Letter to the Editor:

Response to Valenzuela-Galvan et al. 2023: It is not necessary to "create" a new species for the sake of conservation: the case of the Cozumel's coati

Although we acknowledge the conservation efforts of McFadden et al. (2008), we suggest caution in suggesting the existence of a new species without providing sufficient evidence. Recall the work of Zachos (2016), and Zachos et al. (2013a, b), which stated that species are such fundamental units that they should not be introduced carelessly and that descriptions and splitting of species based on simple morphometric differences (even significant ones) or phylogenetic relationships derived from limited molecular datasets (for instance, only one or few mtDNA genes) should be strongly discouraged. They may serve to support conclusions derived from larger and more complete datasets, but are not enough on their own. The case of the Cozumel's coati (Nasua nelsoni, Nasua narica nelsoni, or a special population of Nasua narica) is an emblematic example in which multiple authors have considered a taxon to be a full species without providing strong justification.

From our perspective, there are two relevant points to consider in clarifying the issue of whether or not Cozumel's coati is a full species: the null hypothesis we presented and which definition of species should be used. The null hypothesis is that the Cozumel coati and the coati living in the nearby continental México (Campeche, Quintana Roo, Yucatán) are the same species (Cozumel island is separated from the Mexican mainland by 18 km). In Jaramillo and Ruiz-García (2022), we discuss the analysis of a complete mitogenome of a road specimen sampled on Cozumel Island. The specimen was a male with developed testicles, but its overall size was smaller than the coatis we had observed on the Yucatán Peninsula. Based on analysis, the specimen's mitogenome was not significantly different from the mitogenomes of specimens of *N. narica* sampled in the Yucatán Peninsula. In other words, we couldn't reject the null hypothesis and concluded that Cozumel's coati is not a different species from the Yucatán and Quintana Roo mainland coati. Authors claiming that this island coati is a different species, must reject the null hypothesis and demonstrate that the alternative hypothesis —that there are two different species—is more acceptable. However, based on the paper by McFadden et al. (2008) and others, there is insufficient evidence to reject the null hypothesis. Next, we discuss the shortcomings of the paper authored by these authors.

1. Valenzuela-Galvan et al. (2023) wrote "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." and "In McFadden et al. (2008) we start by considering N. nelsoni as a distinct species based on Merriam (1901)." These quotes confirm that the study by Valenzuela-Galvan et al. (2023) was initially based on the typological species concept similar to studies conducted by many zoologists during the 19th century as well as the beginning of the 20th century. Because the quote specifically mentions Merriam (1901), we need to determine the criteria Merriam used in distinguishing different species. We find the answer in Merriam (1918). In this study, Merriam distinguished 86 different bear species just in North America. He based these "species" by noting minimal differences in the pelage and skulls of the sample that he analyzed. Today, the majority of these 86 bear taxa are forgotten. As Osgood (1943) claimed, Merriam ordered his findings and labeled them without any effort to interpret them. These zoologists ignored the within phenotypic and genetic variability which naturally occurred within a species, especially if a species has a wide geographical distribution. This mentality was eventually overcome with the arrival of synthetic neo-Darwinism (Dobzhansky 1937, 1970; Mayr 1942, 1963, 1970; Simpson 1944, 1953). Nevertheless, many biologists, including molecular ones, maintain a typological view of biological processes.

There are many examples of island dwarfism in mammals that are not representative of different species (anagenesis or phyletic evolution). For example, the extinct Japanese wolf (*Canis lupus*) and the extinct Balis's tiger (*Panthera tigris balica*) were significantly smaller than their continental counterparts. Mitochondrial DNA analysis showed that the Japanese wolf was indifferentiable from North American wolf lineages (Ishiguro et al. 2010; Matsumara et al. 2014). Similarly, mtDNA evidence indicates that the Balis's tiger is indifferentiable from the Sumatra tiger (*Panthera tigris sondaica*; Kitchener et al. 2017). There are other examples too, such as the goats (*Capra hircus*) introduced into the Juan Fernández archipelago during the 16th and 18th centuries by conquerors and pirates. These goats are half of the size of the goats from which they were derived (Muñoz-Pedreros et al. 2003). Recently, Ruiz-García et al. (2022) showed that the agouti from the Roatán Island (*Dasyprocta ruatanica*; Honduras), also considered a different species by its size, is molecularly indifferentiable from the Centro-American agouti, *Dasyprocta punctata*. Indeed, both forms of agoutis can breed without problem (Ruiz-García, unpublished observations). It has been shown that artificial, natural, and sexual selection can drastically change the mor-

phology of different organisms without speciation in a just a small number of generations (the foxes of Belyaev, Dugatkin 2018; Podarcis sicula, Herrel et al. 2008; or Poecilia reticulata, Endler 1980, 1983, 1986; Reznick et al. 1997). Additionally, the average height of people within indigenous populations of different Southeast Asian islands (Andaman, Luzon, Panay, and Mindanao islands) is considerably less than that of humans from the Asian continent (Stock 2013; Endicott et al. 2003; Deng et al. 2022; Zhang et al. 2022). However, no one, considers each one of these insular dwarf human populations as different species. Thus, insular dwarfism in mammals is not a sufficient requirement to differentiate a species as is the case of the Cozumel's coati.

2. McFadden et al. (2008) claimed the Cozumel's coati as a full species, but they did not demonstrate this affirmation (i. e., they never defined a possible species concept). None of the analyses they carried out showed the Cozumel's coati as a full species (This holds true whether we use the Biological Species Concept, BSC, Mayr 1942, 1963, 2004; or different variants of the Phylogenetic Species Concept, PSC: PSC1, Cracraft 1989, Wheeler and Nixon 1990; PSC2, De Queiroz and Donoghue 1988; PSC3, Baum and Donoghue 1995; Shaw 1998).

McFadden et al. (2008) analyzed mt control region data and calculated genetic heterogeneity statistics comparing the Cozumel's coati and the coati found in the Yucatán Peninsula. None of the statistical values they presented were less than 0.05; in other words, the statistical results were not significant ($F_{ST} = 0.166$, p = 0.061; $F_{ST} = 0.089$, p = 0.050). Therefore, the results presented by McFadden et al. (2008) are insufficient to suggest that there is a new species. Additionally, McFadden et al. (2008) stated that a global AMOVA supported a significant difference between the Cozumel coati population and other Mexican mainland coati populations. Yet again, statistical evidence did not support a clear difference ($F_{cr} = 0.108$, p = 0.050). The genetic distance that they obtained between the Cozumel's coati and the Yucatán counterpart was 0.51 %. Kartavtsev (2011) analyzed sequences of mt COI from 20,731 vertebrate and invertebrate animal species and obtained 0.89 $\% \pm 0.16 \%$ for populations within species, 3.78 % ± 1.18 % for subspecies or semispecies, and $11.06\% \pm 0.53\%$ for species within a genus. Bradley and Baker (2001) claimed, for mt Cytb, that values less than 2 % would equal intra-specific variation, values between 2 % and 11 % would merit additional study, and values greater than 11 % would be indicative of specific recognition. Avise (1994) determined 5 to 7 % of differences at the mt control region for different species and around 2 % for subspecies in mammals. Thus, the genetic distance between the Cozumel's coati and the Yucatan's coati is at most typical of intra-specific variation. Additionally, McFadden et al. (2008) estimated the average temporal split between the Cozumel coati population and the populations of coati from Yucatán peninsula and Belize, respectively. They yielded a temporal value of 6,300 years ago (ya) with the population of Yucatán and around 12,000 ya with the population of Belize. Even, with the most rapid mutation rates, these temporal splits should be around 1,300 ya and 2,400 ya, respectively, which agree well with Decker (1991), Glaston (1994), and Zeveloff (2003) that the coati was introduced when Mayas colonized Cozumel Island around 2,500 years ago. In fact, the bones of Cozumel's coati found in excavations in the island were dated to the Mayan Classic Period (ca. 1,300 to 1,700 years bp; Hamblin 1984). In that period, the Cozumel's coati was widespread. Archaeological excavations have found them to be located at several sites on the island with a high number of coati remains found at each site (Hamblin 1984). However, we don't have information about older skeletal remains of this coati. Thus, it is possible that humans introduced this coati taxon in historical times.

Based on text and statistical data in McFadden et al. (2008) and McFadden (2004) there is no compelling evidence of a new species. For example, McFadden et al. (2008) wrote "...it is difficult to make conclusions about this population's haplotype diversity or species level uniqueness...", and, "...our analyses suggest that the Cozumel taxa are most closely related to their Yucatan and Belize conspecifics...", and "...we believe small sample size and low level of population differentiation are responsible for the insignificant Fst value." Additionally, McFadden (2004) analyzed sequences of a nuclear gene (CHRNA1) which were indifferentiable for Yucatán and Cozumel coatis. Despite of this, the authors considered that the Cozumel's coati was a full species for conservation considerations. Again, based on the presented data and statistical analysis there is insufficient information to reject the aforementioned null hypothesis.

3. Nigenda-Morales et al. (2019) carried out a very interesting research project on N. narica. They included nine specimens of coatis sampled on Cozumel island. All of the analyses that they carried out (Phylogenetic tree based on maximum likelihood and Bayesian inference of 2,201 base pairs of concatenated mt sequences from three genes, Cytb, NADH5, and 16S rRNA; timetree showing divergence times among the 21 mtDNA haplotypes that they found within N. narica; median-joining network with these 21 haplotypes of N. narica; genetic clustering [Structure] and neighborjoining tree based on DA distance based on genotypes of 11 microsatellite loci in 85 specimens) showed the same results. The Cozumel's coati specimens formed a group together with the specimens from Yucatán, Belize, and part of Guatemala. This group is clearly differentiated from the groups of coatis from Morelos, Jalisco, and Arizona-New Mexico, which are all well differentiated from each them. It is interesting to note that if we follow the reasoning of Valenzuela-Galvan et al. 2023 these other N. narica clusters should be named as full new species of coatis. The rebuttal letter by Valenzuela-Galvan et al. 2023 cited Nigenda-Morales et al. (2019) and quoted several sentences: "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". However, Valenzuela-Galvan et al. (2023) curiously forgot to comment on the following paragraph in the work of Nigenda-Morales et al. (2019): "We did not find significant evidence indicating coatis from Cozumel Island represent a distinct lineage and therefore a different subspecies (N. n. nelsoni) from those on the Yucatan peninsula (N. n. yucatanica; table A.4, A.9; figure 1B, 3, 6). These results are largely consistent with the findings of McFadden et al. (2008) based on mtDNA control region sequence data that coatis on Cozumel Island may have colonized the island during the Late Pleistocene or Holocene (possibly through human-mediated dispersal)". Therefore, Nigenda-Morales et al. (2019) also did not reject the null hypothesis that was mentioned earlier. It is always possible that the two specimens of McFadden (2004) and McFadden et al. (2008), the nine specimens of Nigenda-Morales et al. (2019), and the specimen of Jaramillo and Ruiz-García (2022), although sampled in the Cozumel island, do not represent the "true" Cozumel's coati because all of them may have been derived from the mainland and transported to the island, but the likelihood of that happening would have been extremely low.

4. When we wrote the article of <u>Jaramillo and Ruiz-Gar-</u> cía (2022), we ignored the existence of the work of Flores-Manzanero et al. (2022). Flores-Manzanero et al. (2022) analyzed samples from 46 individuals of the Cozumel's coati (that were captured alive on site) and performed an analysis of mt Cytb and nuclear microsatellites. The authors obtained two main results. By using the Structure Program applied to microsatellite data, the authors demonstrated that the Cozumel's coati population was differentiated from the populations in Jalisco and Morelos (figure 2b). However, this result was not new. Nigenda-Morales et al. (2019) had already communicated this result to the scientific community. Curiously, the analysis by Flores-Manzanero et al. (2022) did not include coati specimens from Quintana Roo, Yucatán, and Campeche, which were genetically similar to the Cozumel's coati (Nigenda-Morales et al. 2019; Jaramillo and Ruiz-García 2022). Obviously, if they had introduced specimens from these nearby Mexican mainland areas, the differentiation of the Cozumel's coati would have been less conspicuous (conservation prejudice). On the other hand, multiple populations of the same species can significantly diverge for a set of microsatellites but this does not mean that they are different species. For instance, populations of pink river dolphins (Inia geoffrensis) from two Colombian rivers (Putumayo and Caquetá) and from different Peruvian rivers are significantly different based on microsatellite data (Ruiz-García 2010; Ruiz-García et al. 2018). Similarly, different populations of the Andean cat (Leopardus jacobita) from

Perú, Bolivia, and Argentina are considered significantly different based on seven microsatellites (Cossíos et al. 2012; Ruiz-García et al. 2013a). Additionally, different populations of ocelots (Leopardus pardalis) throughout South American are significantly different at 10 microsatellites (Ruiz-García et al. 2013b). The last example we mention here from a very long list of examples is humans. Indigene human populations in South America are significantly different for 15 microsatellites (Demarchi 2009). Does this mean that they are different species because they have significant differences for nuclear microsatellites? The answer is no. In reference to the mtCytb, Flores-Manzanero et al. (2022) showed a phylogenetic tree in figure 3. The authors found that the specimens from Jalisco and Morelos conformed well differentiated clades from the clade of the Cozumel's coati. Again, the scientific community had already been informed about this finding three years earlier (Nigenda-Morales et al. 2019). Within the Cozumel's coati clade, Flores-Manzanero et al. (2022) found three sub-clades. One of them included a few specimens from Quintana Roo that the authors included in their analysis. Therefore, no reciprocal monophyly exists between the Cozumel's coati and the coatis from the nearby Mexican mainland. Additionally, if Flores-Manzanero et al. (2022) had enclosed more specimens from Quintana Roo, Yucatán, or Campeche, surely more specimens of these Mexican mainland areas would have been clustered with the Cozumel's coati group. In other words, similar to McFadden (2004), McFadden et al. (2008), Nigenda-Morales et al. (2019), and Jaramillo and Ruiz-García (2022), Flores-Manzanero et al. (2022), did not reject the null hypothesis that *N. narica* and the Cozumel's coati are different species. In fact, Flores-Manzanero et al. (2022) indicated that they knew it was not a full species when they wrote "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)". Notwithstanding, the Cozumel's coati does not fit the definition of an ESU (sensu Moritz 1994). An ESU should be reciprocally monophyletic for mtDNA and this is not the case with the Cozumel's coati. The Cozumel's coati is, more likely, an example of a management unit (MU). Moritz (1994) originally defined an MU as a population that showed significant differences in allele frequencies at nuclear or mtDNA loci. This can show that their population dynamics depend more on local birth and death rates than on immigration (demographical independence; Bentzen 1998).

There are many definitions of a species besides those previously cited (BSC, PSC1, PSC2, PSC3) such as the Genotypic Cluster Species Concept (GCSC; Mallet 1995), Recognition Species Concept (RSC; Paterson 1985; Lambert et al. 1987), Cohesion Species Concept (CSC; Templeton 1989), etc. The Cozumel's coati does not meet most of these definitions. But, the most universally accepted concept is the BSC, in part because it is the one we apply to our own species. We agree with the explanation about concepts offered by Mayr (2004): "it is very important to understand what the word concept means when combined with the word species. It implies the meaning of the species in nature. A population, or group of populations, is a species, according to BSC, if it configures a reproductive community and does not reproduce with members of similar communities. BSC, as defined, plays a concrete role in nature and differs in this respect from all those other so-called concepts of species that are nothing but instructions, based on human judgment, about how to delimit specific taxons." Clearly, the Cozumel's coati is not "above" the BSC. It would not be surprising that the authors who support the Cozumel's coati as a full species, have some doubts about their suggestion. Why? The N. narica from the Mexican mainland is known to have been brought over to Cozumel (historically as pets), and it is suspected that they may have interbred with the island coati (Gomper, and others). There are no reproductive isolation barriers between the Cozumel's coati and the nearby Mexican mainland coati. In other words, the Cozumel's coati is the same species of coati that is found in the Mexican mainland.

Nevertheless, conservation biological prejudice exists (see McFadden 2004): "Depending on how strictly one interprets the biological species definition (O'Brien and Mayr 1991), the island taxa of N. nelsoni could thus be classified as the same species as the mainland taxa of N. narica. If the criterion of reproductive isolation is generally applied, the taxa of dwarf carnivores may go unrecognized as evolutionarily differentiated populations and thus separate conservation units." But it is not necessary to define new species to conserve "special" populations such as the Cozumel's coati (even biological conservation can be endangered by partition of a species in different supposed species; see Zachos et al. 2013b). For example, the US Endangered Species Act (ESA) of the United States has been a major stimulus to develop criteria for identifying intraspecific population units for biological conservation. The ESA provides full legal protection for subspecies and for Distinct Population Segments (DPSs) of vertebrate species, as if they were full species.

In conclusion, we agree with <u>Valenzuela-Galvan et al. 2023</u> that the Cozumel's coati should be actively and urgently protected by Mexican institutions, but we urge caution in accepting the existence of a new species unless statistical analysis (p value less than 0.05) rejects the null hypothesis (i. e., no difference).

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