A case of pseudohermaphroditism in *Mauremys leprosa* (Sauropsida: Geoemydidae)

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Resumen: Aunque raros, se conocen en vertebrados varios casos de hermafroditismo y pseudohermafroditismo ligados a la presencia de contaminantes o ambientes estresantes. En este trabajo describimos el primer caso de pseudohermafroditismo en un ejemplar de *Mauremys leprosa* colectado en estado salvaje y mantenido en cautividad durante 20 años. Externamente, el ejemplar presenta rasgos congruentes con ambos sexos: pseudopene y morfología general femenina. Mediante un examen interno se detectaron ovarios funcionales, pero ausencia de aparato reproductor masculino. El presente reporte es congruente con un proceso de masculinización posiblemente desarrollado a consecuencia de las condiciones de prolongado cautiverio a las que se encontraba sometido el ejemplar, como ya ha sido puesto en evidencia para otras especies de quelonios.

Among Vertebrata, sperm and egg production usually takes place in separate (gonochoric) individuals. However, hermaphrodite (or intersex) individuals have been also reported. A broad expression of breeding systems of this zoological group is exhibited by fishes, which include sex reversal (i.e. protandry and protogyny) or simultaneous hermaphroditism, to name a few (Bortone & Davis, 1994; Biran & Levavi-Sivan, 2018). By contrast, intersexuality in taxa such as amphibians and sauropsida, in which most species are gonochoric, constitutes a poorly reported phenomenon generally identified through the presence of (1) ovotestis (ovaries within testicular tissue) or (2) functionally separated testes and ovaries within a single individual (Hansen, 1943; Dournon *et al.*, 1990; Reeder *et al.*, 2005; Goldberg, 1989). Some species display a natural hermaphroditism. For example, at metamorphosis, all individuals of *Rana temporaria* Linnaeus, 1758 appear to have ovary-like gonads, and some of them, during the juvenile life period, become males through an intersexuality phase in which ovary-like gonads transform into male gonads (Witschi, 1929; Brande-Lavridsen *et al.*, 2008). However, several cases of hermaphroditism or sex reversal in herpetofauna have been documented under scenarios of chemical environmental pollution or under captive or laboratory conditions (Grafe & Linsenmair, 1989; Dournon *et al.*, 1990; Bergeron *et al.*, 1994; Guillette *et al.*, 1994; Reeder *et al.*, 2005; Moresco *et al.*, 2014). Nevertheless, identifying hermaphrodite individuals based only on external traits is a complex matter, as pseudohermaphroditism (an organism with primary sexual characteristic of one sex, but secondary sexual traits of the other) can blur the true sexual condition (Guix *et al.*, 2001).

In chelonians, both hermaphroditism and pseudohermaphroditism have been documented. Guix *et al.* (2001) suggest that inadequate captive conditions could be related to endocrine disruptions that drives masculinization (both in

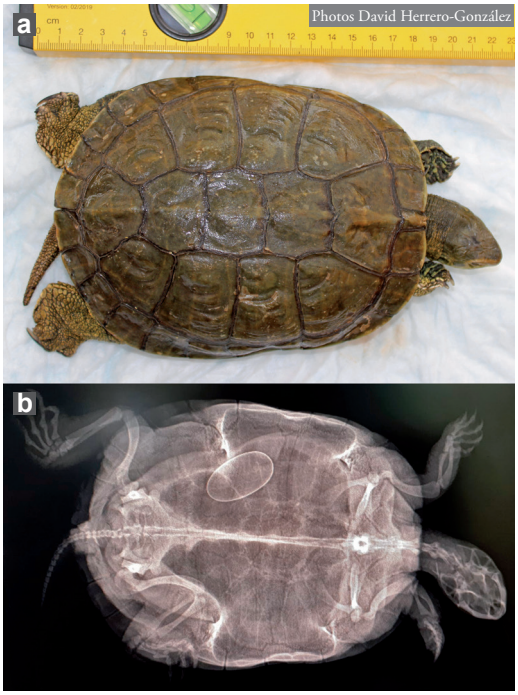

Figure 1: Pseudohermaphrodite specimen of *Mauremys leprosa*. a) Dorsal view with a ruler used for size measurements. b) X-Ray image of the same specimen; noted the ossified egg in the left side.
Figura 1: Individuo pseudohermafrodita de *Mauremys leprosa*. a) Vista dorsal junto a la regla usada para medir el tamaño. b) Imagen de rayos X del mismo ejemplar; nótese el huevo osificado en el lado izquierdo.

behavioural and morphological traits) in some female individuals of *Chelonoidis carbonaria* (Spix, 1824), but unlike the true hermaphroditism, they lacked male gonad development despite the presence of pseudopenis, that could be also interpreted as a hypertrophy of clitoral structure. Thus, distinguishing between hermaphroditism and pseudohermaphroditism from chelonian specimens usually requires gonads examination.

In this work, we revised the sexual condition of a morphologically strange specimen of the Mediterranean pond turtle *Mauremys leprosa* (Schweigger, 1812), which represents, to our knowledge, the first record of pseudohermaphroditism for the species. An adult specimen of *M. leprosa* was received in the Hospital Veterinario el Bosque of Madrid, Spain, in June 2019 (Figure 1). It is a wild caught specimen (likely from the province of Madrid) that has been kept in captivity by at least 20 years in a house backyard with a small pond (according to the owner's description). After a period with apathy and no feeding, the owners contacted to the mentioned veterinary hospital. However, soon after, the animal dies and it was subsequently examined. The specimen, with a straight carapace length of 18 cm, presented external female morphological traits: plane plastron, short tail and cloaca opening not surpassing the carapace (Díaz-Paniagua *et al.*, 2015). A first examination was performed by using X-rays in the veterinary hospital, detecting a single ossified egg of 3.7 cm (Figure 1b). Additional scrutiny uncovered a structure similar to a penis or a clitoris bigger than normal in aquatic turtles (Perpiñán *et al.*, 2016), which was everted by applying pressure over the inguinal region (Figure 2). The plastron was latter removed from the specimen in order to examine the reproductive system and to elucidate if this case belong to a hermaphrodite or to a pseudohermaphrodite individual. Only ovaries (with different sizes) were found (Figure 3). The

Figure 2: Everted pseudopenis of the pseudohermaphrodite specimen.
Figura 2: Pseudopene evertido del ejemplar pseudohermafrodita.

Figure 3: Ovarian follicles.
Figura 3: Folículos ováricos.

lack of a pair of functional testicles or any trace of male reproductive system and the presence of clitoral hypertrophy allow us to classify this case as pseudohermaphroditism. The rest of organs, including thyroid, were morphologically normal, except for the liver, which was fat and slightly damaged. However, in general, the specimen presented a poorly health condition with a marked deficit in body fat, mostly limited to the intestinal mass. Also, signs of pyramiding were noticed.

Alarcos *et al.* (2009) suggest that females of *M. leprosa* from Salamanca (Spain) reach the sexual maturity between 15 and 19 years old, and a little less for the males (13-18 years). These authors estimate the age through counting the growth rings of the left pectoral shield, considering a year by ring. However, this relationship is not fully verified in *M. leprosa* (Díaz-Paniagua *et al.*, 2015). In general, most studies recognise a minimal range of reported size for species maturation in females between 14 and 19 cm of straight carapace length, whereas in males it varies from 6-9 cm (Díaz-Paniagua *et al.*, 2015). Thus, the size of the described specimen is in agreement with the range of the minimal reported size of species maturation for females. It could suggest that the maturation of the female reproductive system drives their death as a dystocia. However, this hypothesis is tentative as the growth rates could be affected by the captivity management. Unfortunately, we lack information about the nutrition and captive conditions in which the specimen lived during the last 20 years. As reported by Guix *et al.* (2001) who described masculinization in captive chelonians, the presence of pseudopenis in this reported specimen of *M. leprosa* could be driven by its long period under stressful captivity conditions. The specimen is currently housed in the Herpetological Collection of the MNCN and labelled as MNCN 50508.

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