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Descriptive osteology of an imperiled amphibian, the Luristan newt (*Neurergus kaiseri,* Amphibia: Salamandridae)

Hadi Khoshnamvand[1], Mansoureh Malekian[1,*], Yazdan Keivany[1], Mazaher Zamani-Faradonbe[1], Mohsen Amiri[2]

[1] *Department of Natural Resources, Isfahan University of Technology, Isfahan, Iran. *Corresponding author.* Email: mmalekian@cc.iut.ac.ir
[2] *Department of Basic Sciences, Islamic Azad University of Hamedan, Hamedan, Iran*

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Abstract. Osteological structures are important biological features which provide valuable biological and ecological information. Luristan newt (*Neurergus kaiseri*), is an endemic salamander, inhabiting the southern part of Zagros Mountains of Iran. The current study was conducted to describe the osteological characteristics of the Luristan newt which might be important in understanding the evolutionary process of newt species. The skull of *N. kaiseri* has a dense structure, severely ossified elements and a low amount of cartilaginous elements, only in mobile facets. Vertebral number in the axial skeleton of the species equals 50. The cervical, abdominal and caudal parts of the vertebral column have two, 16 and 32 vertebrae, respectively. Each hand and foot consisted of four fingers, having three or four phalanxes. The metacarpal includes seven bones and the number of metatarsus bones is eight. Hands are connected to humur through ulnare and radius and then connected to scapulocoracoid. Each leg includes two bones (fibula and tibia) which are connected to femur. The head of the femur articulates with the acetabulum in the pelvic bone, while the distal part of the femur articulates with the tibia.

Keywords. Salamanderidae, newt, *Neurergus kaiseri*, skull, descriptive osteology.

INTRODUCTION

The vertebrate skeletal system and its elements are important in evolutionary biology. Vertebrate skeletons can be regarded as combinations of apparently discrete units (namely bones) which have attracted the interest of comparative anatomists (Simpson, 1944). Osteological data can be used to identify different taxa and phylogenetic relationships (e.g., Hill, 2005) and to understand biological features of animals such as feeding, respiration, swimming and movement (Eastman, 1980; Helfman et al., 2009). In addition, the skeletal structure contains biological information that can be used to distinguish species type, age, sex, size, and even environmental conditions of their habitats (Helfman et al., 2009).

In Iranian freshwater basins live three genera of salamanders including, *Triturus, Salamandra* and *Neurergus*, all belonged to Salamandridae. The latter genus has a relatively vast geographic distribution, ranging from Zagros Mountains (western Iran) to Iraq and southern Turkey (Baloutch and Kami, 1995). The Luristan newt (*Neurergus kaiseri*, Schmidt 1952) is endemic to the southern Zagros Mountains of Iran, with a distribution area of approximately 900 km^2 (Mobaraki et al., 2016). The species inhabits first order rivers and ponds in open woodlands dominated by oak trees (*Quercus brantii*). This newt is classified as vulnerable (VU) by the IUCN Red List because of its small range, illegal trading, habitat loss and climate induced drought (IUCN, 2018). It is also amended to the Appendix I of the convention to the international trade to endangered species (CITES, 2010).

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Current knowledge on *N. kaiseri* is restricted to some aspects of the ecology such as distribution, including reports of new localities for the species (Sharifi et al., 2013; Mobaraki et al., 2014), and demography (age structure, longevity and growth patterns) of a local population (Farasat and Sharifi, 2015). A genetic study reported the presence of two genetically distinct clades within the Luristan newt (Farasat et al., 2016). Sexual dimorphism in *N. kaiser* was evaluated using head- and body- related characters (Khoshnamvand et al., 2018). Skeleton structure of the species is yet to be described, which can provide additional data and a basis for understanding the remarkable and adaptive variation in bone-forms among newt species. Osteology also helps in understanding the important taxonomic characters for identification and classification of a species.

In Salamandridae, a number of studies include osteological characters in the description of a new taxon (Min et al., 2005; Wu et al., 2009; Wu et al., 2010; Wake et al., 2012) or provide comparative descriptions of closely related taxa (Wake and Özeti, 1969; Venczel, 2008; Wu et al., 2012). Amongst *Neurergus* species, little information is available on descriptive osteology. Akia et al., (2010) described the cranial osteology of a closely related species, *Neurergus microspilotus* and compared it with *Salamandra infraimmaculata semenovi*. The current study aims to describe skull characteristics of *N. kaiseri* and compare size, shape and connections of bones with other newts, where possible. Such data provide a basis for further investigations on the subject and help to understand the biological features of Iranian salamanders.

MATERIAL AND METHODS

Field surveys were conducted in late July 2015, when the Luristan newt breeding season was over to minimize disturbances to the species. The Luristan newt habitats are mainly streams and springs, located at high elevations (800 to 1500 m a.s.l), separating from each other by steep and rocky mountains. Further, the species is rare, occurring in low density in a limited number of sites, as many of the ponds and springs in the region have dried out due to the drought during the past few years (IUCN, 2018). The vulnerable (VU) status of the species also entails sampling restrictions for capturing live individuals. Therefore only deceased carcasses of *N. kaiseri* could be taken and used for osteological analyses.

Therefore, we accessed to the newt habitats by climbing the mountains and camping in the region for 10 days, with the assistance from the environmental guards. Due to the water flow, which could wash away the possible carcasses, 2 mm fish nets were installed in down streams. Fish nets were regularly checked during the day and removed from each site before the dark. Two dead individuals were captured by the fish net at two localities in Korki region, Lorestan province, at the elevation of 957 and 1100 m a.s.l. respectively.

Both specimens were adult male with the body length of 132 and 130 mm respectively. Samples were preserved in 96% ethanol in the field prior to the lab experiment. We followed the standard protocols of cleaning and staining bones (Taylor and Van Dyke, 1985; Torres and Ramos, 2016). The carcasses were fixed in 500 ml of 10% neutral formalin for 4 days. Before proceeding with the staining procedure, the specimens were washed thoroughly under running tap water for at least one hour to remove excess of formalin. The specimens were then placed in 1% alizarin red solution, added drop by drop to the freshly prepared 5% KOH solution. About 15 ml of the staining solution was utilized, holding the specimens for three days or until the medium (KOH) showed pinkish violet color. The specimen were then transferred into alkaline blue stain solution for two days. To clean bones, specimens were transferred into trypsin solution at ~37 °C, changing enzyme solution and washing the specimens in distilled water every 3 days to avoid bacterial digestion (repeated three times). The stained specimens were examined using a stereomicroscope (HP SNP 120), and different skeletal elements were dissected and scanned in lateral, dorsal and ventral view, using a scanner (HP Scanjet G4050). For each specimen or view, a series of images were taken to produce a single image with maximum depth of field. The final image was drawn using CorelDrawX7 software. The terminology of the skeletal elements follows Vassilieva et al. (2015).

RESULTS

The skull of *N. kaiseri* has a dense structure, severely ossified elements and a low amount of cartilaginous elements, only in mobile facets. The premaxillae are unpaired and nearly arched dorsally. The pars dorsalis of premaxillae are distinctly and widely separated by a midline fontanelle. A groove that starts in the top of nasal opening is separating the pars dorsalis of premaxillae and continues forward to pars dentalis. This groove further goes toward the antroventral part of the palate and form a pore in the vomer bone. The premaxilla contacts the prefrontal and nasal posteriorly, and contribute to the nasal cavity laterally. The maxilla are paired and complete the arch of the upper jaw that borders the nostril at the anterior part and meet the nasal bones medially (Fig. 1). The paired nasal bones are nearly subrectangular and bordered anteriorly by the premaxillae. Anteriorly, they border the nostril and laterally connected to the prefrontals. The prefrontals are relatively triangular, anteriorly connected to the pars dorsalis, anterolaterally to the nasals and posteromedially to the frontal (Fig. 1). The lacrimal is a small triangular bone enclosed by maxilla, prefrontal, and nasal bones. This bone participates in forming the orbit cavity. In the right half of the head, it has a distinct boundary on both sides, but in the left half

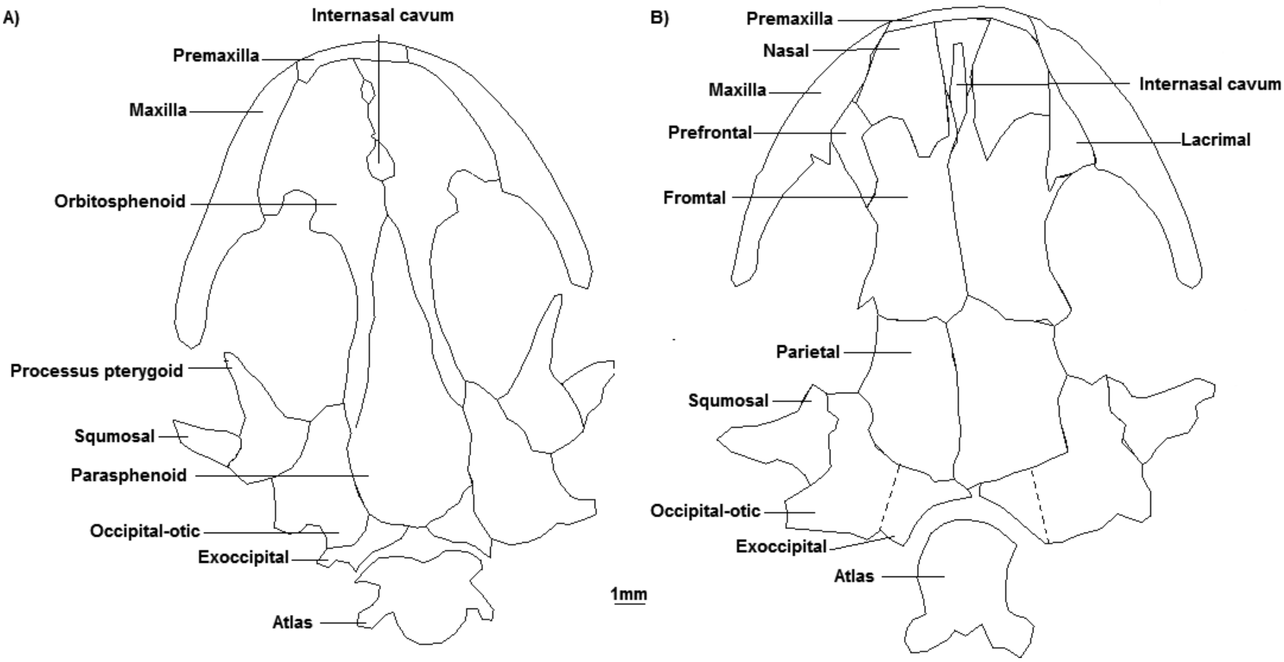

Fig. 1. Explanatory drawings of Dorsal (A) and ventral (B) view of *N. kaiseri* skull. Scale bar: 1 mm

of the head, this bone is fused to frontal with no boundary (Fig. 1). The frontals are flattest paired bones in the skull and trapezoid that makes a posterolateral projection. A very tiny cartilage binds this projection to squamosal to form a ring. The frontals meets the prefrontal anterolaterally, premaxillae anteriorly and parietal posteriorly. The parietals are relatively flat with small curvature in the posterolateral region towards to the orbit cavity. The parietals are rather smaller than the frontals and together complete the roof of the neurocranium. Two parts of parietal, having an overlap toward the foramen magnum. The squamosal is almost triangular that lying on the lateral side of exoccipital, becoming closely fixed with it to form a lateral projection. The base of the skull is ossified.

The orbitosphenoid articulates with the premaxillae (pars dentalis1) and maxilla anteriorly and laterally, respectively. The orbitosphenoid is located in the orbital region and makes an optic foramen posteriorly to pass off the optic nerves into the cranial cavity. Near the premaxilla, the orbitosphenoid has a medial foramen that opens into the braincase. Pterygoids bear relatively triangular depression on ventral surface from basicranial to orbital foramen. The unpaired and oval-shaped parasphenoid is the largest bone in middle part of ventral view of the skull. The quadrate is a relatively small bone located in anterior side of squamosal. The exoccipital forms the posteriormost part of the skull base, encircling the foramen magnum and articulating via the occipital condyles with the first vertebra. Exoccipitals have trapezoid shape ventrally and paired but never meet together at midline. Exoccipitals are anteriorly in contact with the parietal and laterally with squamosal (Fig 1). Further, exoccipital can be seen at the top of the skull. Squamosal is connected to the pterygoids bone and both pterygoids and squamosal are connected to the occipital.

Mandibles are paired and include mentomandibulars, dentaries and pre-articulars. The mandible is separated and symmetrical on both sides. The shape of the mandible (lower jaw) is a simple solid bony arch and dentary is the main element of the mandible (Fig. 2).

Phalanxes are well developed in all fingers, showing a tendency toward bifurcation, and are consistently seen to have small cartilaginous tips (Fig. 3). Further, each hand has four fingers. The biggest finger has four phalanxes, while the other fingers have only three phalanxes. The metacarpal includes seven bones. One of them is larger than the others and is connected to the ulnare and radius. The hands connected to humur through ulnare and radius and then connected to scapulocoracoid (Fig. 3).

Feet have five fingers and each finger has three phalanges (Fig. 3). The number of metatarsus bones is eight and four of them are bigger than the others. Each leg includes two bones (fibula and tibia) which are connect-

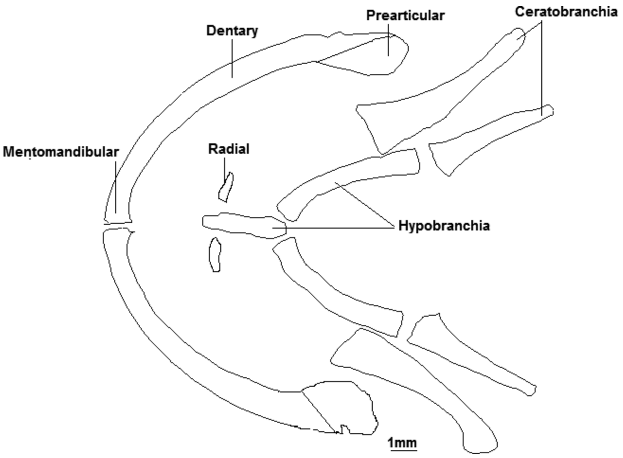

Fig. 2. Explanatory drawing of the lower jaw and hyobranchial apparatus of *N. kaiseri*. Scale bar: 1 mm

ed to femur. The head of the femur articulates with the acetabulum in the pelvic bone, while the distal part of the femur articulates with the tibia (Fig. 3).

Vertebral number in the axial skeleton equals 50. The cervical, abdominal and caudal parts of the vertebral column have two, 16 and 32 vertebrae, respectively. Also hands are connected to the body at the vertebrae number 2-3 and legs are connected at vertebrae number 16-17.

DISCUSSION

We described here, for the first time, the skeletal structure of *N. kaiseri*, an endemic and threatened newt in the southern Zagros Mountains of Iran. The species inhabits mountainous streams at high altitudes, which makes its habitat hard to access Due to the rarity and vulnerability of the species, only two deceased individuals of *N. kaiseri* could be obtained and used for osteological analyses. The two specimens examined here were similar (adult females with the total length of ~130 mm), which minimizes differences in ossification due to age. Body length is linearly correlated with age in many salamandrid species until maximum length is reached (Lima et al., 2000; Üzüm, 2009).

The skull of *N. kaiseri* is very compact and mineralized with low amounts of cartilaginous elements. Within the *Neurergus* genus, skull characteristics has only studied in *N. microspilotus* (Akia et al., 2010). The cranial characters and the number of characters of *N. kaiseri* and *N. micropilotus* show a general similarity. Posterior part of the skull in both species is wide and the snout being short and the entire skull parts are almost fully mineralized in the adults. The frontal and premaxillae of both *N. kaiseri* and *N. microspilotus* is relatively the same. However, it seems that the skull of *N. kaiseri* being smaller compared to *N. micropilotus*. Other noteworthy differences between the skulls of *N. kaiseri* and *N. microspilotus* include differences in the shape and size of

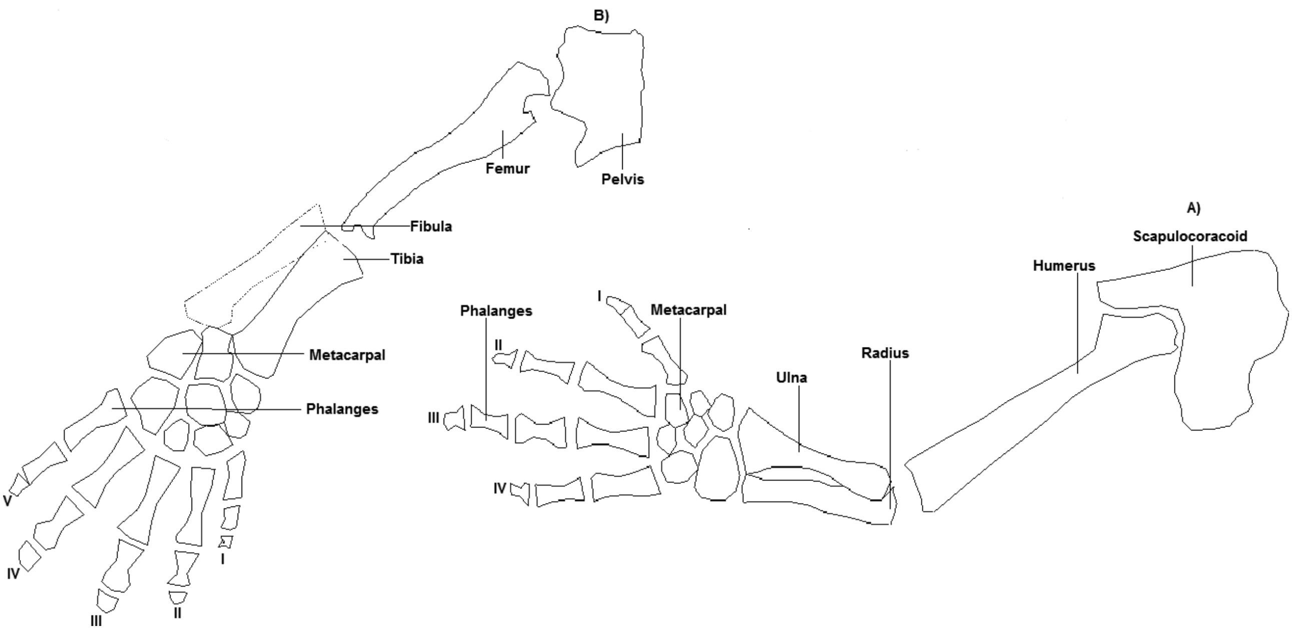

Fig. 3. Hand (A) and leg (B) of *N. kaiseri*. I-IV: digits I-IV. Scale bar: 1 mm.

maxilla, squamosal, exooccipital and cavum internasale. The pterygoids in *N. kaiseri* is shorter and weakly cartilaginous, whereas in *N. microspilotus* is larger, narrower with a great cartilaginous part. Squamosal is connected to the pterygoids bone and both pterygoids and squamosal are connected to the occipital. The premaxillae in *N. kaiseri* is distinct and well ossified. Less ossification, however, has been observed in other salamandrids (e.g., *Salamandra infraimmaculata semenovi* (Akia et al., 2010), which may agree with the primitive position of this taxon (Steinfartz et al., 2007) compared to more derived taxa such as *Neurergus* species. Skull morphology of *N. kaiseri* may be an adaptation for living in mountainous streams. Skulls in plethodontine salamanders that live in the mountain habitats (usually at altitudes above 900 m) tend to be harder and stronger (Buckley et al., 2010) compared to those inhabiting the flatter areas. Maxillary bones in *N. kaiseri* are more flattened and thicker than *N. micropilotus* and nasal bones are long and elongated in the length of skull roof. Naylor (1978) has suggested that the squamosal arch of newts is an anti-predator adaptation designed to add structural support to the skull and protect retracted eyes. Ehmcke and Clemen (2006) showed that the skull of the plethodontid salamanders is membranous and flexible.

Similarities are observed between the osteological structures of the hands, legs, lower jaw and hyobranchial apparatus of *N. kaiseri* with other newt species (Ghosh et al., 1994). Metacarpal establishes a connection between fingers and forearm bones which facilitate the animal movement (Fabrezi and Barg, 2001).

In general, similarities are observed between the skeleton structure of *N. kaiseri* and *N. micropilotus*. Both species are mountains species, however, there are some interspecific differences which could be related to the different total size of the adult individuals. However, there are other differences, which apparently only depend on the phylogenetic position of a species. Further research with a larger sample size and including other *Neurergus* species is required to investigate the remarkable and adaptive variation in bone-forms among these newt species.

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