). Given the central role that subspecies can play under the ESA in serving as surrogates for ESUs (National Research Council 1995), testing subspecies limits is a vital component of conservation biology.

Genetic studies also provide perspective on the recent demographic history of populations. For example, Slatkin and Hudson (1991) and Rogers (1995) use recent advances in coalescence theory to show how population expansion can be distinguished from a history of long-term constant population size. Estimates of gene flow can also be derived (Wright 1931). Such inferences can complement information about patterns of genetic variation. For example, lack of ESUs might result from recent population expansion with insufficient elapsed time for differentiation.

We sequenced the rapidly evolving mtDNA control region (Taberlet 1996) and part of the ND6 gene. We sampled populations throughout the range to clarify genetic patterns of threatened and "healthy" populations. Our goals were to investigate the recent demographic history of this species, to test for the existence of ESUs and hence the validity of various subspecies schemes, and to comment on the relevance of genetic information to the conservation of this species.

Methods

We collected gnatcatchers in Mexico and plucked feathers from nestlings in the United States. Specimens are housed at the American Museum of Natural History, New York, Museo de Zoologia, Universidad Autonoma de Mexico, and the J. F. Bell Museum, University of Minnesota. Sample size at each of the 13 localities (Fig. 1) was five, except for San Diego County ($n = 3$), Riverside County ($n = 4$), and San Telmo ($n = 7$). The mtDNA was isolated from tissue or feather pulp, amplified via the polymerase chain reaction, and sequenced manually following standard protocols (Hillis et al. 1996). We used several pairs of primers (Tarr 1995; Zink et al. 1997) to obtain a sequence for the mtDNA control region, t-RNA^{Glu}, and part of ND6 (ND6E, HCR4, LCR4, HPHE-1; LMCR CCAGTACAGGAGTAATGTCG; and LCCR2M CTCTTCACAGATACAAGTGG). As a check on the control-region results, we also sequenced parts of two other mtDNA genes (318 base pairs [bp] of ND3 and 275 bp of ND2) from 12 specimens spanning the entire geographical range. We used the program PAUP* (Swofford 1999) to estimate a haplotype tree based on maximum parsimony (heuristic search, bases equally weighted); a haplotype of the Black-tailed Gnatcatcher (*P. melanura*), sister species of the California Gnatcatcher (Zink & Blackwell 1998), was used to root the tree. We bootstrapped the data set 250 times using random additions. We tested for departure from a molecular clock by performing a log-likelihood ratio test (HKY85 model with gamma correction) of the difference in likelihood for a minimum-length haplotype tree with and without a molecular clock enforced (Huelsenbeck & Rannala 1997). The significance of two times the difference in log likelihoods was assessed by a chi-square table.

We computed the amount of genetic variation within each population sample, nucleotide diversity (π), following standard equations (Nei 1987). In addition, we

computed the amount of genetic variation distributed among populations, a quantity estimated by N_{ST} (Lynch & Crease 1990). The N_{ST} analysis resembles a hierarchical ANOVA, partitioning genetic variation into among- and within-population components. The N_{ST} tends to 0 with no population subdivision, whereas a value nearing 1 indicates that populations share no recent gene-flow events or common history. We computed Tajima's (1989) D statistic to assess whether sequence evolution appeared consistent with neutral expectation.

We computed the mismatch distribution (Rogers & Harpending 1992), defined as the number of nucleotide differences between all pairs of individual California Gnatcatcher mtDNAs ($n = 64$). We calculated the mean of these differences and, following Slatkin and Hudson (1991), used the mean value to fit the observed distribution to an expected Poisson distribution; the distributions were compared with a Kolmogorov-Smirnov one-sample test. In a subsequent analysis, we divided the gnatcatcher samples into two subsets that corresponded to the 25 individuals from the five localities south of latitude 28°N and the 39 individuals from the eight localities north of latitude 28°N. We computed the mismatch distributions for these two subsets and again fit the means to Poisson distributions and performed Kolmogorov-Smirnov tests. Harpending's (1994) raggedness statistic was estimated for the distribution of pairwise differences. This statistic has been used to distinguish between stationary and growing populations of humans.

Nee et al. (1995) have shown that, for a population of approximately constant size, a plot of the logarithm of the number of lineages versus their branching times will have a characteristic concave shape, whereas an exponentially growing population exhibits a convex shape. We arbitrarily used one of the alternate minimum-length estimates of phylogenetic relationships for individual gnatcatchers and estimated the time of origin of each of the nodes on that tree. For each node, we computed the number of nucleotide substitutions to each terminal stemming from that node. The average of these substitutions over all paths from the internal node to sampled individuals was taken as the "age" of the node. This was plotted against the number of lineages segregating prior to that estimated time. For the shallowest, most terminal nodes of the tree, estimated ages are not precise because the number of substitutions along the short-terminal and near-terminal branches was frequently zero, one, or two. This sampling error can result in estimated branch lengths that are slightly negative. For such cases we collapsed the negative branches into the next most basal nodes. This circumstance arises only in the analysis of log-lineage plots for which the average age of nodes must be estimated; minimum-length parsimony trees have no negative branch lengths (for a worked example, see Barrowclough and Groth 1999).

We constructed an expected plot of log lineages versus coalescent times using the equations reviewed by Hudson (1991). The expected time between coalescent events is $T(j) = (2N_{ef})/(j(j - 1))$, where j is the number of lineages left to coalesce and N_{ef} is the effective number of females in the population. The total expected time to coalescence is $2N_{ef}(1 - 1/n)$, where n is the number of individuals sampled; for 64 individuals this is $1.97N_{ef}$. We therefore calibrated the log-lineages plot by letting the depth, in substitutions, of the gnatcatcher tree equal $1.97N_{ef}$. To compare the observed distribution of lineages versus time with the pattern expected for a population of constant size, we computed the expected time of each coalescent event $T(j)$ and set the estimated age of the earliest node on the tree equal to the expected coalescence time for a sample of 64 individuals from a stationary population.

An alternative to the graphical techniques discussed above for drawing inferences about the demographic history of populations was developed by Kuhner et al. (1998). This approach yields maximum-likelihood estimates of population size and growth rates based on a simple model of DNA evolution and a maximum-likelihood, as opposed to parsimony, estimate of the haplotype tree. Using the program FLUCTUATE (Kuhner et al. 1998), we estimated population growth rates for the entire sample of 64 gnatcatchers, as well as for the sample of 25 individuals from the five localities south of 28°N latitude and the 39 individuals from the eight localities north of 28°N latitude. In estimating the growth rate with FLUCTUATE, we used a transition-to-transversion ratio of 10.0 and a two-rate substitution model in which 90% of the sites were invariant and 10% had the same substitution rate. In this procedure, the search for the maximum-likelihood estimate over the likelihood surface was initiated with Watterson's estimate of theta(θ). To determine if the resulting estimates were stable, we iterated the search for maximum-likelihood estimates of growth and θ, but in successive iterations we used the point estimates from the previous iteration as a starting point. This procedure provides information about whether the likelihood surface is sufficiently smooth and has sufficient relief so that estimates are consistent.

Results

A total of 1399 bp, including some indels, was obtained for all 64 gnatcatchers. We analyzed data with and without deletions, and our overall conclusions are unchanged; omitting deletions results in lower resolution of the pattern of haplotype relationships. We found similar levels of variation for the 12 individuals surveyed additionally for ND2 and ND3 gene regions and no geographically segregating differences, so these data are not discussed further. Because these genes are not contiguous with the

control region, we inferred that our sequence data were mitochondrial and not derived from a nuclear homologue. Furthermore, our control-region sequences contained the "landmarks" found in other avian control regions (Baker & Marshall 1997). Also, the large number of closely related haplotypes we found argues against nuclear copies, which tend to be less variable owing to mutation repair mechanisms.

Direct sequencing of the control region revealed 26 variable positions (17 transitions, 2 transversions, 7 deletions), of which 14 were parsimony-uninformative. Of the 64 California Gnatcatchers examined, 33 exhibited unique haplotypes (Appendix). All haplotypes were closely related, with a maximum (uncorrected) interhaplotype divergence of 0.64% and an average of 0.27%. Twenty-three individuals (35.9%), representing 12 of 13 localities, shared a single haplotype, whereas the next most frequent haplotype was found in four individuals (6.3%). The N_{ST} of 0.074 suggests a lack of population subdivision; 92.6% of the genetic variation was common to populations and only 7.4% was distributed among them. The lack of structure among gnatcatcher haplotypes was confirmed by phylogenetic analysis (Fig. 2), which does not support any subspecies scheme, either previously described (Fig. 1) or unforeseen. That is, haplotypes did not form exclusive clusters that conformed to recognized subspecies or to any other geographically restricted areas. A feature common to the minimum-length trees was the basal position of several haplotypes from southern locations. A likelihood ratio test (LRT) (Kishino & Hasegawa 1989) significantly ($p = 0.01$) rejected a tree in which haplotypes were constrained to match the subspecies limits proposed by Atwood (Fig. 1). A LRT for a haplotype tree (one of the minimum-length trees) with and without a molecular clock enforced was not significant, indicating a lack of rate heterogeneity. Tajima's D statistic was significant in only 1 of 13 population samples.

The most striking genetic pattern observed (Fig. 3) was a transition in level of genetic diversity (π) between the San Ignacio and El Rosarito locales, with populations north of San Ignacio showing π values approximately 25% of those to the south of 30°N latitude.

The mismatch distribution (Fig. 4) had the overall shape associated with growing rather than constant populations (Slatkin & Hudson 1991; Rogers & Harpending 1992). Superimposed on the distribution was the Poisson distribution for a sample with the same mean, 2.33, as the observed distribution. The observed and expected distributions differed significantly (Kolmogorov-Smirnov test, $p < 0.05$). Dividing the population samples at 28°N latitude, which corresponded to the observed discontinuity in π (Fig. 3), we found that neither mismatch distribution (Fig. 5) deviated significantly ($p > 0.05$) from the Poisson expectation (mean for northern samples, 1.17 substitutions; mean for southern samples, 3.81). Harpending's (1994) raggedness value for the overall distribution of pairwise differences, 0.032, resembled those associated with growing populations. Populations with stationary sizes usually had raggedness values of 0.05–0.5, with a mode of 0.1 in their study.

Comparison of observed and expected plots of the distributions of lineages versus time requires calibration of the expected curve. The earliest (deepest) node on the tree corresponded to 6.25 substitutions, which we took as the expected coalescent time for a sample of 64 individuals, $1.97N_{ef}$. Thus, we calibrated the two curves by assuming that $1.97\mu N_{ef} = 6.25$; that is, letting $\mu N_{ef} = 3.17$, where μ is the nucleotide substitution rate. In the resultant plot (Fig. 6), we indicated the expected position of the first 10 coalescent events and used a curve to indicate the shape of the distribution for the remaining 52 closely spaced events. The observed plot (Fig. 6) was consistently to the left of the curve expected for a population of constant size (the result found by Nee et al. [1995] for a growing population).

The maximum-likelihood estimates of growth rates (+1 SD) for the entire sample of 64 California Gnatcatchers, for the five southern populations, and for the eight northern populations were 1025 + 23, 583 + 18, and 1853 + 191, respectively. (These estimates of growth rate are standardized by the mutation rate [e.g., Kuhner et al. 1998]). The three estimates were all positive and significantly different from zero, thereby rejecting a population of constant size. In three successive iterations using the program FLUCTUATE, the estimates of growth rate obtained were stable and consistent.

Discussion

Population History

The most common haplotype (36% of individuals) was found in 12 of 13 population samples. Based on the rooted haplotype tree (Fig. 2), this most common haplotype arose relatively recently. This suggests that gene flow among localities must be substantial; that is, a relatively recently arisen haplotype has spread throughout the range of the California Gnatcatcher compared to the common ancestor of all extant haplotypes. The shape of the phylogenetic tree (Fig. 2) reinforces this conclusion. Although some old, relict haplotypes were confined to the southern portions of the Baja California peninsula, there was no geographic structuring of the more recent branches. If there were substantial barriers to gene flow, one might expect that "families" of related haplotypes would be found in geographically contiguous or proximal locations. This has been found for other birds from Baja California such as LeConte's Thrasher (*Toxostoma lecontei*; Zink et al. 1997), for which mutually exclusive clades of haplotypes were found in two disjunct geographic regions. The haplotype

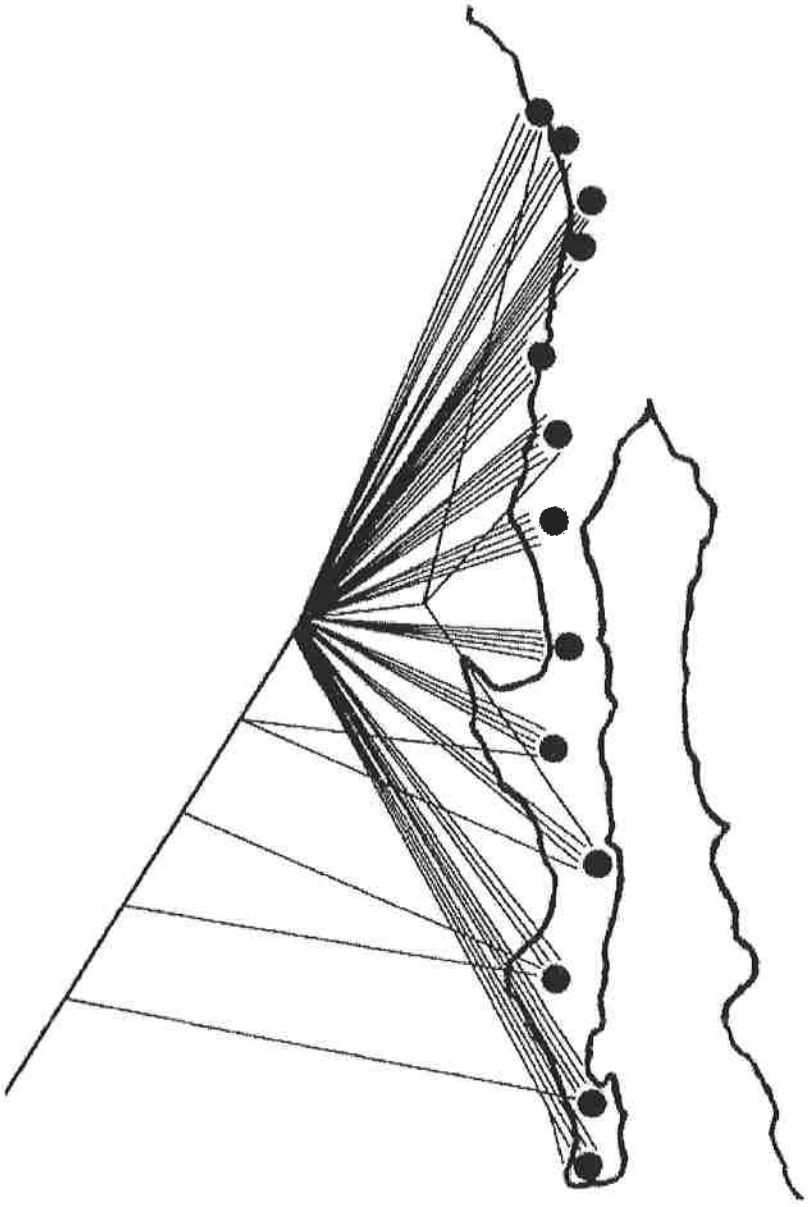

Figure 2. Strict consensus tree of haplotypes derived from 10,751 equally parsimonious trees (length 84, consistency index [ci] = 0.4, excluding uninformative characters; rescaled ci = 0.5) showing no geographic structure among 64 individuals. There is a single node at the top of this tree. No nodes were present at >65% in 250 bootstrap replicate trees. Only two nodes had bootstrap values >50%; each included one pair of haplotypes from different localities.

tree for California Gnatcatchers does not support recognition of ESUs or subspecies.

The N_{ST} value also reinforces the conclusion that there are no ESUs within the California Gnatcatcher. Species including two or more ESUs would have an N_{ST} value an order of magnitude greater than 0.07; in the case of LeConte's Thrasher, for example, N_{ST} was 0.75. Elementary but relatively robust models in population genetics (Neigel 1997) allow one to obtain an estimate of the amount of gene flow necessary to maintain an N_{ST} or F_{ST} value for an isolation-by-distance model or island model of population structure at equilibrium. The estimate of N_{ST} we obtained was equivalent to an exchange of between three and four individuals per generation among populations. Wright (1931) showed that if the amount of gene flow among populations was greater than approximately one individual per generation, the entire population could be thought of as one large panmictic unit. Thus, the pattern of distribution of the most common haplotype, the shape of the phylogenetic tree (phylogeography), and the low N_{ST} estimate all suggest that gene flow among the gnatcatcher populations has been substantial.

The sudden geographic shift in π can be attributable to two alternate phenomena, namely a range expansion

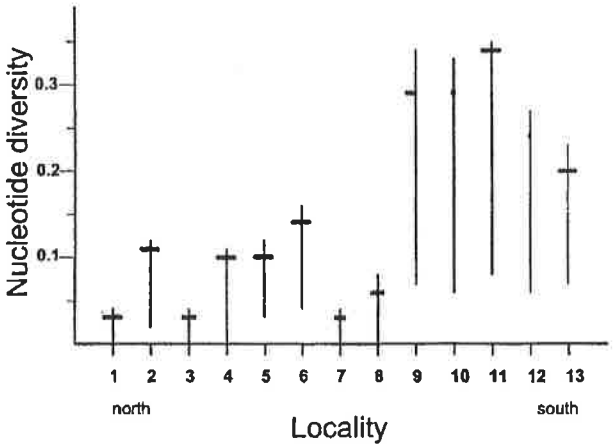

Figure 3. Pattern of geographic variation in nucleotide diversity (π; estimate and 95% bootstrap confidence intervals) showing shift between El Rosarito (locality 8) and San Ignacio (9) at 28°N latitude. The pattern is significant with a run test ($p < 0.05$)

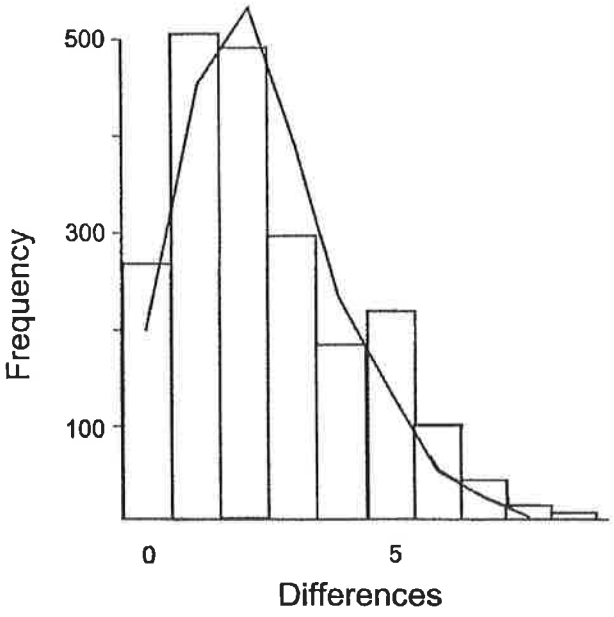

Figure 4. Mismatch distribution for 64 California Gnatcatcher sequences (histogram) and the expected Poisson distribution (line) for a growing population with the same mean.

from a southern refugium or a "selective sweep." If the northern part of the range only recently became inhabitable or otherwise available to gnatcatchers, northward emigrants dispersing from a southern refugium might represent only a part of the species' genetic diversity, leading to decreased π in the north (Hewitt 1996). Alternatively if a new advantageous mutation makes a northern haplotype selectively superior, it can spread southward rapidly and increase in frequency at the expense of older and less fit southern haplotypes. The observed pattern of variation in π is consistent with either hypothesis. Four lines of evidence support the hypothesis of recent and northward population expansion. First, phylogenetic analysis (Fig. 2) revealed several basal (i.e., oldest) haplotypes that occurred only in Baja California Sur. Older (basal) haplotypes are expected to occur disproportionately in previous refugia. Baja California south of 30°N latitude was thought to be a refugium (Magdalena Refugium) during the late Pleistocene (Hafner & Riddle 1997). Other avian (Zink et al. 1997) and nonavian (Upton & Murphy 1997) species also show genetic breaks between 28°N and 30°N latitudes. Second, the plot of the number of lineages versus the estimated age of the haplotype tree suggests an expanding population.

Third, the overall mismatch distribution (Fig. 4) was basically unimodal and had the characteristic shape associated with a growing population (Rogers & Harpending 1992). Such distributions from constant populations are often ragged (Slatkin & Hudson 1991; Harpending et al. 1993; Harpending 1994)—that is, bimodal or multimodal—unlike that for the California Gnatcatcher. In addition, the plot was quite different from those reported by Barrowclough and Groth (1999) for three populations of owls that they interpreted to be stationary in size. The results of the Kolmogorov-Smirnov test, however, indicated that our observed distribution differed from the Poisson expectation for a growing population. These tests must be interpreted with caution because the samples were not independent (Slatkin & Hudson 1991). Visual comparison of the observed and expected mismatch distributions for the 64 gnatcatchers (Fig. 4) suggests that the tail of the distribution is too long for a Poisson curve. This overall distribution, however, was for 13 population samples taken over 1000 linear km. Rogers and Harpending (1992) showed that an exponentially growing population can be characterized by a Poisson-like distribution with a mode that starts at an average pairwise difference of zero at the time of initial growth; the distribution shifts to larger values of pairwise differences as time increases while maintaining the characteristics of a Poisson distribution (for example, variance equal to mean).

Our interpretation of the topology of the sequence relationships and the geographical pattern of nucleotide diversity is that populations of California Gnatcatchers have been expanding their range northward from southern Baja California. If this were true, then the expansion in population size would be older in the southern part of the gnatcatcher range and more recent in more northern parts of the range. Therefore, we divided the gnatcatcher samples into two subsets that corresponded to the regions defined by the pattern of π (Fig. 3). Mismatch distributions (Fig. 5) fit to each subset did not differ significantly from expecta-

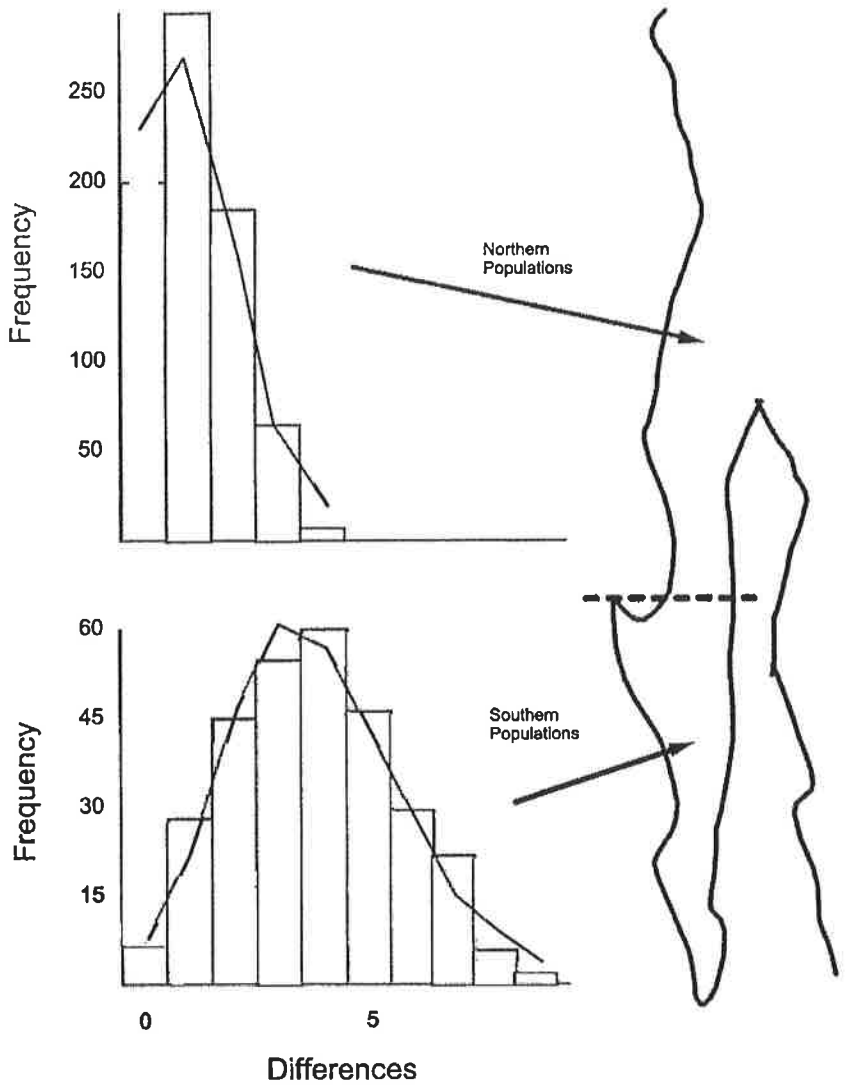

Figure 5. Mismatch distributions for northern and southern samples of California Gnatcatchers with expected Poisson distributions for growing populations with the same mean (lines).

tion. The shallow, wide distribution with the mode removed from the vertical axis in the south, and the tall, narrow distribution close to the axis in the north reflect what would be anticipated given the expansion process we suggest. Although mismatch distributions could be computed separately for each of the 13 populations samples, small sample sizes precluded this procedure.

Fourth, it might be argued that the log-lineage plot and the mismatch distributions are simply qualitative or heuristic techniques. The quantitative, maximum-likelihood method of Kuhner et al. (1998) obtains an estimate of growth rate by integrating over all possible tree topologies, rather than a single parsimony tree. In addition, the maximum-likelihood approach does not require an outgroup for rooting purposes. Consequently, it is in many ways an independent technique for addressing the question of a stable versus growing population. Using the maximum-likelihood method, we obtained estimates of growth rates for the gnatcatchers that were all positive and significantly different from zero. In addition, the growth rate for the northern eight populations was estimated to be greater by a factor of three than that for the southern five populations; this is consistent with our hypothesis that the predominant region of population expansion is the northern part of the range, emanating from a possible southern refugium.

Taken together, the tree of haplotype relationships, the geographical pattern of π, the shape of the mismatch distributions, the log-lineage plot, and the maximum-likelihood results favor a hypothesis of a relatively recent expansion of California Gnatcatcher populations from southern Baja California northward throughout the peninsula and into southwestern California. Such a recent population expansion likely explains the lack of phylogeographic pattern. Given that range expansion has recently occurred, it is unlikely that any current iso-

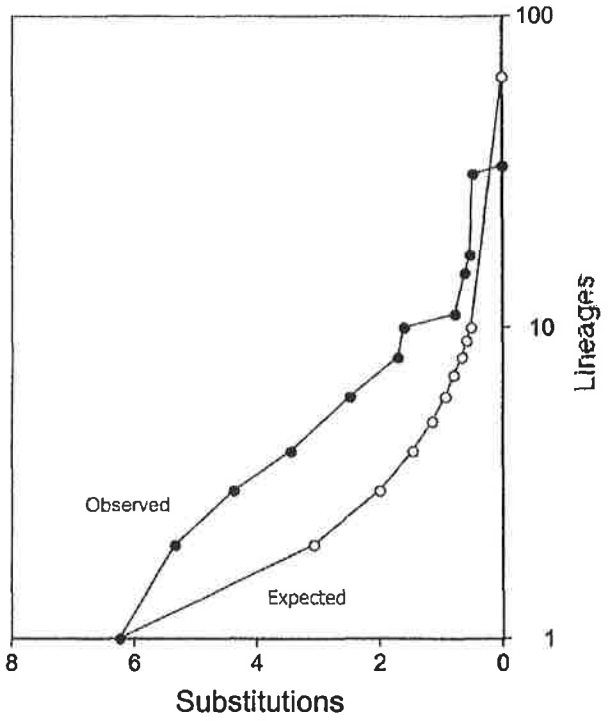

Figure 6. Number of lineages (logarithmic scale) versus estimated age (measured as number of nucleotide substitutions) of coalescent events for hypothesized relationships among California Gnatcatcher sequences (solid circles) and the expected distribution for a sample of 64 from a stationary population (open circles).

lating barriers (except distance) will result in future genetic division of the northern populations.

Conservation Implications of Population History

Our study provides a geographically thorough genetic analysis of a threatened species, encompassing both areas where populations are not threatened (providing a baseline) and where they are. Our genetically based interpretation of the recent history of the California Gnatcatcher provides perspective on current conservation questions. Put simply, based on mtDNA data, northern populations do not appear to constitute a unique component of gnatcatcher biodiversity. Nevertheless, several caveats merit consideration. One might argue that loss of northern populations could be unfortunate because populations at the margins of species' ranges might be "evolutionary laboratories" for novel genetic types (Erwin 1991). Although a few novel haplotypes exist in the coastal sage scrub populations, only an extreme view would support preservation of each unique haplotype, given their minor differences; every individual likely possesses at least one unique mutation. Second, other molecular techniques might reveal more fine-scaled geographic structuring than we have found. Third, local phenotypic adaptations might be maintained by natural selection in different parts of the range. Our data indicate, however, that no particular segment of the range of California Gnatcatchers has been evolving independently long enough (i.e., $2N_{ef}$ generations on average; Avise 1994) to have developed an exclusive set of mtDNA haplotypes (Fig. 2), and if geographic structure were found with another molecular marker (such as microsatellites), in our opinion it would be evolutionarily less "significant" than evolutionary divisions found in other species (Table 1).

Although extrapolations from our gnatcatcher study about the general significance of avian subspecies must be made with caution, our results are consistent with other studies (Ball & Avise 1992). We summarized data (Table 1) for phylogeographic surveys of 17 avian species in North America. For these species, the average number of subspecies is 6.5, and the average number of ESUs is 1.7. The data set itself is biased because the average number of subspecies per North American passerine species is 3.3 ± 3.9 (SD, n = 234, Klicka & Zink 1999); hence, species studied to date have tended to be those recognized as highly polymorphic based on classical taxonomic criteria. Nonetheless, it is likely that most biological species of birds will contain two or fewer ESUs and that subspecies on average will not be equivalent to ESUs (Avise & Walker 1998). Our findings for the California Gnatcatcher are therefore consistent with those for other bird species (Table 1).

Subspecies limits might not be predictive of ESUs in gnatcatchers and other birds (Ball & Avise 1992) because such limits are often based on single characteristics, such as plumage coloration, size, and shape, that are probably controlled by relatively few genes and influenced individually by different selective pressures. In contrast, neutral genetic characters are more likely to reflect overall demographic events and population history. Based on our mtDNA results, we predict that reanalysis of gnatcatchers will show that inconsistent patterns of variation among single morphological characters caused conflicting taxonomic opinions (Fig. 1) because different authors emphasized different characters. Our finding of no significant genetic divisions explains prior controversy among subspecies schemes: there probably is no general pattern of variation in morphological characters consistent with historical isolation and independent evolution of populations. Thus, preservation of biodiversity in California Gnatcatchers can be considered independent of subspecies designations.

Although northern populations of California Gnatcatchers do not represent discrete elements of biodiversity, our results must be interpreted in a broader context. In recent years, concern over single species has been complemented by ecosystem or community perspectives (Murphy et al. 1994). Our study reinforces this

Table 1. Molecular support for evolutionarily significant units (ESUs) in avian songbirds.[a]

Species	Number of subspecies examined	Number of ESUs
Polioptila californica	3–5	1
Passerella iliaca	15	4[b]
Melospiza melodia	19	1[c]
Spizella passerina	3	1[b]
Geothlypis trichas	6	2[d]
Molothrus ater	2	1[b]
Toxostoma curvirostre	6	2[c]
Pipilo fuscus	7	2[c]
Auriparus flaviceps	3	1[c]
Agelaius phoeniceus	10	1[b]
Campylorhynchus brunneicapillus	7	2[c]
Parus carolinensis	4	2[b]
Parus atricapillus	5	1[b]
Parus hudsonicus	4	2?[b]
Dendroica petechia	2–3	2[b]
Ammodramus maritimus	6–7	2[b]
Ammodramus caudacutus	5	2[b]

[a] *Includes North American studies (Ball & Avise 1992; Zink 1997; Avise & Walker 1998) that include two or more named subspecies for which mtDNA restriction-site or sequencing studies were performed. These studies show that the genetic structure of the California Gnatcatcher is consistent with that of other birds examined with similar molecular approaches.*
[b] *Restriction fragment studies.*
[c] *R. M. Zink, unpublished mtDNA sequence data.*
[d] *J. Klicka, personal communication*

trend because, although the gnatcatcher's widespread distribution, visibility, and legal status make it a good "flagship species" for regional conservation efforts, our genetic data show that the species poorly reflects the endemism of the coastal sage scrub community. Other species are restricted to coastal sage scrub and are relatively unstudied, and many do not share the gnatcatcher's extensive distribution to the southern tip of the Baja peninsula (Atwood 1993). Hence, further loss and fragmentation of coastal sage scrub in the United States might entail a large genetic cost, if not extinction, for other species. Programs focused at the ecosystem or community level, such as the State of California's Natural Community Conservation Planning process (O'Connell & Johnson 1997), appear most relevant to conservation of coastal sage scrub and other threatened ecosystems. That is, preservation of the California Gnatcatcher should be coupled to preservation of the coastal sage scrub ecosystem, rather than the reverse.

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Literature Cited

American Ornithologist's Union. 1989. Thirty-seventh supplement to the American Ornithologists' Union check-list of North American birds. Auk 106:532–538.

Atwood, J. L. 1988. Speciation and geographic variation in Black-tailed Gnatcatchers. Ornithological Monographs 42.

Atwood, J. L. 1991. Subspecies limits and geographic patterns of morphological variation in California Gnatcatchers (*Polioptila californica*). Bulletin of the Southern California Academy of Sciences 90:118–133.

Atwood, J. L. 1993. California Gnatcatchers and coastal sage scrub: the biological basis for endangered species listing. Pages 149–169 in J. E. Keeley, editor. Interface between ecology and land development in California. Southern California Academy of Sciences, Los Angeles.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Avise, J. C., and D. Walker. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the Royal Society of London, Series B 265:457–463.

Baker, A. J., and H. D. Marshall. 1997. Mitochondrial control region sequences as tools for understanding evolution. Pages 51–82 in D. P. Mindell, editor. Avian molecular evolution and systematics. Academic Press, New York.

Ball, R. M., Jr., and J. C. Avise. 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. Auk 109:626–636.

Barrowclough, G. F., and N. Flesness. 1996. Species, subspecies, and races: the problem of units of management in conservation. Pages 247–254 in D. G. Kleiman et al., editors. Wild mammals in captivity: principles and techniques. University of Chicago Press, Chicago.

Barrowclough, G. F., and J. G. Groth. 1999. Demographic inferences from coalescent patterns: mtDNA sequences from a population of Mexican Spotted Owls. Pages 1914–1921 in N. Adams and R. Slotow, editors. Proceedings 22nd International ornithological congress. BirdLife South Africa, Johannesburg.

Cronin, M. A. 1997. Systematics, taxonomy, and the endangered species act: the example of the California Gnatcatcher (*Polioptila californica*). Wildlife Society Bulletin 25:661–666.

Erwin, T. L. 1991. An evolutionary basis for conservation strategies. Science 253:75–82.

Hafner, D. J., and B. R. Riddle. 1997. Biogeography of Baja California peninsular desert mammals. Pages 39–68 in T. L. Yates, W. L. Gannon, and D. E. Wilson, editors. Life among the muses: papers in honor of James S. Findley. Special publication 3. The Museum of Southwestern Biology, Albuquerque, New Mexico.

Harpending, H. C. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Human Biology 66:591–600.

Harpending, H. C., S. T. Sherry, A. R. Rogers, and M. Stoneking. 1993. The genetic structure of ancient human populations. Current Anthropology 34:483–496.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnaean Society 58:247–276.

Hillis, D. M., B. K. Mable, A. Larson, S. K. Davis, and E. A. Zimmer. 1996. Nucleic acids IV: sequencing and cloning. Pages 321–381 in D. M. Hillis, C. Moritz, and B. K. Mable, editors. Molecular systematics. 2nd edition. Sinauer Associates, Sunderland, Massachusetts.

Hudson, R. R. 1991. Gene genealogies and the coalescent process. Oxford Survey Evolutionary Biology 7:1–44.

Huelsenbeck, J. P., and B. Rannala. 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. Science 276: 227–232.

Hunter, M. L., Jr., and A. Hutchinson. 1994. The virtues and shortcomings of parochialism: conserving species that are locally rare, but globally common. Conservation Biology 8:1163–1165.

Kishino, H., and M. Hasegawa. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. Journal of Molecular Evolution 29:170–179.

Klicka, J., and R. M. Zink. 1999. Pleistocene effects on North American songbird evolution. Proceedings Royal Society London B 266:695–700.

Kuhner, M. K., J. Yamato, and J. Felsenstein. 1998. Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149:429–434.

Lynch, M., and T. J. Crease. 1990. The analysis of population survey data on DNA sequence variation. Molecular Biology and Evolution 7:377–394.

Mann, C. C., and M. L. Plummer. 1995. California vs. Gnatcatcher. Audubon 104:39–48, 100–104.

Mellink, E., and A. M. Rea. 1994. Taxonomic status of the California gnatcatchers of northwestern Baja California. Western Birds 25:50–62.

Miller, A. H., H. Friedmann, L. Griscom, and R. T. Moore. 1957. Distributional check-list of the birds of Mexico, Part II. Pacific Coast avifauna number 33. Cooper Ornithological Society, Berkeley, California.

Moritz, C. C. 1994. Defining "evolutionarily significant units" for conservation. Trends in Ecology and Evolution 9:373–375.

Murphy, D., et al. 1994. On reauthorization of the endangered species act. Conservation Biology 8:1–3.

National Research Council. 1995. Science and the Endangered Species Act. National Academy Press, Washington, D.C.

Nee, S., E. C. Holmes, A. Rambaut, and P. H. Harvey. 1995. Inferring population history from molecular phylogenies. Philosophical Transactions of the Royal Society of London, Series B 349:25–31.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Neigel, J. E. 1997. A comparison of alternative strategies for estimating gene flow from genetic markers. Annual Review Ecology and Systematics 28:105–128.

O'Connell, M. A., and S. P. Johnson. 1997. Improving habitat conservation planning: the California natural community conservation model. Endangered Species Update 14:1–3, 14.

Rogers, A. R. 1995. Genetic evidence for a Pleistocene population explosion. Evolution 49:608–615.

Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology Evolution 9:552–569.

Ryder, O. A. 1986. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology and Evolution 1:9-10.

Slatkin, M., and R. R. Hudson. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555–562.

Swofford, D. L. 1999. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0b2. Sinauer Associates, Sunderland, Massachusetts.

Taberlet, P. 1996. The use of mitochondrial DNA control region sequencing in conservation genetics. Pages 125–142 in T. B. Smith and R. K. Wayne, editors. Molecular genetic approaches in conservation. Oxford University Press, New York.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Tarr, C. L. 1995. Primers for amplification and determination of mitochondrial control-region sequences in oscine passerines. Molecular Ecology 4:527–529.

Upton, D. E., and R. W. Murphy. 1997. Phylogeny of the side-blotched lizards (Phrynosomatidae: Uta) based on mtDNA sequences: support for a midpeninsular seaway in Baja California. Molecular Phylogenetics and Evolution 8:104–113.

U.S. Department of the Interior and U.S. Department of Commerce. 1996. Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. Federal Register 61:4722–4725.

U.S. Fish and Wildlife Service. 1993. Endangered and threatened wildlife and plants; threatened coastal California Gnatcatcher. Final rule and proposed special rule. Federal Register 58:16742–16753.

U.S. Fish and Wildlife Service. 1995. Endangered and threatened wildlife and plants; notice of determination to retain the threatened status for the coastal California gnatcatcher under the Endangered Species Act. 50 CFR Part 17, 60 FR 15693.

Waples, R. S. 1995. Evolutionarily significant units and the conservation of biological diversity under the endangered species act. American Fisheries Society Symposium 17:8–27.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97–159.

Zink, R. M. 1997. Phylogeographic studies of North American birds. Pages 301–324 in D. P. Mindell, editor. Avian molecular evolution and systematics. Academic Press, San Diego, California.

Zink, R. M., and R. C. Blackwell. 1998. Molecular systematics and biogeography of aridland gnatcatchers (genus *Polioptila*), and evidence supporting species status of the California Gnatcatcher (*P. californica*). Molecular Phylogenetics and Evolution 9:26–32.

Zink, R. M., R. C. Blackwell, and O. Rojas-Soto. 1997. Species limits in the Le Conte's thrasher. Condor 99:132–138.

Appendix

Variable base positions[a] that define the 33 unique haplotypes of the California Gnatcatcher.

	111111111111 133455588800023333333 125640452390163554444555 88 196661197581981673123014 29	Locality[b] (n)
1. CAGN3LA	TTTGCCATCA-GCTAT-G--T--CTT	LA(4), OR(2), RV(2), SD(1), ES(2), ST(2), MS(4), ER(3), SI(1), MU(1), CL(1)
2. CAGN2LA	TTTACCATCA-GCTAT-G--T--CTT	LA(1), ST(1)
3. CAGN39RV	TTTGCCATCA-GCTAT-A--T--CTT	RV(1)
4. CAGN42RV	TTTGCCATCA-GCTAC-G--T--CTT	RV(1)
5. CAGN6OR	TTTGCCATCA-GCTAT-A-----CTT	OR(2)
6. CAGN7OR	TTTGCCATCA-GCTAT-G--T--ATT	OR(1)
7. CAGN43SD	TTTGTCATCA-GCTAT-G--T--CTT	SD(1)
8. CAGN45SD	TTTGCCATCA-GCCAT-G--T--CTT	SD(1)
9. CAGN48ES	TTTGCCATCA-GCTAT-G--TT--CTT	ES(2), ST(1), ER(1)
10. CAGN51ES	TTTGCCATCA-GCTAT-G----CCTT	ES(1), LP(1)
11. CAGN13ST	TTTGCCATCA-GCTGTCG--T--CTT	ST(1)
12. CAGN14ST	TTTGCCATCA-GCCAT-G--TT--CTT	ST(1)
13. CAGN34ST	TTTGCCATCA-GCTAT-G-----CTT	ST(1), CL(1), MU(1), VI(1)
14. CAGN23MS	TTTGCCATCA-GCTGT-G--T--CTT	MS(1)
15. CAGN17ER	TTTGCCATTA-GCTAT-G-T--CTT	ER(1)
16. CAGN27SI	TTTGCCATCA-GCTGT-G-TT--CTT	SI(1)
17. CAGN28SI	TTCGCCATCAAGCTAT-G--T--CTT	SI(1)
18. CAGN38SI	TCTACCATCA-GCTAT-G----CCTC	SI(1)
19. CAGN56SI	TTTGCCATCA-GCTGT-G-----CTT	MU(1)
20. CAGN64MU	TTTGCTATCC-GCTAT-G----CCTC	MU(1)
21. CAGN65MU	CCTACTATCA-GCTAT-G--T--CTT	MU(1)
22. CAGN68MU	TTTGCCACCA-GCTAT-G--T--CTT	MU(1)
23. CAGN19VI	CCTGCCATCA-GTTAT-G--T-CCTC	VI(1)
24. CAGN57VI	TTTGCCGTCA-GCTAT-G--T--CTT	VI(1)
25. CAGN58VI	TTTGCCATCA-ATTAT-G----CCTC	VI(1)
26. CAGN69VI	TTTGCTATCA-GCTGT-G--T--CTT	VI(1)
27. CAGN21LP	TTTGCCATCA-GCTAT-G-TT-CCTT	LP(1)
28. CAGN22LP	TTTGCCATCA-GCTGT-G----CTC	LP(1)
29. CAGN29LP	CTTGCCATCA-GTTAT-G---CCCTT	LP(1)
30. CAGN31LP	TTTGCCATCA-GCTAT-G--T-CCTT	LP(1)
31. CAGN60CL	TTTGCTATCA-GCTAT-G-TT--CTT	CL(1)
32. CAGN61CL	TTTGCTATCA-GCTAT-GTTT--CTT	CL(1)
33. CAGN62CL	TTTGCCATCA-GCTAT-G-TT--CCC	CL(1)
34. BTGN[c]	CTTGCCATTA-GCCGT-G???CCCTC	OUTGROUP

[a] *Positions correspond to positions in the aligned sequence in Genbank AF246931.*
[b] *LA, Los Angeles County; OR, Orange County; RV, Riverside County; ES, Ensenada; ST, San Telmo; MS, Mision San Fernando; ER, El Rosarito; SI, San Ignacio; MU, Mulege; VI, Villa Insurgentes; LP, La Paz; and CL, Cabo San Lucas (for locations see Fig. 1).*
[c] *Black-tailed Gnatcatcher.*

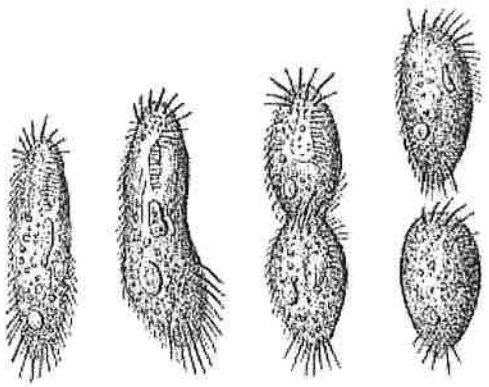

EXHIBIT D

Exhibit D

Reliance on Mitochondrial DNA and Nuclear DNA in Proposed or Final Listings; De-Listings; Findings on Petitions

The U.S. Fish and Wildlife Service (Service) and the National Marine Fisheries Service (NMFS) have relied on evidence provided by mitochondrial DNA (mtDNA_ and nuclear DNA in dozens of decisions to list and delist species as threatened or endangered and in its findings on petitions for the same. *See, e.g., Determination of Endangered Status for the Sonoma County Distinct Population Segment of the California Tiger Salamander*, 68 Fed. Reg. 13,498 (Mar. 19, 2003) (using mitochondrial DNA to support listing of the species); *12-Month Finding for a Petition to List the West Coast Distinct Population Segment of the Fisher* (Martes pennati), 69 Fed. Reg. 18,770 (using mt DNA to find that listing of the species was warranted but precluded); *12-Month Finding on a Petition To List the Northern Leopard Frog in the Western United States as Threatened*, 76 Fed. Reg. 61896 (October 5, 2011) (using mtDNA and nuclear DNA analyses to support conclusion that leopard frog populations are not genetically distinct and finding that listing as DPS is not warranted); *12-Month Finding on a Petition to List the Cactus Ferruginous Pygmy-Owl as Threatened or Endangered with Critical Habitat*, 76 Fed. Reg. 61856 (Oct. 5, 2011) (mtDNA analysis cited to support conclusion that Sonoran desert population of pygmy owl does not satisfy "discreteness" criteria of Distinct Population Segment policy).

The Service has used mtDNA for various reasons, such as to determine whether the species at issue was a subspecies or a distinct population segment or whether the species had hybridized with another species. *See, e.g., Endangered Status for the Rota Bridled White-Eye* (Zosterops rotensis) *from the Commonwealth of the Northern Mariana Islands*, 69 Fed. Reg. 3022 (Jan. 22, 2004) (using mtDNA to establish that the Rota bridled white-eye was a full species rather than a subspecies); *Final Determination of Threatened Status for the Koala*, 65 Fed. Reg. 26,762 (May 9, 2000) (using mtDNA to establish that the Koala was not a distinct population segment); *Determination of Threatened Status for the California Tiger Salamander*, 69 Fed. Reg. 47, 212 (Aug. 4, 2004) (using mtDNA to determine that the California tiger salamander was at risk from hybridization).

The table below is a partial list of listing and delisting matters where the Service or NOAA Fisheries relied upon mtDNA or nuclear DNA in ESA listing/delisting determinations.

Citation	**Species**	**How DNA Used**
79 Fed. Reg. 8656 (February 13, 2014)	Remove the Modoc Sucker From the Federal List of Endangered and Threatened Wildlife	mtDNA used to evaluate extent and role of hybridization of Modoc and Sacramento suckers.
78 Fed. Reg. 61622 (October 3, 2013)	Proposed Threatened Status for the Western Distinct Population Segment of the Yellow-billed Cuckoo (*Coccyzus americanus*)	On basis of mtDNA evaluation, FWS determined that listing as subspecies is not justified.
78 Fed. Reg. 33300 (June 4, 2013)	12-Month Finding and Proposed Endangered Listing of Five Species of Sawfish Under the Endangered Species Act	On basis of mtDNA analysis, NMFS concludes that three species of sawfish should be classified as a single species.
78 Fed. Reg. 24472 (April 25, 2013)	Endangered Status for the Sierra Nevada Yellow-Legged Frog and the Northern District Population Segment of the Mountain Yellow-Legged Frog, and Threatened Status for the Yosemite Toad	mtDNA analysis used to recognize two mountain yellow-legged frog species
77 Fed. Reg. 61938 (October 11, 2012)	Listing Taylor's Checkerspot Butterfly and Streaked Horned Lark and Designation of Critical Habitat	mtDNA used to support subspecies designation of streaked horned lark
77 Fed. Reg. 5880 (February 6, 2012)	Threatened and Endangered Status for Distinct Population Segments of Atlantic Sturgeon in the Northeast Region	mtDNA and nuclear DNA analysis used to identify distinct population segments
76 Fed. Reg. 63720 (October 13, 2011)	12-Month Finding on a Petition to List a Distinct Population Segment of the Red Tree Vole as Endangered or Threatened	mtDNA analysis used to support finding regarding listing of distinct population segment

Citation	**Species**	**How DNA Used**
76 Fed. Reg. 61896 (October 5, 2011)	12-Month Finding on a Petition to List the Northern Leopard Frog in the Western United States as Threatened	mtDNA and nuclear DNA analysis support conclusion that leopard frog populations are not genetically distinct and finding that listing as DPS is not warranted
76 Fed. Reg. 61856 (October 5, 2011)	12-Month Finding on a Petition to List the Cactus Ferruginous Pygmy-Owl as Threatened or Endangered with Critical Habitat	mtDNA analysis cited in conclusion that Sonoran desert population of pygmy owl does not satisfy "discreteness" criteria of DPS policy
76 Fed. Reg. 58868 (September 22, 2011)	Determination of Nine Distinct Population Segments of Loggerhead Sea Turtles as Endangered or Threatened	mtDNA and nuclear DNA relied on to determine "discreteness" and "significance" of sea turtle populations.
76 Fed. Reg. 48777 (August 9, 2011)	12-Month Finding on a Petition to List the Nueces River and Palteau Shiners as Threatened or Endangered	mtDNA analysis used to identify separate species of shiners
76 Fed. Reg. 48721 (August 9, 2011)	Endangered Status for the Cumberland Darter, Rush Darter, Yellowcheek Darter, Chucky Madtom, and Laurel Dace	Recognition of full species status of *E. n. susanae* based on analyses of mtDNA *for E. n. susanae* and *E. n. nigrum.*
76 Fed. Reg. 45130 (July 27, 2011)	12-Month Finding on a Petition to List the Gopher Tortoise as Threatened in the Eastern Portion of Its Range	Analyses of mtDNA and nuclear DNA indicate a long-term population decline since the Pleistocene era of *G. polyphemus* in the western portion of its range
76 Fed. Reg. 40822 July 12, 2011	Endangered Status for the Largetooth Sawfish	NMFS relied on mtDNA to determine species' range is the eastern and western Atlantic Ocean
76 Fed. Reg. 31556 (June 1, 2011)	Endangered Species Act Listing Determination for Atlantic Bluefin Tuna	NMFS referred to mtDNA analyses to conclude that the western Atlantic and eastern Atlantic/Mediterranean populations represent two DPSs of Atlantic bluefin tuna

Citation	**Species**	**How DNA Used**
76 Fed. Reg. 14210 (March 15, 2011)	Withdrawal of Proposed Rule to List the Flat-Tailed Horned Lizard as Threatened	mtDNA and nuclear DNA data used to identify three distinct population segments and to determine that fourth population did not satisfy "discretensess" and "significance" criteria of DPS policy
75 Fed. Reg. 70169 (November 17, 2010)	Proposed Endangered Status for the Hawaiian Insular False Killer Whale Distinct Population Segment	NMFS relied on mtDNA and nuclear DNA to identify DPS
75 Fed. Reg. 65239 (October 22, 2010)	Threatened Status for the Southern Distinct Population Segment of the Spotted Seal	NMFS relied on mtDNA to identify DPS
75 Fed. Reg. 61872 (October 6, 2010)	Proposed Listing Determinations for Three Distinct Population Segments of Atlantic Sturgeon in the Northeast Region	NMFS relied on mtDNA and nuclear DNA to support identification of DPS
75 Fed. Reg. 39656 (July 12, 2010)	90-Day Finding on Petitions to List the Porbeagle Shark under the Endangered Species Act	NMFS relied on mtDNA to support finding that there was insufficient evidence to support listing DPS
74 Fed. Reg. 23376 (May 19, 2009)	12-Month Finding on a Petition to List the Coaster Brook Trout as Endangered	Service concludes that brook trout in the upper Great Lakes, including all life forms, do not differ markedly from other populations of the species in their genetic characteristics population is not a listable entity
72 Fed. Reg. 43560 (August 6, 2007)	Final Rule to Remove the Idaho Springsnail (*Pyrgulopsis* (*=Fontelicella) idahoensis*) from the List of Endangered and Threatened Wildlife	Service relied on mtDNA and nuclear DNA to conclude that Idaho springsnail no longer constitutes a distinct species

Citation	**Species**	**How DNA Used**
71 Fed. Reg. 56228 (September 26, 2006)	12-Month Finding on a Petition to List the Northern Mexican Gartersnake (*Thamnophis eques magalops*) as Threatened or Endangered with Critical Habitat	Service relies on absence of genetic information indicating differences between Mexican and U.S. populations to determine that U.S. population is not a DPS
70 Fed. Reg. 5404 (February 2, 2005)	12-Month Finding on a Petition To Delist the Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*) and Proposed Delisting of the Preble's Meadow Jumping Mouse	mtDNA used to support lack of separate subspecies/delisting of species
69 Fed. Reg. 76673 (December 22, 2004)	Proposed Threatened Status for Southern Resident Killer Whales	MtDNA and microsatellite DNA used to support DPS of species
69 Fed. Reg. 47212 (August 4, 2004)	Determination of Threatened Status for the California Tiger Salamander	MtDNA used to support threatened status of species
69 Fed. Reg. 43664 (July 21, 2004)	Removing the Eastern Distinct Population Segment of the Gray Wolf From the List of Endangered and Threatened Wildlife	MtDNA used to determine if animals captured and killed in the Northeastern US were gray wolves
69 Fed. Reg. 21151 (April 20, 2004)	90-Day Finding on a Petition To List the Colorado River Cutthroat Trout	MtDNA used to support non-threatened or endangered status of species
69 Fed. Reg. 18770 (April 8, 2004)	12-month Finding for a Petition to List the West Coast Distinct Population Segment of the Fisher (Martes pennanti)	mtDNA used to support DPS of species

Citation	**Species**	**How DNA Used**
69 Fed. Reg. 16944 (March 31, 2004)	90-Day Finding for a Petition to Delist the Preble's Meadow Jumping Mouse in Colorado and Wyoming	mtDNA used in petition to delist species
69 Fed. Reg. 13326 (March 22, 2004)	90-Day Finding on a Petition To Delist the Pacific Coast Population of the Western Snowy Plover	mtDNA discussed in petition to delist species (finding that delisting may be warranted)
69 Fed. Reg. 6600 (February 11, 2004)	Proposed Rule Listing the Southwest Alaska Distinct Population Segment of the Northern Sea Otter (*Enhydra lutris kenyoni*) as Threatened	mtDNA and microsatellite DNA used to support DPS of species
69 Fed. Reg. 3022 (January 22, 2004)	Endangered Status for the Rota Bridled White-Eye (*Zosterops rotensis*) From the Commonwealth of the Northern Mariana Islands	mtDNA used to support finding of no subspecies (it is a full species)
69 Fed. Reg. 933 (January 7, 2004)	90-day Finding for a Petition To List the Eastern Subspecies of the Greater Sage-Grouse as Endangered	mtDNA used to support finding of no subspecies
68 Fed. Reg. 53947 (September 15, 2003)	12-Month Finding on a Petition to List the Northern and Florida Panhandle Loggerhead Sea Turtle (*Caretta caretta*) Subpopulations as Endangered	mtDNA and microsatellite DNA used to support finding of no DPS
68 Fed. Reg. 46989 (August 7, 2003)	Reconsidered Finding for an Amended Petition To List the Westslope Cutthroat Trout as Threatened Throughout Its Range	mtDNA used to support non-threatened or endangered status of species

Citation	**Species**	**How DNA Used**
68 Fed. Reg. 34628 (June 10, 2003)	Status Review and 12-Month Finding for a Petition To List the Washington Population of the Western Gray Squirrel	mtDNA and microsatellite DNA used to support finding of no DPS
68 Fed. Reg. 28648 (May 23, 2003)	Proposed Listing of the Central California Distinct Population Segment of the California Tiger Salamander	mtDNA used to support finding of DPS
68 Fed. Reg. 20228 (April 24, 2003)	Determination of Distinct Vertebrate Population Segment for the California Gnatcatcher (*Polioptila californica*)	mtDNA used to question whether species was subspecies
68 Fed. Reg. 13498 (March 19, 2003)	Determination of Endangered Status for the Sonoma County Distinct Population Segment of the California Tiger Salamander	mtDNA used to support finding of DPS
68 Fed. Reg. 11574 (March 11, 2003)	12-month Finding for a Petition To List the Lower Kootenai River Burbot (Lota lota-) as Threatened or Endangered	mtDNA used to support finding of no DPS
68 Fed. Reg. 10388 (March 5, 2003)	Final Rule to List the Columbia Basin Distinct Population Segment of the Pygmy Rabbit (*Brachylagus idahoensis*) as Endangered	mtDNA used to support finding of DPS
68 Fed. Reg. 7580 (February 14, 2003)	12-Month Finding for a Petition To List the California Spotted Owl (*Strix occidentalis occidentalis*)	mtDNA used to support finding of subspecies

Citation	**Species**	**How DNA Used**
68 Fed. Reg. 6500 (February 7, 2003)	90-day Finding on a Petition To List the Western Sage Grouse	mtDNA used to support finding of no subspecies
68 Fed. Reg. 4433 (January 29, 2003)	12-Month Finding on a Petition to List North American Green Sturgeon as a Threatened or Endangered Species	mtDNA used to support finding of separate species and of DPS
68 Fed. Reg. 2283 (January 16, 2003)	12-Month Finding for a Petition To List the Sierra Nevada Distinct Population Segment of the Mountain Yellow-legged Frog (*Rana muscosa*)	mtDNA used to support finding of DPS
67 Fed. Reg. 75834 (December 10, 2002)	12-Month Finding for a Petition to List the Yosemite Toad	mtDNA used to support finding of one species
67 Fed. Reg. 47726 (July 22, 2002)	Listing the Sonoma County Distinct Population Segment of the California Tiger Salamander as Endangered	mtDNA used to support finding of DPS
67 Fed. Reg. 44382 (July 2, 2002)	Determination of Endangered Status for the Southern California Distinct Vertebrate Population Segment of the Mountain Yellow-Legged Frog (*Rana muscosa*)	mtDNA used to support finding of DPS
67 Fed. Reg. 44133 (July 1, 2002)	12-Month Finding for a Petition To List Southern Resident Killer Whales as Threatened or Endangered Under the Endangered Species Act (ESA)	mtDNA and microsatellite DNA used to support finding of no DPS

Citation	**Species**	**How DNA Used**
67 Fed. Reg. 40790 (June 13, 2002)	Listing of the Chiricahua Leopard Frog (*Rana chiricahuensis*)	mtDNA discussed in finding of threatened species
67 Fed. Reg. 38459 (June 4, 2002)	90-day Finding for a Petition to Reclassify the Northern and Florida Panhandle Subpopulations of the Loggerhead as Distinct Population Segments with Endangered Status and to Designate Critical Habitat	mtDNA used to support finding of DPS
67 Fed. Reg. 21586 (May 1, 2002)	Range Extension for Endangered Steelhead in Southern California	mtDNA used to support range extension
66 Fed. Reg. 59734 (November 30, 2001)	Emergency Rule To List the Columbia Basin Distinct Population Segment of the Pygmy Rabbit (*Brachylagus idahoensis*) as Endangered	mtDNA used to support finding of DPS
66 Fed. Reg. 50383 (October 3, 2001)	Proposed Endangered Status for the Rota Bridled White-Eye (*Zosterops rotensis*-) From the Commonwealth of the Northern Mariana Islands	mtDNA used to support finding of separate species
66 Fed. Reg. 38611 (July 25, 2001)	12-Month Finding for a Petition To List the Yellow-billed Cuckoo in the Western Continental United States	mtDNA used to support finding of DPS
66 Fed. Reg. 22984 (May 7, 2001)	12-Month Finding for a Petition To List the Washington Population of Western Sage Grouse (*T4Centrocercus urophasianus phaios*-)	mtDNA used to support finding of DPS

Citation	**Species**	**How DNA Used**
66 Fed. Reg. 15643 (March 20, 2001)	Final Rule To Remove the Aleutian Canada Goose From the Federal List of Endangered and Threatened Wildlife	mtDNA used to support finding of need for delisting
65 Fed. Reg. 79328 (December 19, 2000)	Proposed Range Extension for Endangered Steelhead in Southern California	mtDNA used to support finding of range extension
65 Fed. Reg. 57242 (September 21, 2000)	Final Rule To List the Santa Barbara County Distinct Population of the California Tiger Salamander as Endangered	mtDNA used to support finding of DPS
65 Fed. Reg. 35033 (June 1, 2000)	Proposed Endangered Status for the Buena Vista Lake Shrew	mtDNA used to support finding of subspecies
65 Fed. Reg. 26762 (May 9, 2000)	Final Determination of Threatened Status for the Koala	mtDNA used to support finding of no DPS
65 Fed. Reg. 26438 (May 5, 2000)	Final Rule To List the Alabama Sturgeon as Endangered	mtDNA used to support finding of separate species
65 Fed. Reg. 25867 (May 4, 2000)	Reclassification of Yacare Caiman in South America From Endangered to Threatened	mtDNA used to support finding of one species
65 Fed. Reg. 3096 (January 19, 2000)	Emergency Rule To List the Santa Barbara County Distinct Population of the California Tiger Salamander as Endangered	MtDNA used to support finding of DPS
65 Fed. Reg. 20 (January 3, 2000)	Final Rule To List the Sierra Nevada Distinct Population Segment of the California Bighorn Sheep as Endangered	mtDNA used to support finding of DPS

Citation	**Species**	**How DNA Used**
64 Fed. Reg. 62627 (November 17, 1999)	Proposed Endangered Status for a Distinct Population Segment of Anadromous Atlantic Salmon (*Salmo salar*) in the Gulf of Maine	mtDNA used to support finding of DPS
64 Fed. Reg. 50394 (September 16, 1999)	Threatened Status for Two Chinook Salmon Evolutionarily Significant Units (ESUs) in California	mtDNA used to support finding of ESU
64 Fed. Reg. 42058 (August 3, 1999)	Proposal To Remove the Aleutian Canada Goose From the List of Endangered and Threatened Wildlife	mtDNA used to support finding of need for delisting
64 Fed. Reg. 33816 (June 24, 1999)	Proposed Rule to Remove the Northern Populations of the Tidewater Goby From the List of Endangered and Threatened Wildlife	mtDNA used to support finding of DPS
64 Fed. Reg. 14676 (March 26, 1999)	Proposed Rule To List the Alabama Sturgeon as Endangered	mtDNA used to support finding of separate species
63 Fed. Reg. 56596 (October 22, 1998)	Proposed Threatened Status for the Gulf of Maine Population of Harbor Porpoise	mtDNA used to question whether species was DPS
63 Fed. Reg. 50850 (September 23, 1998)	Proposed Reclassification of Yacare Caiman in South America From Endangered to Threatened	mtDNA used to support finding of one species
63 Fed. Reg. 26517 (May 13, 1998)	Final Rule to List the Preble's Meadow Jumping Mouse as a Threatened Species	mtDNA used to support finding of subspecies
63 Fed. Reg. 11798 (March 10, 1998)	Proposed Threatened Status for Two ESUs of Steelhead in Washington and Oregon	mtDNA used to support finding of ESU

Citation	**Species**	**How DNA Used**
62 Fed. Reg. 66325 (December 18, 1997)	Withdrawal of Proposed Rule to List a Distinct Population Segment of Atlantic Salmon (*Salmo Salar*) as Threatened	mtDNA used to support finding of DPS
62 Fed. Reg. 24345 (May 5, 1997)	Change in Listing Status of Steller Sea Lions Under the Endangered Species Act	mtDNA used to support finding of DPS
62 Fed. Reg. 14093 (March 25, 1997)	Proposal To List the Preble's Meadow Jumping Mouse as an Endangered Species	mtDNA discussed in finding of subspecies
62 Fed. Reg. 665 (January 6, 1997)	Determination of Endangered Status for Three Wetland Species Found in Southern Arizona and Northern Sonora, Mexico	mtDNA used to support finding of subspecies
61 Fed. Reg. 41541 (August 9, 1996)	Proposed Endangered Status for Five ESUs of Steelhead and Proposed Threatened Status for Five ESUs of Steelhead in Washington, Oregon, Idaho, and California	mtDNA used to support finding of ESU
60 Fed. Reg. 51968 (October 4, 1995)	Change in Listing Status of Steller Sea Lions Under the Endangered Species Act	mtDNA used to support finding of DPS
60 Fed. Reg. 47338 (September 12, 1995)	12-Month Finding for a Petition To List the Southern Population of Walleye as Endangered	mtDNA used to support finding of DPS
60 Fed. Reg. 38011 (July 25, 1995)	Proposed Threatened Status for Three Contiguous ESUs of Coho Salmon Ranging From Oregon Through Central California	mtDNA used to support finding of ESU

Citation	**Species**	**How DNA Used**
60 Fed. Reg. 16836 (April 3, 1995)	Proposal To Determine Endangered Status for Three Wetland Species Found in Southern Arizona and Northern Sonora	mtDNA used to support finding of subspecies
60 Fed. Reg. 13397 (March 13, 1995)	90-Day Finding and Initiation of Status Review for a Petition To List the Southern Population of the Walleye as Endangered	mtDNA used to support finding of DPS
59 Fed. Reg. 64794 (December 15, 1994)	Withdrawal of Proposed Rule for Endangered Status and Critical Habitat for the Alabama Sturgeon	mtDNA used to support finding of one species
59 Fed. Reg. 31970 (June 21, 1994)	Extension of the Final Decision To List the Mobile River System Population of the Alabama Sturgeon as an Endangered Species With Critical Habitat	mtDNA used to support finding of separate species
58 Fed. Reg. 65325 (December 14, 1993)	Notice of 90-Day Findings on Petitions To List Three Southern Arizona Cienega Species	mtDNA used to support finding of subspecies
58 Fed. Reg. 3108 (January 7, 1993)	Listing of the Gulf of Maine Population of Harbor Porpoise as Threatened under the Endangered Species Act (ESA)	mtDNA used to support finding of DPS
57 Fed. Reg. 43676 (September 22, 1992)	Finding on Petition to List the Paddlefish	mtDNA used to support finding of no DPS
57 Fed. Reg. 28167 (June 24, 1992)	Notice of 90-Day Findings on Petitions to List the Corral Beach Sand Dune Weevil and to Delist the San Joaquin Kit Fox	mtDNA used to support finding of DPS

Citation	**Species**	**How DNA Used**
57 Fed. Reg. 1246 (January 13, 1992)	Finding on a Petition to Delist the Red Wolf (*Canis rufus*)	mtDNA used to support finding that species is not a hybrid
57 Fed. Reg. 588 (January 7, 1992)	Threatened Status for the Louisiana Black Bear and Related Rules	mtDNA used to support finding of subspecies
56 Fed. Reg. 56325 (November 4, 1991)	Determination of Experimental Population Status for an Introduced Population of Red Wolves in North Carolina and Tennessee	mtDNA used to support finding that species is not a hybrid
56 Fed. Reg. 47732 (September 20, 1991)	Extension of Proposed Rule To List the Louisiana Black Bear as Threatened	mtDNA used to support finding of subspecies
Status 55 Fed. Reg. 51506 (December 12, 1990)	Reclassification of the Aleutian Canada Goose From Endangered to Threatened	mtDNA used to support finding of subspecies
55 Fed. Reg. 51112 (December 12, 1990)	Final Rule to Delist the Dusky Seaside Sparrow and Remove its Critical Habitat Designation	mtDNA discussed in finding of subspecies
55 Fed. Reg. 49656 (November 30, 1990)	Notice of Finding on a Petition to Delist the Gray Wolf (*Canis lupus*)	mtDNA discussed in finding that delisting is not warranted
55 Fed. Reg. 25341 (June 21, 1990)	Proposed Threatened Status for the Louisiana Black Bear. Proposed Designation of Threatened by Similarity of Appearance of all Bears of the Species *Ursus americanus* Within the Historic Range of U. a. luteolus	mtDNA discussed in finding of subspecies
55 Fed. Reg. 17555 (April 25, 1990)	Proposed Rule to Delist the Dusky Seaside Sparrow and to Remove its Critical Habitat Designation	mtDNA discussed in finding of subspecies

Citation	**Species**	**How DNA Used**
55 Fed. Reg. 12178 (April 2, 1990)	Determination of Threatened Status for the Mojave Population of the Desert Tortoise	mtDNA used to support finding of DPS
54 Fed. Reg. 40142 (September 29, 1989)	Proposed Reclassification of the Aleutian Canada Goose From Endangered to Threatened	mtDNA used to support finding of subspecies
54 Fed. Reg. 32833 (August 10, 1989)	Notice of Finding on Petition To List the Louisiana Black Bear	mtDNA discussed with respect to subspecies