## **Letter to the Editor:**

## A single sample is not enough to claim systematic conclusions, much less for taxa of conservation concern: comments on Jaramillo and Ruiz-García (2022)

<u>Jaramillo and Ruiz-García (2022)</u> provide new information on aspects of the phylogeography of *Nasua narica*, particularly for Central America and northern South America. Nevertheless, we disagree with these authors on some fundamental points and emphatically reject their conclusion that "the molecular evidence seems to reject the validity of Nasua narica nelsoni (N. nelsoni)." Here we point out errors in interpretation and scope that the authors attribute to their data, as well as to previously published information on the Cozumel dwarf coati, *Nasua nelsoni*.

Since conservation efforts in México, and worldwide, tend to prioritize species and, to a lesser extent, subspecies, or populations (<u>Garner et al. 2005</u>; <u>Gippolliti and Amori 2007</u>; <u>Ceballos et al. 2017</u>), such errors of interpretation are not trivial. In fact, they can have negative consequences because their conclusions are directly linked to the assessment and attention, in terms of conservation, that can and should be given to a taxonomic entity whose geographic distribution is extremely restricted and has a critically endangered population scenario.

When <u>Jaramillo and Ruiz-García (2022)</u> point out that their evidence does not support the validity of the specific identity and even of the singularity at the subspecies level of the Cozumel dwarf coati (*Nasua nelsoni*) they make an interpretation and an assertion that is too categorical for the amount and quality of their data. Moreover, it is based on an incorrect and incomplete consideration of the previously published evidence in this regard, as we demonstrate here.

A single sample is not enough. The categorical conclusions of <u>Jaramillo and Ruiz-García</u> (2022) regarding *N. nelsoni* are based on data from a single specimen. Moreover, they do not specify the origin of this individual, including the specific collection locality. That is, the authors do not present the most basic information in mammalogical research, including whether this specimen was captured alive and belonged –or not– to a wild population; or if it was an opportunistic sampling of a dead animal, was a captive individual or a museum specimen. Furthermore, the genetic data (mitochondrial sequence) of this specimen is not publicly available (as of the time we submitted this letter), while the GenBank accession numbers they provide (page 4; MT587713-MT587788, MW410859-MW410914, and MW419814-MW419853) are of 306 bp length (not the 2,153 and 16,200 bp they claimed were analyzed). In addition, it should be noted that captive mainland coatis (*N. narica*) have been introduced and released onto Cozumel Island (Cuarón et al. 2004; J. C. González Malpica, pers. com.). Genetic evidence supports this assertion (Flores-Manzanero et al. 2022). Thus, it cannot be discarded that <u>Jaramillo and Ruiz-García (2022)</u> may have analyzed a sample of a *N. narica* specimen on Cozumel Island. In fact, *N. narica* is among the species of mammals with highest cultural value in México, and very commonly used as pet, particularly in southern México (Cuarón 1997; Ávila-Nájera et al. 2018; Herrera-Flores et al. 2019). Some of these animals are taken to places well beyond their site of capture within the country (Cuarón 2005).

Jaramillo and Ruiz-García (2022) cite Nigenda-Morales et al. (2019), indicating that we used nine coati specimens from Cozumel and that we did not find obvious differences (at the molecular level) between these individuals and other samples from Yucatán or northern Guatemala. However, in Nigenda-Morales et al. (2019) we made a rather relevant precision to avoid over interpretation of the data: "Nonetheless, the number of samples from Cozumel was low (eight samples) and seven of the samples were from pet or captive raised animals that may have been derived from the mainland and transported to the island. Therefore, we suggest caution in interpreting our results regarding the genetic status of the coati samples from Cozumel. Given the uncertainty about the taxonomic status of the coati population on Cozumel, a more extensive analysis, including more samples and additional loci, will be required to reach any conclusions that could affect the conservation efforts of this population".

Furthermore, <u>Jaramillo and Ruiz-García (2022)</u> incorrectly cite and interpret <u>Glatston (1994)</u> when they say: "We agree with Glatston (1994) that this coati was introduced to Cozumel by the Mayans, although we do not agree that N. nelsoni is a full species". What <u>Glatston (1994)</u> states is: "It has been suggested that these animals were introduced to Cozumel by the Maya (Bixler, unpubl.)", although she does not present empirical evidence in this respect.

Regarding the possible introduction of *N. nelsoni* to Cozumel, by the early Mayas, there is crucial information that <u>Jaramillo and Ruiz-García (2022)</u> do not consider. On the one hand, in <u>McFadden et al. (2008)</u> we clearly indicate that our data (with n = 2) do not enable asserting whether *N. nelsoni* colonized Cozumel before or after the Maya. On the other hand, there is a critical reference on this matter, not considered by <u>Jaramillo and Ruiz-García (2022)</u>. That study is <u>Hamblin (1994)</u>,

in which the author presents detailed information on the remains of wild fauna found in Mayan archaeological sites at Cozumel Island. The author reports relevant data on the size of the bones and on the abundance of the remains of the different species found, to derive conclusions about the possible pre-Maya colonization of the island. She found that N. nelsoni bones accounted for 92 % of all procyonid bones and estimated that these remains represented at least 88 individuals with dimensions consistent with very small animals (adults and juveniles); she also found evidence that they were hunted locally and were well distributed, suggestive of their settlement on the island prior to the Mayan colonization of Cozumel.

Finally, our recent key study, Flores-Manzanero et al. (2022; available online since September 2021) is, to date, the most updated and complete on the genetic diversity and divergence, both on a historical and contemporary scale, of the Cozumel dwarf coati. Thus, the statement by Jaramillo and Ruiz-García (2022) about "... an original population persists in the island, which had not been sampled...", is wrona.

In Flores-Manzanero et al. (2022) we analyzed samples from 46 individuals of the Cozumel dwarf coati (that were captured alive on site) and performed a comprehensive battery of analyzes based on both mitochondrial (cytochrome b) and nuclear (microsatellite) information. The results show a clear genetic differentiation between Cozumel dwarf coatis and Nasua narica individuals from the mainland. These results support that, at least following the precautionary principle, the Cozumel dwarf coati should be considered and managed as a Significant Evolutionary Unit (sensu Moritz 1994). Thus, we presented evidence that supports its uniqueness and our urgent proposal to change its conservation status in the IUCN Red List of Threatened Species to Critically Endangered (see the Appendix in Flores-Manzanero et al. 2022).

Based on all of the above, it is relevant to emphatically reiterate that the conclusion of Jaramillo and Ruiz-García (2022) about the invalidity of Nasua narica nelsoni (N. nelsoni) does not hold.

Other considerations. Jaramillo and Ruiz-García (2022) indicate, in the introduction of their article, that N. n. nelsoni is a recognized subspecies, but they incorrectly associate that point with the work of Merriam (1901). It was Decker (1991) who suggested categorized the Cozumel dwarf coati as a subspecies (N. n. nelsoni). This author analyzed cranial morphometric characteristics for Nasua narica, N. nelsoni and N. nasua. She considered data from more than 100 individuals of N. narica and N. nasua, but only 11 of N. nelsoni. In addition, in most of the statistical comparisons made, the cranial morphometric data of N. narica and N. nasua were compared against data from only 1 to 3 N. nelsoni individuals, significantly reducing the statistical power of the results that sustains **Decker's** (1991) conclusions. Merriam (1901) described the Cozumel dwarf coati (Nasua nelsoni) as a distinct species, based on the minute size of Cozumel coatis in comparison to those from the mainland. This dwarfism pattern is observed with several other taxa from Cozumel Island (Martínez-Morales 1996; Cuarón et al. 2004, 2009; McFadden and Meiri 2013), a pattern consistent with Foster's Rule (McFadden and Meiri 2013).

Jaramillo and Ruiz-García (2022) specify, "McFadden et al. (2008) designated the insular subspecies as a different species (N. nelsoni) based on a morphometric analysis". This statement is incorrect. In McFadden et al. (2008) we start by considering N. nelsoni as a distinct species based on Merriam (1901). Later, based on the molecular analyzes carried out, and being always cautious with our assertions (particularly due to the small sample size used for N. nelsoni, n = 2), we specify that some of the analyzes used support a population differentiation between individuals from the Yucatán peninsula and those from Cozumel (e. q. the global AMOVA), but other results did not (*Fst* was not significant). We clearly stated too in McFadden et al. (2008) that "... although our data do not unequivocally suggest unique species status, we believe that the body of evidence does suggest a precautionary approach which allows these taxa (allopatric populations or species) to be managed as distinct management units", based on a holistic perspective, where we consider estimated divergence time and haplotype diversity data, among others.

Taxonomic uniqueness and conservation. Any conclusions about the identity and taxonomic uniqueness of Nasua nelsoni (and any other species, particularly those facing conservation threats) should be based on the most robust information and on careful interpretation of it. If N. nelsoni is again recognized as a distinct species, as we stress it should be, along with its categorization as Critically Endangered, better attention and resources for its conservation will be feasible. The currently available evidence supports that the Cozumel dwarf coati is genetically distinct (especially in mitochondrial markers) with respect to continental congenerics, that its population has been historically small since its foundation and that the current total number of individuals is extremely small and decreasing due to anthropogenic pressures. Finally, that it faces population, ecological and evolutionary challenges that threaten its persistence.

Indeed, the Cozumel dwarf coati faces serious threats derived from the increase of tourism infrastructure, landcover change and habitat fragmentation, from the introduction of exotic species and continental congeneric individuals to the island, and from a greater anthropization of Cozumel Island, which increases the negative effects on this and many other endemic species (e.g., deaths by car run over, pathogens and disease spillover from feral and domestic animals, risks of genetic introgression and hybridization; Cuarón et al. 2004, 2009; Flores-Manzanero et al. 2022).

Paradoxically, Ruiz-García et al. (2021) argue that having an exhaustive sampling including as many individuals as possible is the most important condition for both delineating conservation units and developing management

and conservation plans for many species. Therefore, the conclusion by Jaramillo and Ruiz-García (2022) regarding Cozumel's Dwarf coati based on one sample only, is totally contradictory and lacking scientific support.

Therefore, we consider it of the utmost importance to be cautious, concise and objective in using adequate evidence to support the taxonomic and genetic uniqueness of the Cozumel dwarf coati and to generate the most appropriate scenario for the conservation of this procyonid. Systematics requires robust data and interpretations, while effective conservation requires robust systematic data and conclusions.

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