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Comparative morphology and histology of Hemipeneal structure of *Laudakia nupta* (De Filippi, 1843) and *Paralaudakia caucasia* (Eichwald, 1843)

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Abstract
The morphology and structure of the hemipenis play a significant role in the recognition of species, and could be an excellent indicator of the phylogenetic relationships among male taxa**.** The hemipenes have value in distinguishing taxa**.** *Laudakia nupta* and *Paralaudakia caucasia* are sympatric in Kalekan Neck (Northeastern regions of Kermanshah Province, 34° 42' N, 47° 17' E, Elevation: 1880 m). In this study, we investigated and compared hemipeneal structure of *Laudakia nupta* and *Paralaudakia caucasia* as character displacement in sympatric populations. Hemipenes of eight adult collected males (four from each species) were removed and examined morphology and histology. The results indicate that there are differences in the structure of their hemipenis between two species. In *Laudakia nupta*, hemipenes are black organs, approximately smooth tubular with groove and split in tips but in *Paralaudakia caucasia*, hemipenes are pink organs, bilobed, branched and bifurcated structure. It seems that the difference between the structure of hemipenises in two species can be a factor in the separation of male species in Kalekan Neck (Northeastern regions of Kermanshah Province). The structure of hemipenis is potentially important for distinguishing male species.

Key words: *hemipenis, character displacement, Paralaudakia caucasia, Laudakia nupta, sympatric populations.*

INTRODUCTION

In biology, two species are considered sympatric when they exist in the same geographic area and thus regularly encounter one another (Futuyma, 2009). Character displacement is the phenomenon in sympatric areas that two species show greater differences in some character (Geyer & Palumbi, 2003). Reproductive character displacement, more specifically, refers to divergence in characters involved in reproductive isolation (Blair, 1955; Waage, 1979). Low fitness of hybrids drives the change of characters in sympatric populations for reproductive character displacement process (Geyer & Palumbi, 2003). The morphology and structure of the hemipenis play a significant role in the recognition of species, and could be an excellent indicator of the phylogenetic relationships among taxa (Maduwage & da Silva 2012). Hemipenes, the male external genitalia of reptiles, is one character for divergence of reproduction of *L.*

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nupta and *P. caucasia* in areas of sympatry. Hemipenes were first used as a systematic tool by Cope (1894, 1895), who noted the systematic value of the structure of this organ. In this regard, some taxonomists even consider hemipenial structure as one of the major morphological characters (Maduwage & da Silva 2012).

In northwestern regions of the Iranian plateau, limits of *L. nupta* distribution in Kermanshah and Kurdestan Provinces along the central regions of the Zagros Mountains were determined, where its range comes in contact with *P. caucasia* (pers. observ.) (Rastegar-Pouyani & Nilson, 2002). *L nupta* and *P. caucasia* are sympatric in Kalekan Neck (Narrow area in northeastern region of Kermanshah Province, N 34° 42' N, 47 17' E, Elevation: 1880 m). In this region, they exist in the same geographic area and thus regularly encounter one another. In Agamid lizards, hemipenes have been rarely described (Semenov & Dunaev, 1989; Baig & Böhme, 1997). Difference in the structure of their hemipenis may prevent them from mating with each other and creating hybrid species in the region of sympatry. The structures of hemipenis potentially important for distinguishing species, therefore, we examined hemipeneal structure of two species based on this character. Here we present descriptions and illustrations of the hemipenes of two above-mentioned species of Agamidae. Based on the results of this study, we concluded that hemipeneal structure can be considered as one of the major morphological characters for identification of two species.

MATERIAL AND METHODS

Four adult males of *Laudakia nupta* and four adult males of *Paralaudakia caucasia* were collected from Kalekan Neck (Northeastern region of Kermanshah Province, 34° 42' N, 47° 17' E) in western Iran during May to September 2018. The collected lizards were identified based on identification key. The males were identified by the abdominal perineum under the abdomen. The collected lizards were transferred to the laboratory. Hemipenes of four adult captive males of *Laudakia nupta* and *Paralaudakia caucasia* were removed. For macroscopic studies we used the camera. For histological studies the hemipenes were carefully cut off at their base and fixed in 10 % neutral buffered formalin for at least 24 hours, embedded in paraffin, sectioned at 5-7 µm thick by a rotary microtome, and stained with Hematoxylin–Eosin for light microscopic examination.

RESULTS

Morphology and Histology of Hemipenis in *L. nupta*

Morphology

In *Laudakia nupta*, hemipenes are moist, fleshy, black organs, approximately smooth tubular with groove and split in tips (Fig. 1). The whole surface of hemipenis is irregular due to the presence of a venous network, nearly. The hemipenes have external groove, the sulcus spermaticus, to transport the semen from the cloaca to its tip.

Histology

Histologically a pleated epithelium covers the hemipenis. This epithelium covers a fibrous connective tissue which is provided with a network of venous sinuses. The superficial epithelium of hemipenis is stratified squamous with projections and depressions which has a connective tissue axis. Near the base of epithelium, black pigments are observed continuously and uniformly (Figs. 2, 3 and 6). The surface of the epithelium is covered by the keratin layer, which is indicator of superficial epithelium keratinization. The

base of hemipenis associated with a suspensory ligament. Suspended ligaments have transverse lines. This ligament has different types of fibers in different directions for moving the hemipenis inside and outside the base of tail. Movement of hemipenes directed by a pair of ipsilateral muscles in the rostral region of the tail (Arnold, 1984). The connective tissue in tip of hemipenis is associated with ligament in muscular part (Figs. 4, 6). Eversion through the cloacal vent is caused by contraction of the transversus penis (TPN) muscles, and after copulation, retraction of the hemipenes back into the tail occurs via contraction of the retractor penis magnus (RPM) (Arnold, 1984).

FIGURE 1. Morphology of hemipenis structure in *L. nupta*.

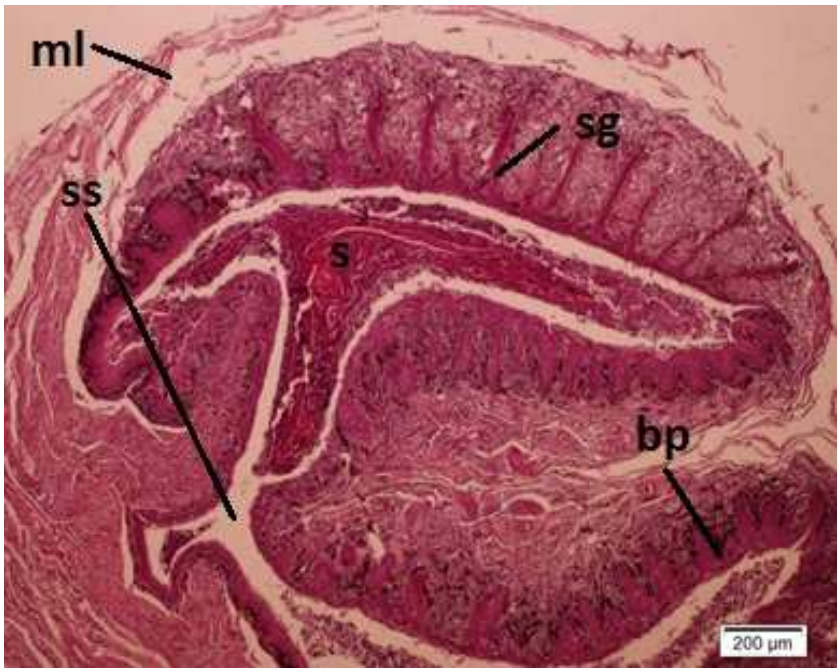

FIGURE 2. Cross section of the Hemipenis of *L. nupta* with magnification of 200 µm. Aabbreviation, ml: outer mucous layer, SS sulcus spermaticus, s: secretions, bp: black pigments, sg: secretory glands.

Hemipenis in tissue section, consists of two parts including the proximal and distal parts (Fig. 7). The outer surface of hemipenis is covered by a mucus layer. The proximal part is a secretion and glandular portion that has two or three glandular tube with mucoid secretions being connected to the cloaca and it looks like a foamy section (Figs. 4, 5). The proximal part of hemipenis covered with regular connective tissue and collagen fibers. The collagen fibers encompassed by reticular sheath and constituted collagen bundles. In distal part of hemipenis, longitudinal bundle covered by a connective sheath completely. There are thin bundles had more branches and developed blood vessels between proximal and distal part of hemipenis.

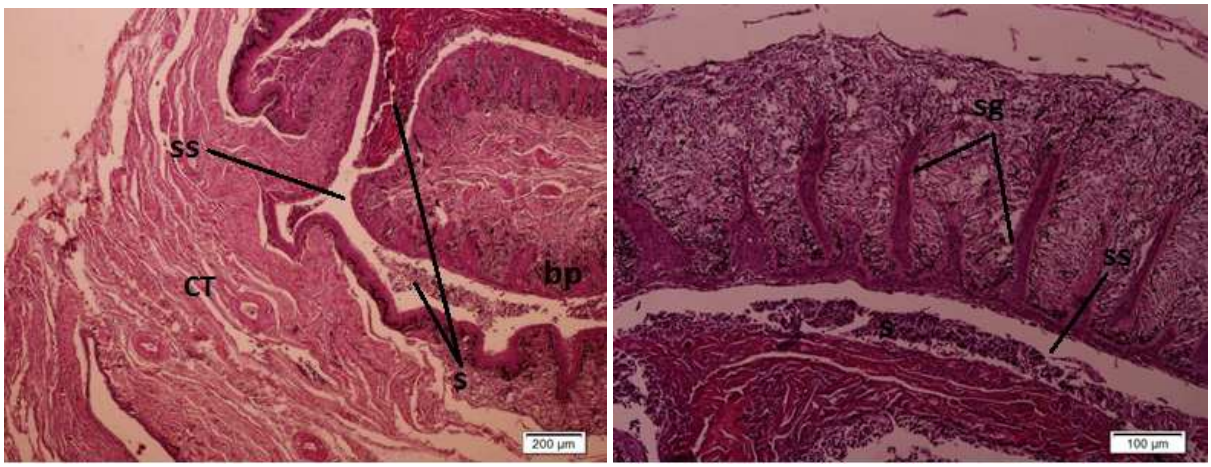

FIGURE 3. Tissue cross section of the proximal hemipenis of *L. nupta* with magnification of 200 and 100 µm. Aabbreviation, SS: sulcus spermaticus, s: secretions, bp: black pigments, CT: connective tissue, sg: secretory glands.

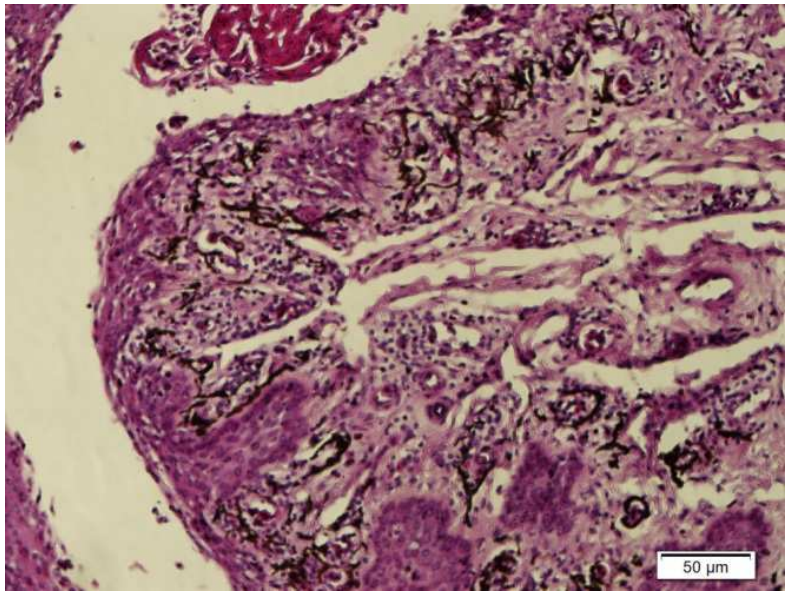

FIGURE 4. Cross section of hemipenis tip of *L. nupta* with magnification of 50 µm.

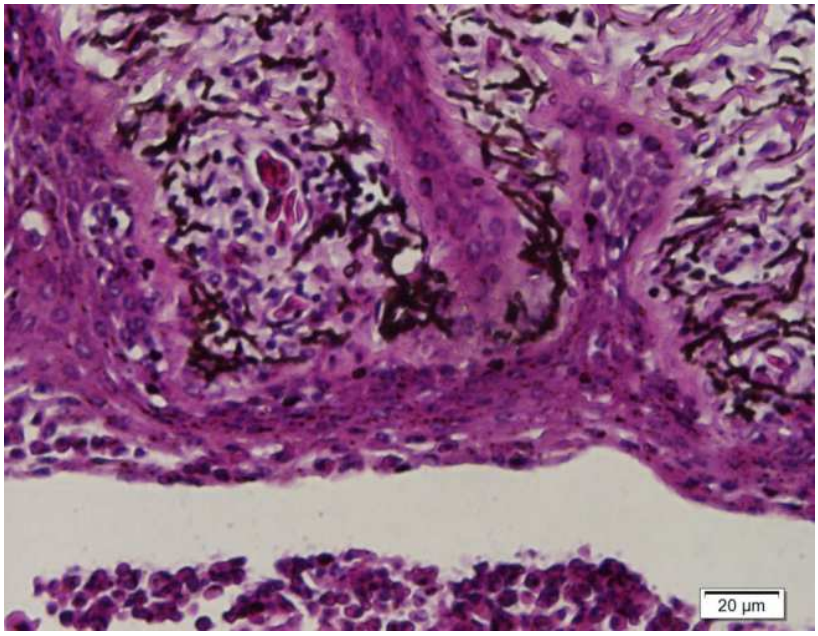

FIGURE 5. Cross section of secretory glands of hemipenis of *L. nupta* with magnification of 20 µm.

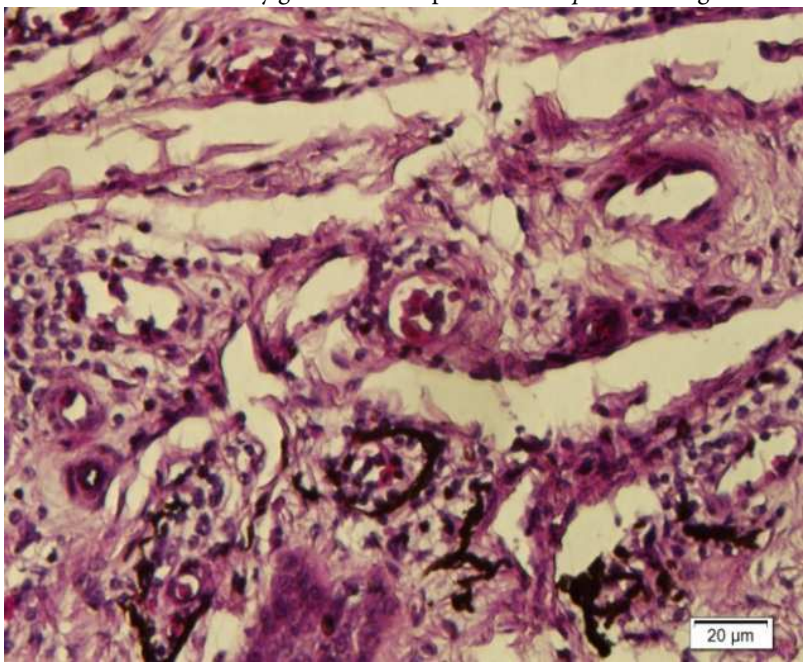

FIGURE 6. Cross section of pigmentation accumulation and blood vessels in the hemipenis tip of *L. nupta* with magnification of 20 µm.

Morphology and Histology of hemipenis in *P. caucasia*

Morphology

In *Laudakia caucasia*, hemipenes are moist, fleshy, pink organs, bilobed and branched structures (Fig. 8). The surface of the hemipenes is adorned with sharp spines and projections, arranged in rosettes. Nearly, the surface of hemipenis is irregular due to the presence of a venous network in this region. The two lobes are equal in length. The two lobes of hemipenis provided bifurcated structure. The hemipenes have sulcus spermaticus to transport the semen to its tip (Fig. 8).

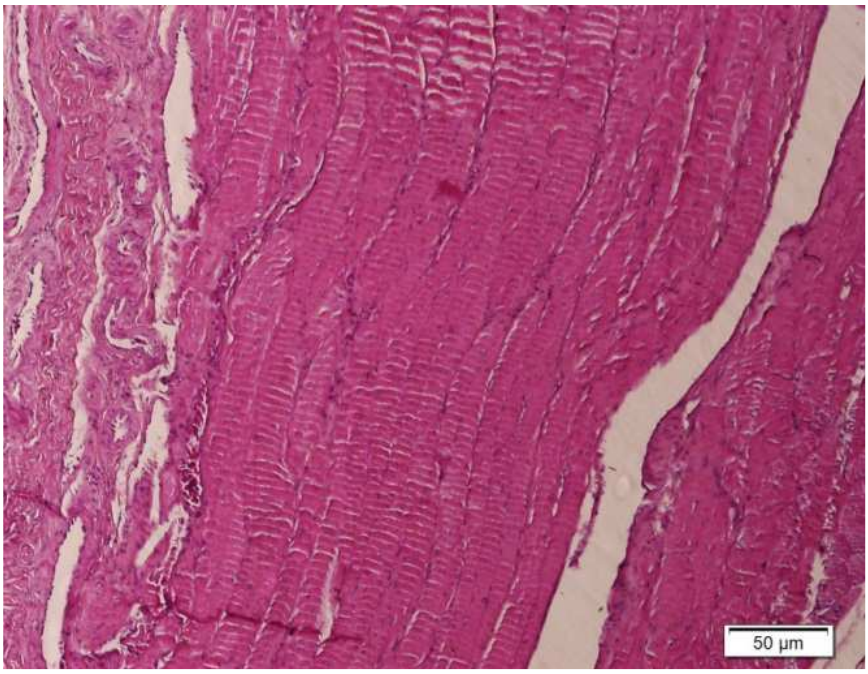

FIGURE 7. Tissue cross-section of the muscles of the distal part of the hemipenis of *L. nupta* with magnification of 50 µm.

FIGURE 8. Morphology of hemipenis structure in *L.caucasia*.

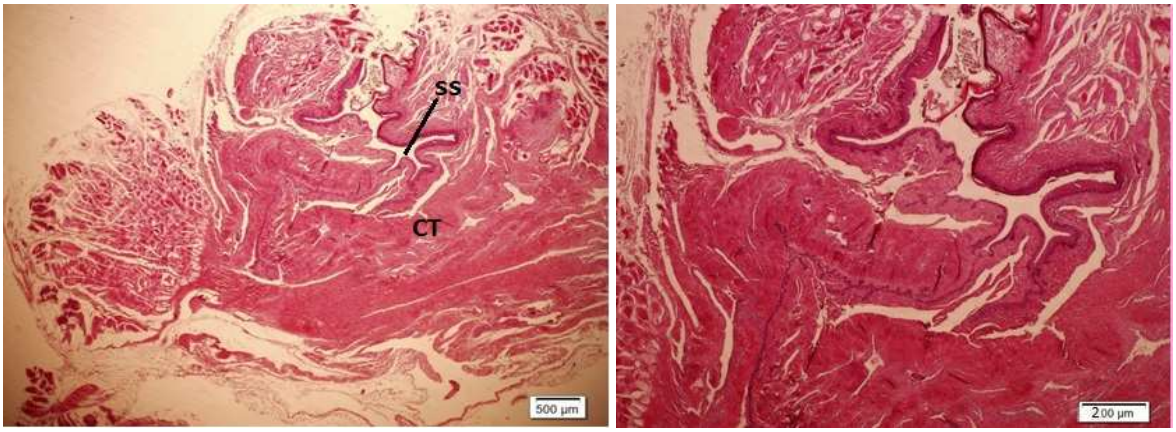

FIGURE 9. Cross section of the hemipenis *L.caucasia* with magnification of 500 and 200 µm. Aabbreviation, SS: sulcus spermaticus, CT: connective tissue.

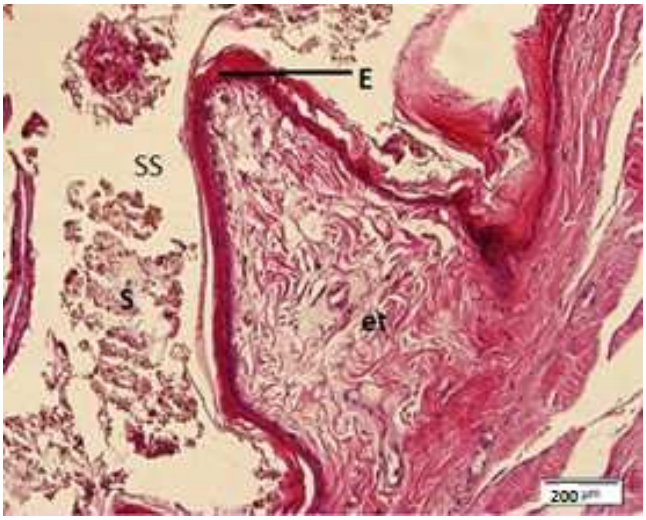

FIGURE 10. Tissue cross section of the hemipenis *L.caucasia* with magnification of 200 µm Aabbreviation, SS: sulcus spermaticus, et: elastic tissue, E: folded epithelium, s: secretions.

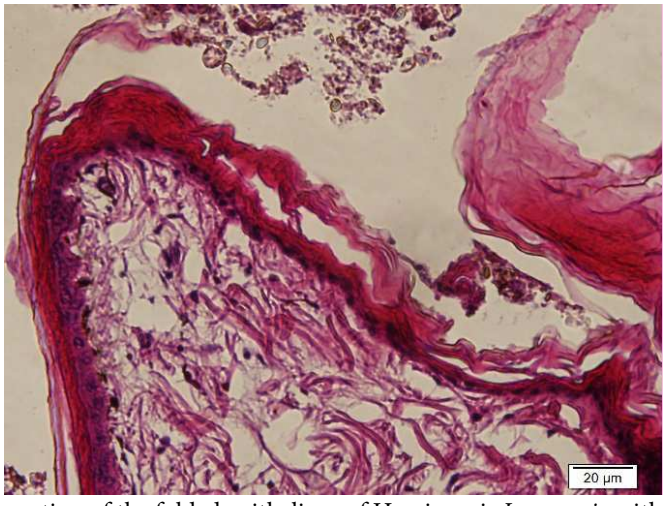

FIGURE 11. Tissue cross-section of the folded epithelium of Hemispenis *L.caucasia* with magnification of 20µm.

Histology

Histologically the superficial epithelium of hemipenis is stratified squamous with projections and depressions. In *Paralaudakia caucasia,* the number of epithelium layers is fewer than the *Laudakia nupta.* The main bulk of hemipenis of *Paralaudakia caucasia* is built of a fibrous connective, rich in elastic fibers. Unlike *Laudakia nupta,* pigment is not seen at the base of the epithelium. Other features of the hemipenis are similar to *Laudakia nupta* (Figs. 9-12).

DISSCUSSION

These are structures of hemipenes which we describe in detail for the first time in Iran. Because we find them to be of considerable taxonomic importance in Agamidae (Cole & Hardy, 1981), we consider morphology and histology of hemipenes of *Laudakia nupta* and *Laudakia caucasia*. The ornamentation and morphology of the hemipenis play an important role in the diagnosis of species, and have demonstrated to be a great indicator of the phylogenetic relationships among taxa (Cope, 1896; Böhme, 1988; Harvey *et al*., 2012; da Silva *et al*., 2013). Hemipenis is potentially important for distinguishing species of the Agamidae. Therefore, we examined hemipenis of two species. Some characters are species specific and useful for recognizing taxa at the species level. Character displacement (Brown & Wilson, 1956; Grant, 1972) is the phenomenon in which two species exhibit greater differences in some characters in areas whose distributions overlap geographically in order to minimize niche overlap and avoid competitive exclusion (Blair, 1955; Waage, 1979). Generally, character displacement refers to morphological differences due to competition (Brown & Wilson, 1956). Character displacement can arise as a secondary effect of environmental adaptation. Reproductive character displacement, more specifically, refers to divergence in characters involved in reproductive isolation (Blair, 1955; Waage, 1979).This pattern results from evolutionary change driven by biological competition among species for a limited resource (e.g. food) (Brown & Wilson, 1956). Low fitness of hybrids drives the change of characters in sympatric populations for reproductive character displacement process (Geyer & Palumbi, 2003).

Ecological character displacement studied in *Typhlosaurus gariepensis* and *Typhlosaurus lineatus*in sympatric area by Huey & Pianka in 1974. They showed that in sympatric areas divergence and difference of characters are greater than allopatric areas (Huey & Pianka, 1974). Also, Character Displacement in *Anolis* Lizards was presented by Yoel Stuart in 2013. According to his study, ecological character displacement an evolutionary process whereby two resource competitors diverge from one another in phenotype and resource use, facilitating coexistence in a community (Stuart, 2013).

The theory of Ecological Character Displacement proposes that sympatric species that compete for the same set of limited resources should be favored by natural selection to diverge in resource use and phenotype (Brown & Wilson, 1956).

As well, character displacement in some *Cnemidophorus* lizards studied by Radtkey et al in 1997. Based on this study, ecological character displacement leading to increased morphological separation (e.g., anoline lizards, Whiptail lizards). *Cnemidophorus tigris* and *Cnemidophorus hyperythrus* demonstrate a biogeographic pattern of morphological variation suggestive of character displacement (Radtkey *et al*., 1997).

Our data show reproductive character displacement exhibiting extreme divergence in hemipeneal structures. These results suggest that reproductive character displacement including hemipenis responsible for the divergence of populations of *L. nupta* and *P. caucasia* in the areas of sympatry in northeast regions of Kermanshah Province.

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