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To: United States Fish and Wildlife Service

Re: Listing decision for Tucson shovel-nosed snake (*Chionactis [occipitalis] annulata klauberi*)

This letter offers comments on facts and interpretations given in the decision by the U.S. Fish and Wildlife Service (the Service, USFWS) not to list the Tucson shovel-nosed snake (TSS) as threatened under the Endangered Species Act (ESA) as presented in the following documents:

- "Not Warranted" Finding: USFWS (2014a: Federal Register / Vol. 79, No. 184 / Tuesday, September 23, 2014 / Proposed Rules: 50 CFR Part 17, [Docket No. FWS–R2–ES–2014–0035: 4500030113]. Endangered and Threatened Wildlife and Plants; 12-Month Finding on a Petition To List the Tucson Shovelnosed Snake as Endangered or Threatened); and
- Species Status Assessment Report ("SSA Report"): USFWS (2014b: Species Status Assessment Report For the Tucson Shovel-Nosed Snake (*Chionactis occipitalis klauberi*), Prepared by the Arizona Ecological Services Field Office, U.S. Fish and Wildlife Service, Date of last revision: August 26, 2014).

The SSA Report is authoritatively cited or the only basis presented by the Service in the "Not Warranted" Finding to sustain statements and interpretations that I question in the present letter. Therefore my comments deal directly with the SSA Report, and thereby impinge on key aspects of the "Not Warranted" Finding. The decision was further based, in substantial part, on recent genetic work in Wood et al. (2014: Wood, D. A., R. N. Fisher, and A. G. Vandergast. 2014. Fuzzy boundaries: color and gene flow patterns among parapatric subspecies of the Western Shovel-nosed Snake (*Chionactis occipitalis*). PLOS ONE 9: 1489–1507), which I also discuss. Wood et al. (2014) newly recognize two species (*C. occipitalis*, the Mojave Desert shovel-nosed snake, and *C. annulata*, the Sonoran Desert shovel-nosed snake) within the western shovel-nosed snake group, or *Chionactis occipitalis* complex, with the TSS now recognized as *Chionactis annulata klauberi*. In this letter, I follow this new taxonomic arrangement as it helps to clarify reality and will contribute to further understanding of the origin, conservation status, and ecology of the group.

In my comments, I address five problems with the agency's "Not Warranted" Finding and the SSA Report:

1. The TSS, especially, as well as *C. annulata* and all members of the *C. occipitalis* complex, are habitat specialists, not generalists as stated by the agency (USFWS 2014 a&b).
2. The TSS has experienced severe declines in the core of its genetic distribution, contradicting statements by the agency (USFWS 2014 a&b).
3. The TSS is vulnerable to habitat fragmentation. Evidence appears to contradict the statement in the SSA Report that, "The limited movement data available suggests that Tucson shovel-nosed snakes do not move far and, therefore, may not require large tracts of Desertscrub habitat."
4. The habitat model in the SSA Report depicting the local distribution of the TSS is erroneous and overestimates the distributional boundaries of the taxon.
5. Most critically, the assumed extent and shape of the geographic distribution for the TSS referenced in the "Not Warranted" Finding and mapped in the SSA Report is arbitrarily large, and inconsistent with the key source referenced (Wood et al. 2014).

I address these five issues in sequence.

Issue 1 – **Specialized ecology of the TSS and *Chionactis occipitalis* complex**

According to the SSA Report (pp. iii, ix, x, and 17), "The Tucson shovel-nosed snake appears to be a habitat generalist." This is a misinterpretation. The *C. occipitalis* complex snakes – all – are well-known habitat specialists, largely to entirely restricted to sand and sandy loam substrates and to valley floors and relatively level lower bajadas. In the Mojave Desert, where massive wind-blown and alluvial sand formations may mix with rocks, *C. occipitalis* may be found among or near rocks, but this represents a mixture of habitat types (see Klauber 1951), rather than habitat selection by a habitat generalist.

Although beyond the scope of this letter to provide a detailed quantitative analysis of habitat specialization in Sonoran Desert region snakes, it is possible to note that few species are 100% restricted – as is *C. annulata* – to desertscrub, not occurring also in adjacent environments (semi-desert grassland, interior chaparral, and Sinaloan thornscrub); and only the sidewinder rattlesnake is similarly confined to fine soils and low slope value areas (i.e., flats and lower bajadas). Yet even the sidewinder extends further upslope or more frequently onto coarser bajada soils than does *C. occipitalis*. Although semi-aquatic and other primarily non-desert snake species can be more restricted in habitat association within the Sonoran Desert, no desert snake species in the region can be considered to specialize as closely to a definable macrohabitat category as does *C. annulata*, for even the rock-dwelling specialist species extensively utilize surrounding bajadas.

Considering only the Sonoran Desert species *C. annulata*, there is even less association with rocky environments. Although one publication reports a population from rocky slopes, as a new species, *C. saxatilis* (Funk 1967), this report has been criticized: the specimens are missing, and the species is no longer recognized. Even in very arid regions where it is abundant, the species is not found in rocky environments (González-Romero and Alvarez-Cárdenas 1989). I have examined all published and museum locality records of the species in Arizona, and none of them are for rocky environments. Where they occur near rocks, these are slopes partially submerged in sediment derived from larger mountain ranges, and thus lacking bajada development with coarse soils including gravel and cobble. The marked specialization of *C. annulata* for utilization of flat to nearly flat desert valley environments of low perennial plant diversity was demonstrated by multivariate methods and detailed by Rosen et al. (1996) for the region of Organ Pipe Cactus National Monument.

Considering the relatively less arid region occupied by the TSS ecotypes, habitat specialization has been found at an even finer scale. In Avra Valley and Santa Cruz Flats, all records of the species were from mid-valley flats, none were from lower bajadas, and within mid-valley flats all records were from sandy soils and none were from clay soils (Rosen 2003, 2004, 2008). The only other area that has been thoroughly surveyed within the region occupied by genetically and morphologically core populations of the TSS is along the highway from Florence to Florence Junction, Pinal County, Arizona. I ground-truthed all records in this region in 2008. The species is absent from south of Florence in coarse sandy loams with arborescent desert, and absent from the Gila River floodplain, occurring only in the creosote-dominated flats north of Gila River. Within the occupied area, records essentially end to the north where the first saguaro is visible along the highway. These specifics indicate how remarkably specialized the species is, and the extreme habitat specialization of the TSS in the core of its range.

While secondary habitats may yield occurrence records, as near Santan Mountains (Wood et al. 2008, 2014), these cannot be expected to provide long-term persistence for the species when its mid-valley flats and lowermost bajada habitats are eliminated or severely compromised, as seen in Avra Valley (see [2] and [3], below). In this sense, the issue of habitat specialization is important for the conservation biology of the TSS. Because the TSS is unable to persist in areas lacking its specialized habitat requirements, the snake will likely be extirpated in areas where these essential habitats are destroyed or fragmented through urbanization or other development.

Issue 2 – **Severe declines of the TSS**

Although USFWS (2014a&b) state that populations of the TSS that have been formally surveyed are stable or show no declines (SSA Report; p. 54: "Throughout the areas that have had systematic surveys, as described above, populations of snakes appear to be stable and persisting"), it is difficult to align this statement with the demonstrated decline and apparent extinction of most or all populations of the TSS in Avra Valley and Santa Cruz Flats, where the most systematic surveys have been done (Rosen 2003, 2004, 2008; all three of which are cited in the SSA Report).

Quoting from Rosen (2003, sentences 1 and 2):

> "Briefly, despite an intensive and productive June survey, we found no Tucson Shovel-Nosed Snakes in Avra Valley. They were last verified in Avra Valley in 1979, and could possibly be regionally extinct."

From Rosen (2004, sentence 2, after an additional year of intensive survey, and referring first to Avra Valley):

> "As previously reported, we found no Tucson Shovel-nosed Snakes (*Chionactis occipitalis klauberi*) in 2003, but one was found near Picacho in 2004, the first regional record since 1979, showing that the species is not regionally extinct, and may yet be found in Avra Valley."

Thus, the nearest record after two years of intensive survey lies 34 km from the core of the population that existed in Avra Valley until at least 1979.

After an additional year of intensive sampling, including drift fence trapping, Rosen (2008, p. 4) reported:

> "The findings strongly confirm the previous indications that the Tucson Shovel-nosed Snake has declined precipitously in Avra Valley, and that the Banded Sand Snake has increased in observable abundance in at least many of the same areas the shovel-nosed snake has declined. We cannot confirm the complete extirpation of the shovel-nosed snake in the Avra Valley based on these sample sizes, but it seems increasingly probable that it does not currently live in eastern Pima County."

It is true that we cannot be certain that the Tucson Shovel-nosed Snake is completely extinct in the large portion of its range in Avra Valley and north into Santa Cruz Flats: but the extent of decline to apparent extinction was highlighted in Rosen (2003, p. 10):

> "… the Shovel-Nosed Snake was reasonably abundant and reliably found, with up to 2-3 being observed per night of road driving, during the 1970's (C. R. Schwalbe, personal communication). This confirms the severe decline of the Shovel-Nosed Snake."

To my knowledge, no other "systematic surveys" examining evidence for long-term stability decline have been reported. During the 2003 survey alone, all 17 other snake species previously recorded or reasonably expected in Avra Valley were observed: only the Tucson Shovel-nosed Snake was not found, nor has it been reported since then, an absence of 35 years in an intensively studied region where other snake collectors have also been very active.

Whereas the taxon persists in other areas, there is no evidence pertaining to whether it is stable, declining, or increasing in abundance in those areas, few of which have any measure of protection. Persistence of the species in currently less impacted areas than Avra Valley and in two local areas on the periphery of Santa Cruz Flats is certainly encouraging, but given the known declines and apparent extinctions, this is weak evidence upon which to base a critical conclusion of non-decline.

Further, Rosen (2008) demonstrated that other species with similar habitat requirements, including lizards, birds, and mammals, appear to also be declining in Avra Valley and Santa Cruz Flats, which suggests that the decline of the Tucson Shovel-nosed Snake may be only the best-known symptom of collapse of the regional valley floor ecosystem.

Issue 3 – **Vulnerability to habitat fragmentation**

Statements related to home range or movements in USFWS (2014b) that, (1) "The limited movement data available suggests that Tucson shovel-nosed snakes do not move far and, therefore, may not require large tracts of Desertscrub habitat." (p. 56); and (2) "Based on this, we assume that both individuals and populations can successfully maintain their redundancy and representation in relatively small areas of suitable habitat;" (p. 22), are unsupported by any substantial data and contradicted by the species' decline in the Avra Valley.

After considering several alternative hypotheses for declines and apparent extinctions of the Tucson Shovel-nosed Snake in Avra Valley, I found (Rosen 2008, p. 14):

> "A reasonable synthetic hypothesis is that habitat destruction and fragmentation (conversion of level, valley floor habitat from Lower Colorado Valley Sonoran Desertscrub to agriculture, along with urban and suburban development and road mortality) has undermined the metapopulation dynamics of species that specialize in that natural habitat in the study region."

Regardless of which hypothesis or combination of hypotheses most nearly represents what happened in Avra Valley, the timing of extirpation presents a troubling prospective. Habitat modification and fragmentation of that region – primarily by agricultural developments – were made during the 1930s to 1940s. TSS then survived for approximately 40-50 years (to ca. 1979 – the last reported record). The time course for decline thus appears to be on the order of several decades, and this reveals a potentially serious concern for many small remnant populations of the TSS that have been documented in recent years in the peripheral sprawl of metropolitan Phoenix.

In context of the disappearance of the TSS in Avra Valley, the other remarkable change in the regional snake assemblage was proliferation of the banded sandsnake (or "bandless sandsnake," *Chilomeniscus cinctus*, also referred to as *C. stramineus*) documented in Rosen (2003, 2004, 2008). This species is ecologically similar to the TSS in diet and microhabitat use, but has a broader macrohabitat niche, occurring in canyon bottoms, along major arroyos, and on bajadas and even, at least locally, on rock slopes. Its center of distribution is in the Arizona Upland Sonoran Desertscrub, and is rare in the core of Lower Colorado River Valley Sonoran Desertscrub; where *Chionactis annulata* is more widespread and abundant, as noted in the SSP Report (p. 42):

> "As a species, the shovel-nosed snake currently persists, often in abundance, within portions of its range (e.g., southwestern Arizona and southeastern California) that experience less precipitation and higher temperatures and are characterized by simpler vegetation communities (Turner and Brown 1982, pp. 190–202) than that found within the range of the Tucson shovel-nosed snake. Hence, if climates dry and become warmer, with concomitant changes in vegetation communities, the Tucson shovel-nosed snake may be able to persist under those conditions."

This optimistic suggestion, however, is contradicted by our surveys in the Avra Valley. According to these and other surveys, the TSS occurs in the less arid transitional region of the Lower Colorado River Valley Sonoran Desert province. Under drought conditions, which are projected to worsen under climate change, surviving species may be those that more successfully utilize the less arid parts of the landscape, such as canyons and major arroyos. Given the rising temperatures and substantial drought episodes from the 1960s through 1970s when the TSS declined and disappeared from Avra Valley, it appears that drought, and thus climate change, may be a threat to the TSS. As conditions worsen, those species in the most arid environments may suffer most; they may become extinct, leaving behind species less adapted to increasing aridity. The subsequent expansion of *Chilomeniscus* in Avra Valley after its first detection in 1981 occurred during wet years from 1977-1984 and 1990-1995, which is consistent with this scenario. These are the kinds of metapopulation dynamics referred to in my quotation above. There is, therefore, little reason to be optimistic that the TSS will survive rapid climate change and, with other arid-adapted species, be available as part of the regional fauna as aridity spreads. This scenario should be alarming, as it is the drought-adapted species that would, in the aftermath of climate change impacts, be the best adapted.

During our Avra Valley surveys in the 2000s we were to some extent hampered by dry conditions. The most striking aspect of drought was in habitat fragments most suitable for the TSS based on vegetation, soils, and historical records. It was difficult to find any snakes at all in these places and appeared, in the field, that these arid local environments were the ones where snakes were most affected by drought. It is thus of concern that climate change was more or less dismissed as a threat in the SSA Report.

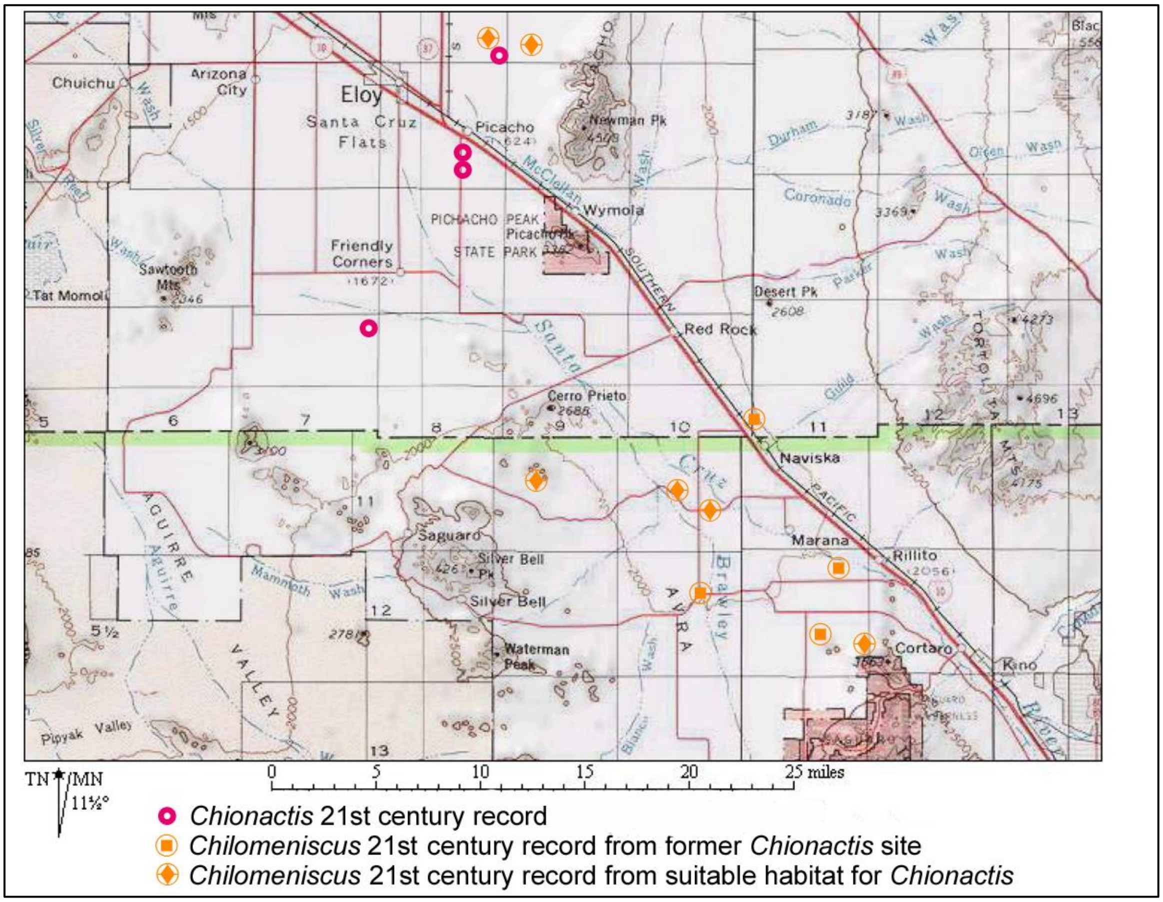

Figure 1. Replacement of the Tucson shovel-nosed snake by the banded sandsanke in the Avra Valley region (from Rosen 2008).

Issue 4 – **Habitat model accuracy**

The habitat model for the TSS presented in USFWS (2014b) is shown below with my annotations. I am only knowledgeable enough about the southeastern one-sixth of the depicted range on the map to offer definitive evaluation herein. However, it is clear the habitat model represents as habitat a large area (which I have enclosed in a black-dashed ellipse) that is non-habitat. A major, heavily collected highway transects the area, with no records obtained. The area has coarser loams than usually occupied by the TSS and is largely covered by rich, arborescent Sonoran Desertscrub not utilized by the taxon. The extent to which this egregious error afflicts the rest of the map is not clear, but it is possible that other non-habitat areas of significant size are also misrepresented as habitat. I am collaborating on a new habitat model that will be available soon, and should help clarify this issue.

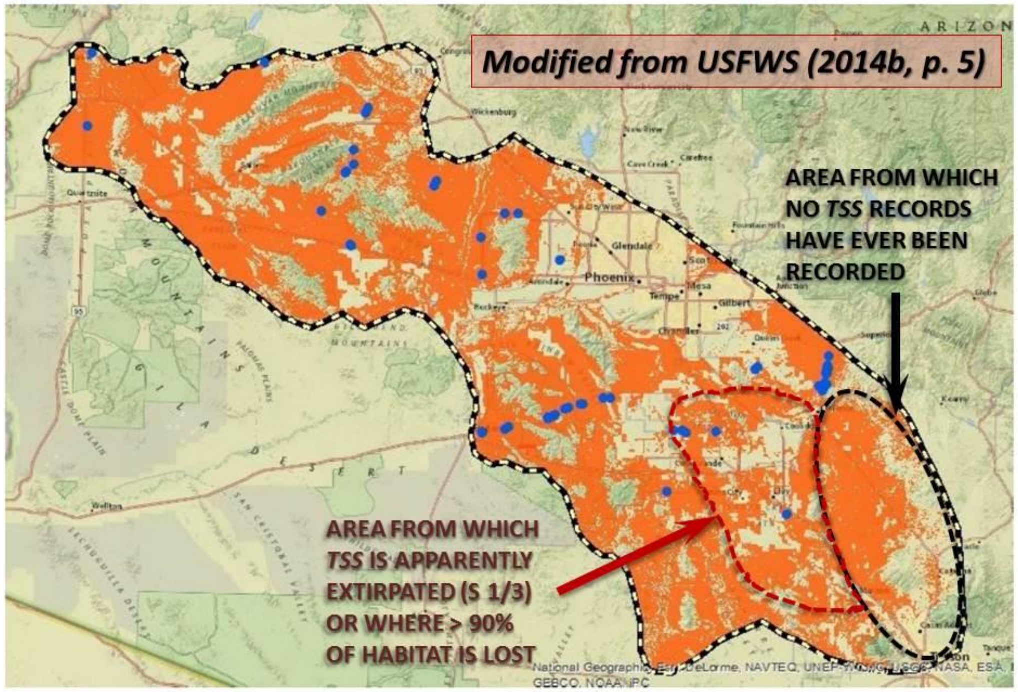

Figure 1. Suitable habitat throughout the estimated range of the Tucson shovel-nosed snake. Orange areas represent suitable habitat below 1,500 m

Figure 2. My annotations (large type, arrows, and dashed lines) of the habitat model of the Tucson shovel-nosed snake according the SSA Report (USFWS 2014b).

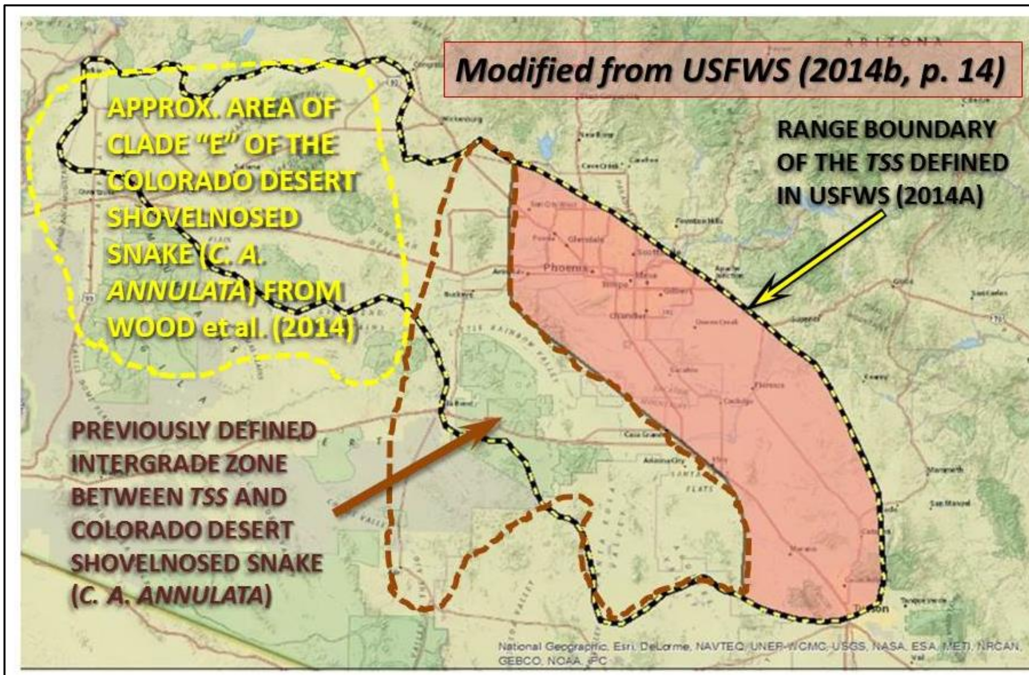

Figure 3. Current estimated range of the Tucson shovel-nosed snake based upon work conducted by Wood *et al*. (2014, in press) and the former representation of the range of the Tucson shovel-nosed snake (red-shaded area) as described by Mahrdt *et al.* (2001, p. 731.2).

Figure 3. My annotations (large type, arrows, and dashed lines) of the distributional map of the Tucson shovel-nosed presented in the SSA Report (USFWS (2014b).

Issue 5 – **Extent and shape of range of the TSS as indicated by genetic studies**

The most essential element in the decision not to list the TSS (USFWS 2014a) was the greatly expanded geographic distribution adopted by the Service (see map below), which the Service based on Wood et al. (2014). The vast extension of the proposed distribution, far to the west of the previously defined distribution and intergrade zone, is indicated on the map below.

Importantly, Wood et al. (2014) never conclude that the snake's range should be expanded to include the area near the California border. Instead, the study at page 9 remarks that the observed phenotypic variation in:

> "northwestern Maricopa county . . . corroborate[s] well with the inferred cluster boundaries" [*of Figure 4 and Figure 5a*], "which suggests that the western boundary of [the Tucson shovel-nosed snake] may be more extensive than previously assumed" (*emphasis added*)."

Thus, the Wood et al. (2014) paper offers some support for expanding the snake's range into that one county but not as far west as the California boundary, as the agency assumed and used to justify the "Not Warranted" Finding.

Specifically, Wood et al. (2014, p. 10) wrote:

> "In the same way, haplotypes of *C. a. annulata* from west-central Arizona (clade E) formed a well-supported mtDNA clade, but could not be grouped with confidence to either the Colorado or Sonoran lineages. However, nuclear genotypes of these same samples were assigned to the genetic cluster containing the majority of all other *C. a. annulata* samples."

Thus, Wood et al. (2014) are clearly stating and supporting a concept of the range excluding almost the entire region west of Maricopa County that the Service included in making its determination, as shown here in my Fig. 3.

Although Wood et al.'s (2014) discussion of the genetic and geographic history of *C. occipitalis* evolution and differentiation is complicated, it unequivocally contradicts the map adopted in the SSA Report. I outlined in yellow-dash on the map (Fig. 3) the area the Service included that should, instead, either (1) be included within the Colorado Desert shovel-nosed snake distribution, or (2) be recognized as a separate area within the complex history of the species but within the TSS.

Thus, the pivotal mapping adopted by the Service for the distribution of the TSS (2014b) doesn't agree with the critical document from the published literature (Wood et al. 2014). Further, morphological data (color pattern) argues strongly against the vastly expanded range proposed by the Service.

The situation involves, as emphasized by Wood et al. (2014 in the title ("Fuzzy Boundaries"), a more complicated phylogeographic history of the *C. occipitalis* complex. Based on mitochondrial DNA (mtDNA) differentiation within the complex began with divergence of a Mojave Desert lineage (now solely carrying the name *C. occipitalis*) and a *sensu lato* Sonoran Desert lineage (now with name *C. annulata* as a full species) comprising a complex lineage history that includes both the Colorado Desert shovel-nosed snake (*C. a. annulata*) and the TSS (*C. a klauberi*). This usage of "Colorado Desert" (which has been used to refer to the Lower Colorado River Valley (LCV) Sonoran Desert in California, west of Colorado River and south of Mojave Desert, is useful but novel in including the LCV of southwestern Arizona. It is also appropriate, as well as useful, at least in this context in reflecting the strong similarity of the herpetofauna of the entire LCV with that of the Mojave Desert and the marked difference between it and the herpetofauna of the Arizona Upland Sonoran Desert, which has strong affinities to Sinaloan Thornscrub (Rosen 2007). Fig. 2 from Wood et al. is shown here as Fig 4.

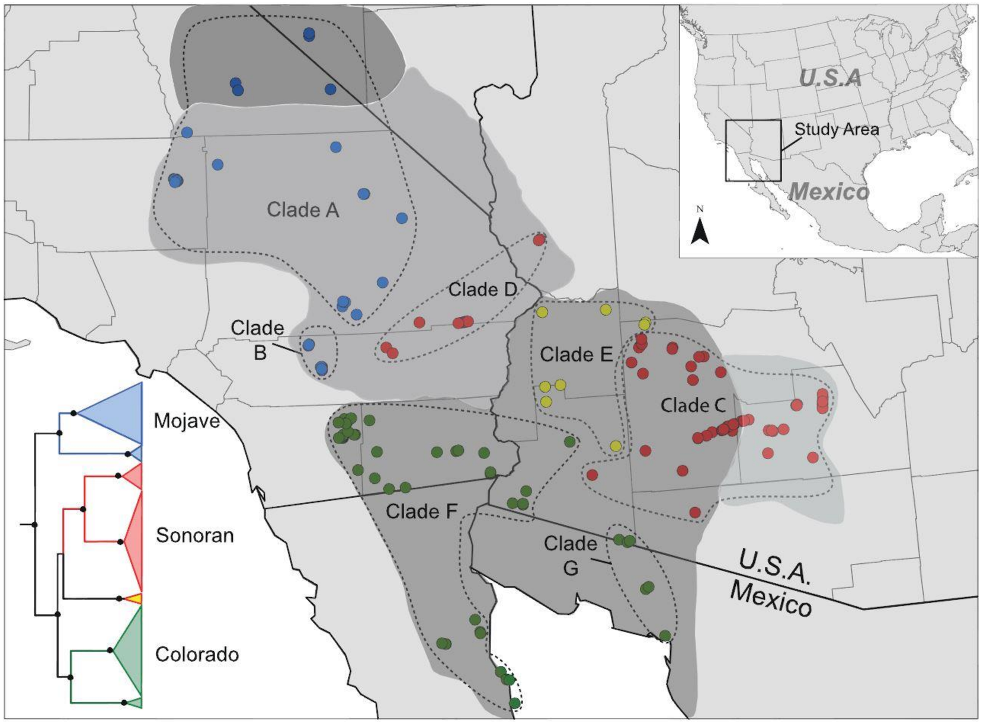

Figure 2. Phylogeny for the western shovel-nosed snake (*Chionactis occipitalis*) based on partitioned Bayesian analysis of mitochondrial DNA sequence data (16S rRNA and ND1 genes) and the geographic distribution of the major lineages (Mojave lineage in blue, Sonoran lineage in red, and Colorado lineage in green). The geographic distribution of clades within each lineage are outlined with a dashed line, clade E (yellow clade and dots) was routinely placed as sister to the Sonoran lineage, but posterior probabilities supporting this relationship were weak ($Pp < 0.50$). Black circles at nodes represent Bayesian posterior probabilities of ≥ 0.95. See Figures S1-3 for more detail within each mtDNA lineage.
doi:10.1371/journal.pone.0097494.g002

Figure 4. mtDNA clades and their distribution in the *C. occipitalis* complex (from Wood et al. 2014).

The relatively more ancient split of Mojave and *sensu lato* Sonoran lineages (mtDNA clades C-G) within the complex is supported strongly by microsatellite (msat) DNA from the nuclear chromosomes (nDNA). However, the situation is more complex. (Phylogenetic splitting sequences can be inferred from mtDNA but not readily from the msat nDNA.) The mtDNA shows a second ancient split between the Colorado Desert lineage and a less arid-associated Sonoran Desert lineage that includes the TSS. However, mtDNA from the latter Sonoran lineage (*sensu stricto*, which may be termed, for convenience, the "Northern Sonoran lineage") also occupies a region of the eastern Mojave Desert as well as the northeastern Sonoran Desert (Fig. 4). And, as noted above, a third clade ("E") within *C. annulata* is interposed between the Northern Sonoran mtDNA clusters in eastern Mojave Desert and northeastern Sonoran Desert, thus helping delineate the distribution of the TSS and its intergrade zone with *C. a. annulata*. Clade "E" represents an ancient split within *C. annulata,* so close to the split between Colorado Desert and Northern Sonoran lineages that it cannot now be known to be closer to one than the other (an "unresolved trichotomy" in phylogenetics-speak; see phylogram at lower left in Fig. 4). Thus, the geographic range the Service used in the SSA report to justify the "Not Warranted" Finding is strongly inconsistent with the phylogenetic history of the *C. occipitalis* complex by including well-resolved mtDNA clades that clearly do not align with TSS.

These unexpected findings by Wood et al. (2014) demonstrate a complex history of *C. annulata* within the Sonoran Desert region, and extending into the eastern Mojave Desert where the two deserts have, and have had, close biogeographic and ecological interaction. The relatively ancient interaction is indicated by the presence of Northern Sonoran (*C. annulata*) mtDNA in the eastern Mojave Desert. This indicates either (1) secondary transgression by the Northern Sonoran lineage across Colorado River into Mojave Desert, with traces of this introgression into the gene pool of *C. occipitalis* remaining only in the mtDNA (and not in the nDNA; see Wood et al. 2014); or (2) an ancient distribution of Northern Sonoran shovel-nosed snakes from eastern Mojave Desert across central Arizona into southeastern Arizona (near Tucson) which was subsequently obliterated (by *C. occipitalis* nDNA in Mojave Desert; and by Clade "E" mtDNA and, largely, by *C. a. annulata* nDNA in northwestern Arizona).

Thus, overlying the patterns shown by the mtDNA are new nDNA data presented by Wood et al. (2014). They analyzed these nDNA data in two ways that produce overlapping insights into the genetic structure of the species *C. annulata*; the results are shown here as Figs. 5 and 6.

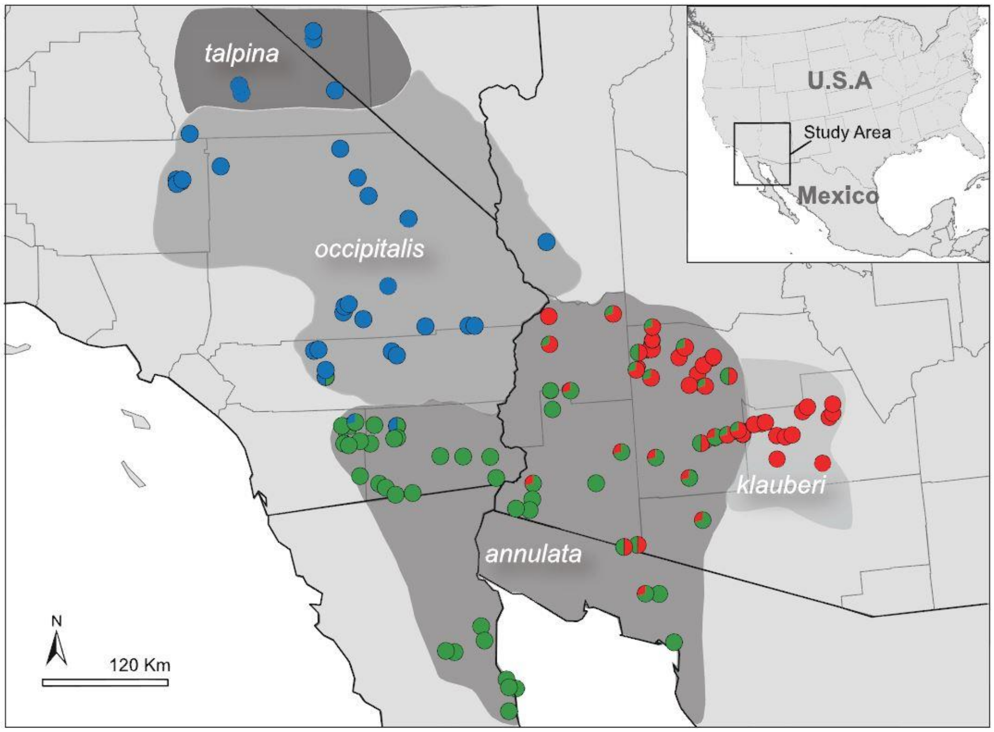

Figure 4. Range-wide geographic distribution of the three clusters inferred by STRUCTURE (*K* = 3) overlaid on the subspecies distributions. Circles on the map are colored proportional to their posterior probability assignment to each of the three clusters.

Figure 5. One of two depictions of geogrpahic variation of microsatellite DNA in the *C. occipitalis* complex (from Wood et al. 2014).

Figure 5 shows that nDNA loosely associated with Northern Sonoran lineage (in red) is found from the northeastern Sonoran Desert (i.e., *C. a. klauberi* as originally defined) west-northwest across the northern part of the Sonoran Desert in Arizona where it is extensively mixed with nDNA from *C. a. annulata* (in green). Although Wood et al. (2014) suggest introgression of TSS genes into *C. a. annulata*, I suggest that the ancient Northern Sonoran mtDNA relict in Mojave Desert tends to indicate introgression from *C. a. annulata* into an earlier wider distribution of the Northern Sonoran lineage. This requires further study. In either case, the SSA Report (USFWS 2014b) defined the distribution of the TSS primarily based only on Fig. 5 (note: this is Fig. 4 of Wood et al. 2014).

Figure 6 shows a second exploration of the nDNA data. This analysis integrates genetic and spatial data to yield a spatially weighted scenario of population genetic structure of *C. annulata*. As in Fig. 5, the most consistent clusters are *C. a. annulata* in southwestern Arizona and *C. a. klauberi* in the northeastern Sonoran Desert. Fig. 6 is thus more similar than Fig. 5 to the existing taxonomy based on color pattern and scalation for the two subspecies, and also indicates the intergrade zone connecting the two subspecies.

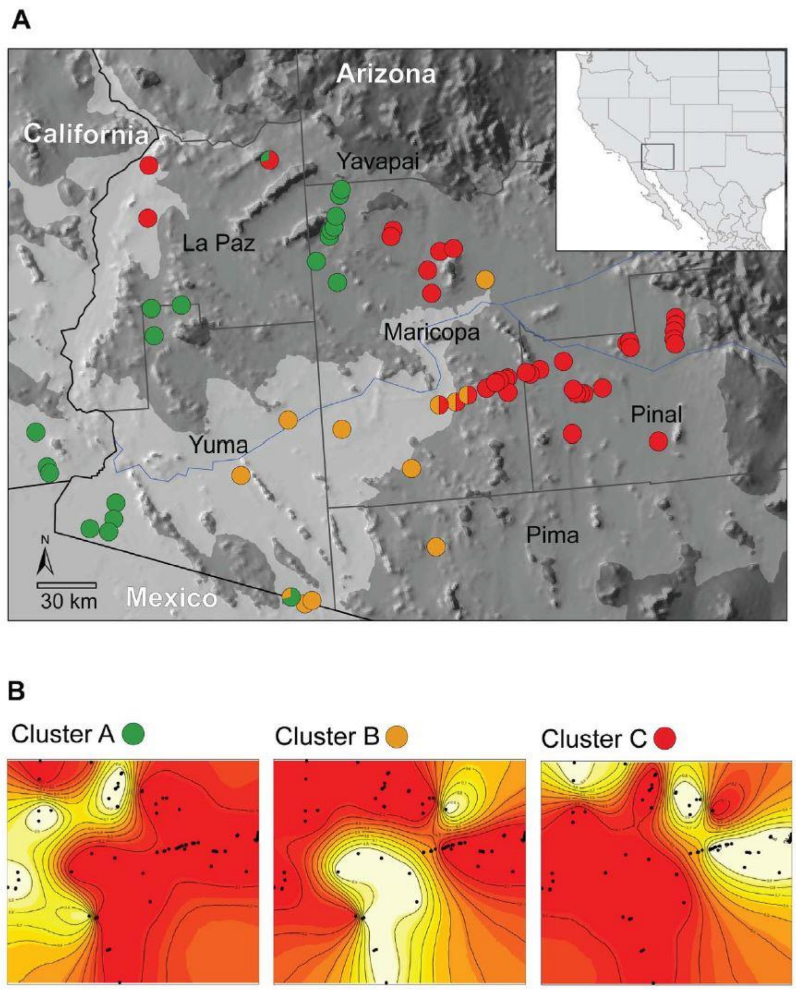

Figure 5. Spatially-explicit estimate of population structure across Arizona based on the GENELAND analysis. a) Map of Arizona and the geographic distribution of sampled individuals colored according to the probability of assignment within each cluster identified using GENELAND (green, orange, and red circles represent individuals assigned to Clusters A, B, and C, respectively). The shading indicates elevations below 300m (light grey), between 300 and 1000m (grey) and above 1000m (dark grey). **b**) Geneland probability contour maps for the three clusters across Arizona. The highest posterior probabilities are in white ($p \geq 0.9$) and the lowest are in red ($p \leq 0.1$) – contour lines within each map depict the spatial change in population assignment probability.

Figure 6. The second depiction of geogrpahic variation of microsatellite DNA in the *C. occipitalis* complex (from Wood et al. 2014).

Considering all three sets of genetic analyses (mtDNA and the two analyses of nDNA; Wood et al. 2014) does not support the Service's mapping of a wide-ranging TSS. The genetic data at this stage of the research do suggest a somewhat wider distribution of the TSS than the earlier mapping (Mardt et al. 2001), extending toward northwestern Maricopa County. Remarkably similar spatial complexity regarding the distribution of the TSS and other taxa in the *C. occipitalis* complex was summarized for color and scalation data by Cross (1979, pp. 403-422), who also noted that populations in in northern La Paz County, Arizona, showed traces of *C. a. klauberi* "influence" but were primarily linked to other populations and taxa within the complex. Therefore, the Service should re-evaluate the status of the TSS based on a narrower concept of the taxon – geographically restricted to northwestern Maricopa County, east-central Maricopa County, and Pinal County and, if the species can be found, in northeastern Pima County.

Such a re-evaluation should assign weight to those portions of the TSS range in which its unique morphology characteristics are most strongly expressed and where genetic data consistently demonstrate it presence as an entity. Based on the complexity Cross (1979) found, further genetic data are likely to significantly improve our ability to understand both the phylogeographic and adaptational history of the Tucson Shovel-nosed Snake, and thus improve our understanding of its conservation status.

Adaptive hypotheses and considerations for legal protection

Here I provide hypotheses and information on the adaptive significance of the entity generally known as the TSS. I relate this to the genetic and distributional facts and hypotheses clarified in the preceding sections of this letter. I then relate these adaptive and historical perspectives to the general validity of attempts to legally protect the Tucson shovel-nosed snake.

Genetic data *in toto* (Wood et al. 2008, 2014) demonstrate patterns much like data from morphology and coloration (Klauber 1951; Cross 1979). Intraspecific variation is extensive within both the *C. occipitalis* complex as a whole and within *C. annulata*. The genetic data demonstrate a deep historical context to this variation. The TSS, in the narrower conception (as in Mardt et al. 2001) is, within the *C. occipitalis* complex, among the most unique, striking, and geographically cohesive examples of such variation. A reasonable working hypothesis for the several relatively cohesive regional populations within the complex is that these entities represent the outcome of adaptive evolution to regional conditions overlain on a historically complex substrate of genetic variation. In this sense, the currently defined taxa (*C. o. occipitalis, C. a. annulata, and C. a. klauberi*) can be conceived of as adaptive syndromes rooted in a more ancient phylogeographic substrate.

This conception raises novel questions in two related areas: (1) what are the regional adaptive phenomena and how important are they for the future of species; and (2) how do we arrive at decisions about protecting such adapted entities? Here I describe hypotheses for the adaptive syndrome of *C. a. klauberi* before turning to the question of what we might consider important to protect.

Broadly understood, the Northern Sonoran lineage and especially the TSS occur in relatively less arid desertscrub than the rest of *C. occipitalis* complex. The TSS does not live in sand dunes, which are absent in its range (and in the intergrade zone as mapped here and by Mardt et al. 2001). In contrast, the other major lineages do occur and are often abundant in sand dunes and associated sand sheets. Overall, these other sand-associated lineages occur on more pallid soils than does the TSS.

In addition, the TSS and, in some areas such as the Organ Pipe Cactus National Monument region, *C. a. annulata* X *klauberi* intergrades, live in relatively complex snake assemblages where Sonoran coralsnakes (*Micruroides euryxanthus*), with which they are part of a mimicry complex, are abundant. The other lineages and intergrade populations generally occur where coralsnakes are rare, local, or absent (see Brennan and Holycross 2006). For example, a search of museum records for the Sonoran coralsnake revealed that only 3 of 142 records from Arizona were from counties (Yuma, La Paz, and Mohave) in the arid desert regions; in Avra Valley and Santa Cruz Flat, my records indicate the coralsnakes comprise ≈ 0.9 % of the snake community sample based on road-driving; at Organ Pipe they are about 1 %; in traps at Organ Pipe, 2.2 %; whereas Jones et al. (2011) recorded no coralsnakes in a road-cruising sample of 572 snakes in the intergrade zone west and southwest of Phoenix that represents the more arid portions of Maricopa County, Arizona. Therefore, in the core of the TSS distribution (*sensu* Mardt et al. 2001) as well as in portions of the intergrade zone best known to support *C. annulata* with clear expression of TSS traits (Klauberi 1951; Cross 1979; pers. obs.) shovel-nosed snake are sympatric with relatively strong populations of coralsnakes.

How might these two ecological factors, darker, less sandy habitat and abundant coralsnakes, likely relate to the unique traits of *C. a. klauberi*? First, in the more heavily vegetated, less sandy soils in the habitat of the TSS, background matching using flicker-fusion is improved by the addition of black pigment that partially obscures the red body bands or rings. Flicker fusion in coral snakes and their mimics is the blending of colors (reds, blacks and yellows or whites) as the snake moves quickly to create a visual mirage of a background-matching brown or tan. On more pallid, sandier and dune backgrounds, the absence or scarcity of black dorsal pigment instead produces a lighter shade of brown with flicker-fusion of the colors.

Although the red coloration can thus contribute to predator escape by crypsis (background matching), it is believed to function primarily for aposematic mimicry of coralsnakes. The hypothesized functionality involves the mimetic red coloration producing a hesitation in or delay or deflection of predator attack, during which the mimetic snake initiates a rapid retreat and “disappears” due to flicker fusion. Aposematic red coloration in snakes, identifying its bearer as potentially dangerous to attack, is almost always carried by fast, agile snakes that can benefit from the flicker fusion effect. This general mimetic system is widespread in snakes, especially in regions where coralsnakes occur, but its effects are understood to occur beyond the geographic range of coralsnakes. Nonetheless, natural selection in mimetic systems is stronger where model and mimic are sympatric.

The red color, therefore, is ultimately thought to be mimetic of dangerously venomous coralsnakes, such as the Sonoran coralsnake *Micruroides euryxanthus* in the Southwest. Therefore, we can hypothesize that the TSS ecotype is more strongly affected by natural selection related to coralsnake mimicry than other lineages within the group, and the Mojave shovel-nosed snakes, which lives in a biotic community where coralsnakes are entirely absent, red coloration in shovel-nosed snakes is weakest. In the Sonoran Desert of Arizona, bright red is best developed in the Sonoran shovel-nosed snake (*C. palarostris*) and is also conspicuous in much of the range of the Colorado Desert shovel-nosed snake (*C. a. annulata*). The latter may also benefit most strongly from flicker fusion because its escape by submergence into the sand of sand sheets and dunes is so fast.

The mimetic coloration of *C. a. klauberi* and intergrade populations (*sensu* Mardt et al. 2011) is hypothesized to involve another feature of mimetic systems, in addition to flicker fusion. A characteristic of many mimicry systems is color variability in context of evolutionary “arms races” between models (tending to evolve to be more distinctive and often thereby to be more conspicuous) and mimics (tending to evolve to resemble the model and often thereby to compromise crypsis). As a result, considerable color variation and polymorphism are often found in both models and mimics in these systems. Both Sonoran coralsnakes and the TSS and intergrades have marked variability in the amount of black pigment suffused dorsally and laterally over the bright red body bands. We can hypothesize that in this evolutionary race, both model and mimic experience natural selection for variable conspicuousness in context of model-mimic coevolutionary and flicker fusion selective forces.

Thus, there are good reasons to believe that the TSS and other regional lineages and populations of shovel-nosed snake display adaptive variation as well as deep historical variation in genetic traits, noting that adaptive hypotheses should be examined further, particularly as genetic methods become available to apply to them. What, therefore, may be considered important for preservation of genetic variation in the species complex? Traditionally in recent decades, systematists have tended to recognize and the Service has tended to protect species (often described subspecies that are elevated to species status) based on ancient historical divergence, which is demonstrated in many cases using presumptively neutral (i.e., non-adaptive) genetic variation.

In this case, the genetic data show a complex (“fuzzy”) pattern sufficient to support continued recognition of *C. a. klauberi* (Wood et al. 2014) but do not yet clear enough to allow us to draw a definitive boundary between taxa or to precisely delineate the zone of intergradation between taxa. These data do provide sufficient guidance to suggest a significantly more limited range for the TSS than that used by the Service in the SSA Report and “Not Warranted” Finding. The geographic range of the adaptive syndrome of the TSS, discussed here, provides further guidance: it also indicates a more restrictive view for the distribution of the taxon. Protection of the significant entity could best be served by adopting a restrictive definition of the distribution based on a correct interpretation of current genetic evidence, and giving special weight to those parts of the range where adaptive phenotypic traits are fully and consistently displayed.

The practical implication of this is that a reasonable distributional area for conservation evaluation of the TSS includes the earlier-mapped distribution of subspecies (Mardt et al. 2001), parts of the intergrade zone (e.g., east of the Maricopa Mountains), an area in northwestern Maricopa County and adjoining southwestern Yavapai County, and, in case the population still exists, the Avra Valley region of northeastern Pima County. The relatively “pure” TSS populations should be accorded special weight. The precise distribution considered would likely best be defined by a group of biologists most experienced with the species, in consultation including the Service.

The decline of the Tucson shovel-nosed snake is indicative of threats to an entire regional ecosystem. Urban sprawl in the specialized habitat of the taxon is rampant throughout most of the affected region and can be expected to expand unchecked and largely unregulated in coming decades. At present, it appears that no law or agency other than the ESA and USFWS have any potential to provide guidance that will avoid the essentially complete destruction of valley floor ecosystems, the TSS, and numerous other specialized species in the northeastern Sonoran Desert. Without ESA listing, this ecological devastation will likely proceed to completion without even serious biological assessment. Based on (1) the problems I have identified with the Service’s SSA Report and “Not Warranted” Finding; (2) the review presented here of the ecology, status, genetics, and adaptive significance of the TSS; and (3) the critical situation of an entire regional ecosystem on which the TSS and many other species are completely dependent; I propose that the Service should re-open the listing decision for *Chionactis annulata klauberi*.

Philip C Rosen

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