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ORIGINAL ARTICLE

Radiographic Anatomy of the Limb Skeleton of the *Neurergus kaiseri*

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ABSTRACT

This study investigates the radiographic anatomy of the forelimb and hindlimb skeletons in the critically endangered Kaiser's Mountain newt (*Neurergus kaiseri*), an endemic Iranian salamander. Using non-invasive digital mammography (32 kVp, 80 mAs), we analyzed the osteology of 10 adult specimens (5 males, 5 females) to characterize appendicular skeletal features. The scapulocoracoid was a single bony unit with a prominent glenoid cavity and supracoracoid foramen. The humerus exhibited flattened cranial and caudal crests, while the radius and ulna remained separate, with distinct proximal/distal width patterns. The carpus comprised seven bones (ulnare, radiale, prepollicis, centrale, distal carpals 3–4, and basale commune), and the manus displayed four digits with a phalangeal formula of 2-3-2-2. The pelvic girdle fused into an ilium-ischiopubic plate, lacking an obturator foramen. The femur was longer than the humerus, featuring a medially oriented head and a hook-shaped trochanter. The tibia and fibula were equal in length but shorter than the femur. The pes had five digits (phalangeal formula: 2-2-3-3-2) and seven tarsal bones, including a fused basale commune (distal tarsals 4–5). Comparative analysis revealed key differences from related species (*Hynobius setouchi*, *Batrachuperus londongensis*), such as carpal/tarsal bone counts and fusion patterns. These findings provide foundational data for ecological, evolutionary, and conservation studies, highlighting mammography as a viable, non-invasive tool for skeletal research in delicate amphibians.

Introduction

Salamanders and newts are a group of amphibians (Class: Amphibia) belonging to the Caudata order. Unlike anurans (frogs and toads), caudatans (salamanders and newts) retain their tails throughout their life cycle, from the larval stage to adulthood. This key trait places them within the tailed amphibians (Caudata), distinguishing them from other amphibian groups.[1]

The *Neurergus kaiseri* Schmidt, 1952 is an Iranian newt species of the family *Salamandridae*, endemic to the mountainous habitats of southern Lorestan and northern Khuzestan provinces in the Zagros Mountains forest steppe range. This species exhibits an elongated body, long tail, and short, webbed limbs adapted for swimming, primarily using its powerful tail for aquatic propulsion. *Neurergus* genus possess four digits on the forelimbs and five toes on the hindlimbs. Specimens with malformations often have reduced or extra digits or limbs, or are missing digits or limbs entirely or partially.[2] Notably, *N. kaiseri* displays significant regenerative capacity, including the ability to regenerate various body parts and certain organs.[1]

Due to the vulnerable status of the Kaiser's Mountain newt and its significant conservation value, research methodologies must prioritize minimal invasiveness

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while still providing valuable scientific data. Anatomical studies and imaging techniques, particularly skeletal imaging, form the foundation for numerous biological and biomedical investigations. While traditional methods like dissection remain economically accessible, their invasive nature renders them unsuitable for studying endangered species and other valuable wildlife. Furthermore, conventional bone preparation techniques often yield suboptimal results when examining delicate skeletal structures like those of salamanders, especially when working with limited specimen availability. In contrast, non-invasive imaging modalities such as mammography overcome these limitations, enabling detailed examination of even the most delicate anatomical structures without compromising specimen integrity. While micro-CT scanning offers superior resolution for skeletal imaging, digital mammography with optimized parameters (<35 kVp) has proven equally effective for visualizing amphibian osteology while being more accessible and less stressful for sensitive species.[2] This makes it particularly suitable for endangered taxa like *N. kaiseri*, where specimen preservation is paramount.

Imaging techniques serve a dual purpose in biological research: they not only provide comprehensive anatomical visualization of bodily structures, but also enable non-invasive injury assessment without causing additional harm or stress to the specimen. This makes them particularly valuable for studying delicate or endangered species.

The skeletal system serves as the fundamental framework of the body, providing the primary reference for the spatial organization of other organ systems. In diagnostic imaging, skeletal structures not only serve as direct subjects of investigation but also function as crucial topographic landmarks for locating other anatomical features. Despite its importance, comprehensive anatomical and diagnostic imaging studies of both the appendicular skeleton and cranium in this species remain lacking. While limited osteological examinations of this salamander species have been conducted, none have employed radiographic techniques, resulting in an absence of detailed structural characterization.[3]

Beyond the aforementioned advantages of radiography for anatomical studies, it is important to emphasize that this technique is significantly more accessible than other imaging modalities such as micro-CT or conventional CT scanning. In the present study, we aimed to investigate the osteology of both forelimbs and hindlimbs in male and female Kaiser's Mountain newt (*Neurergus kaiseri*) using mammography, with subsequent comparative analysis against other salamander species. Specifically, this study focuses on characterizing the detectable anatomical features of the appendicular skeleton in this species through mammographic imaging.

Materials and Methods

Specimens

Ten adult Kaiser's Mountain newt (*Neurergus kaiseri*) (5 males and 5 females; 3.68-5.80 cm) were studied. Due to the special conservation status of this species and its classification as nationally endangered, all necessary coordination and correspondence with relevant organizations was completed prior to sample collection, and their official approval was obtained. The newts were captured during two field visits to the Karkari-Karsar region in southern Lorestan. It should be noted that this study is part of a PhD dissertation in Comparative Veterinary Anatomy at the University of Tehran's Faculty of Veterinary Medicine (ID: 30740/6/12).

For this phase of the study, the specimens were transported to the radiology department of Lorestan Social Security Hospital in Khorramabad. Prior to mammography, each specimen was anesthetized using MS-222 administered through dermal absorption (dose: 10 mg per liter of water) in an anesthetic bath.[4]

Imaging Parameters

The mammographic images were acquired in dorsoventral positioning using a Hologic digital mammography system (manufactured in the USA; Figure 1), with imaging parameters set at 32 kVp and 80 mAs. Due to the inherent compression requirement of mammography imaging systems, lateral views could not be obtained, and the dorsoventral images were acquired with minimal applied compression to minimize specimen stress. Given this species' sensitivity, the imaging procedures were performed rapidly, and specimens were not held for extended periods. Sex differentiation during specimen selection was accomplished using established morphological identification keys from published literature.[5]

Release of Specimens

Following completion of imaging procedures, all salamanders were transported back to their original habitat for release.

Results

Scapular Girdle

The scapulocoracoid bone existed as a single bony unit. This bone did not have a particularly wide or extensive structure. In the Kaiser's Mountain newt, the scapulocoracoid bone had three edges: cranial, caudal, and lateral. Both the cranial and caudal edges were prominent, and in the anterior portion of the lateral edge, a structure was observed that was more radiopaque than other parts. Near the posterior edge of the lateral side of

Figure 1. The Hologic digital mammography system used in this study.

the bone, the glenoid cavity structure was located. Slightly cranial to the glenoid cavity, the supracoracoid foramen was observed (Figure 2).

Humerus

The humeral head was not particularly rounded and appeared mostly flattened. Slightly distal to the head, the bone narrowed to form the anatomical neck. Further distally, both the crista caudalis and crista cranialis were observed. The crista cranialis was larger and faced cranially, while the crista caudalis was smaller and faced caudally. Additionally, the crista cranialis was positioned more proximally than the crista caudalis (Figure 3).

Radius and Ulna

The radius and ulna in *Neurergus kaiseri* were approximately equal in length. These two bones remained separate, with no fused segments between them. The distal end of the radius appeared wider than its proximal end, while the opposite pattern was observed in the ulna, the proximal end was broader than the distal end. However, the marked difference in width between proximal and distal ends seen in the radius was not present in the ulna (Figure 3).

Carpal Bones

The carpus was composed of seven bones arranged in proximal and distal rows. The proximal row (from lateral to medial) consisted of the following elements: the ulnare, the radiale, and the prepollicis. Between the ulnare and radiale, slightly more distal, lay a single centrale. The distal row (from lateral to medial) included: distal carpal 4, distal carpal 3, and the basale commune. Among the carpal bones of the Kaiser's Mountain newt (*Neurergus kaiseri*), the ulnare was the largest. This polygonal bone articulated with the distal end of the ulna. In contrast, the smallest carpal bone was the prepollicis (Figure 3).

Metacarpal Bones

In all examined specimens, the forelimb consistently exhibited four digits. Each digit contained a single metacarpal bone. The first metacarpal was shorter than the others, while the second and third metacarpals were nearly equal in length and longer than the remaining two. All four metacarpals were morphologically similar, displaying a dumbbell-like shape (Figure 3).

Digits

As mentioned in the previous section, the number of digits in all samples of the forelimb was four. In the *N.*

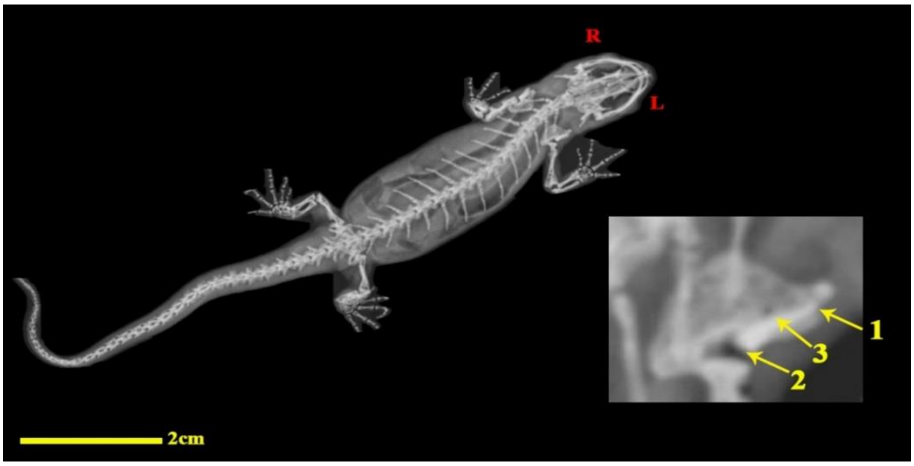

Figure 2. Dorsoventral radiograph of the *Neurergus kaiseri* full-body view. The right panel shows an enlarged view of the scapulocoracoid region. Roman numerals label the digits, though the phalangeal joints are not clearly discernible due to non-standard positioning of the digits. 1. More radio opaque area on lateral border of the scapulocoracoid, 2. Glenoid cavity, 3. Supracoracoid foramen.

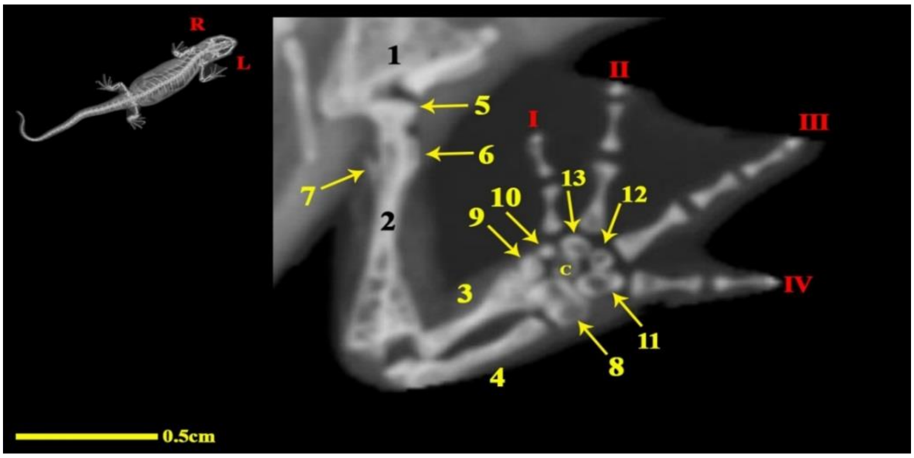

Figure 3. Dorsoventral radiograph of the *Neurergus kaiseri* full-body view (left panel). The right panel shows an enlarged view of the forelimb skeletal structures. Roman numerals mark the digits, though the phalangeal joints are not clearly discernible due to non-standard digit positioning. 1. Scapulocoracoid, 2. Humerus, 3. Radius, 4. Ulna, 5. Head of the humerus, 6. Crista cranialis (humerus), 7. Crista caudalis (humerus), 8. Ulnare, 9. Radiale, 10. Prepollicis, C. Centrale, 11. Distal carpal 4, 12. Distal carpal 3, 13. Basale commune.

kaiseri, the third digit was longer than the others. Digits one, two, and four had two phalanges, while digit three had three phalanges. Thus, the phalangeal formula from lateral to medial was observed as 2-3-2-2 (Figure 4).

Pelvic Girdle Bones

The pelvic girdle in *Neurergus kaiseri* consisted of three bones - the ilium, ischium, and pubis. No distinct boundary was observed between the ischium and pubis, which together formed a quadrilateral plate-like structure, consistent with findings in other studies. The proximal end of the ilium articulated with the sacral rib, while its caudolateral portion exhibited a prominent ischial spine (Figure 5). This configuration, characterized by the fused ischiopubic plate and ilial-sacral connection, reflects the species' adaptations for semi-aquatic locomotion. The absence of a clear obturator foramen and the robust ischial spine represent distinctive osteological features of this salamandrid species.

Femur

The femur of the Kaiser's Mountain newt exhibited several distinctive anatomical features. Notably longer than the humerus, this bone displayed a proximal head structure that was distinctly oriented medially and caudally. Below the head region, the femoral neck showed minimal constriction, maintaining nearly uniform shaft diameter. A characteristic hook-shaped femoral trochanter projected medially from the proximal shaft, with a distinct proximal orientation. The distal femoral extremity expanded significantly, forming prominent articular surfaces for tibial and fibular articulation (Figure 5). These morphological adaptations collectively facilitate powerful limb movements in both aquatic and terrestrial environments, with the elongated structure providing mechanical advantage during propulsion while the robust articular surfaces ensure stable limb articulation. The observed trochanter morphology suggests strong attachment sites for pelvic musculature, particularly important for this species' unique locomotor demands in its mountainous stream habitat.

Tibia and Fibula

In *Neurergus kaiseri*, the tibia and fibula were nearly equal in length but shorter than the femur (Figure 5). The tibia exhibited a broader proximal end compared to its distal portion, while the fibula showed the opposite pattern with distal expansion. Craniocaudally, the tibia was positioned cranial to the fibula.

Tarsal Skeletal Structure

The tarsus in *Neurergus kaiseri* consisted of seven bones organized into functional groups (Figure 6). The

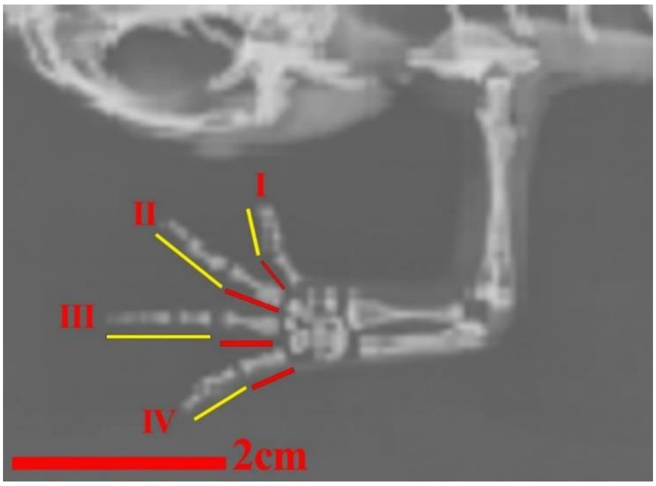

Figure 4. Dorsopalmar radiograph of the left manus in *Neurergus kaiseri*, with digits labeled using Roman numerals (I-IV). The phalangeal regions are delineated by yellow lines, while the metacarpal zones are marked with red lines along their abaxial margins. 1. Scapulocoracoid, 2. Humerus, 3. Radius, 4. Ulna, 5. Head of the humerus, 6. Crista cranialis (humerus), 7. Crista caudalis (humerus), 8. Ulnare, 9. Radiale, 10. Prepollicis, C. Centrale, 11. Distal carpal 4, 12. Distal carpal 3, 13. Basale commune.

proximal row included the tibiale (articulating with the tibia), fibulare (connecting to the fibula), and an intermedium positioned between them. A single centrale bone occupied the central tarsal region. Distally, the basale commune -formed by fusion of distal tarsals 4 and 5- articulated with metatarsals IV and V, while three separate distal tarsals (1-3) connected to metatarsals I-III. This arrangement, particularly the derived basale commune fusion, provides both structural stability for weight-bearing and flexibility for aquatic propulsion, representing an adaptive balance for this semi-aquatic species' locomotor needs. The precise articulation patterns between all tarsal elements and their corresponding long bones are clearly demonstrated in Figure 6.

Metatarsal Bones

In *Neurergus kaiseri*, five metatarsal bones (I-V) were observed in the hindlimbs, each exhibiting a characteristic dumbbell shape (Figure 6). Metatarsal I was the shortest, while metatarsals II, III, and IV were significantly longer and of nearly equal length, demonstrating a clear morphological gradient that likely enhances weight distribution and locomotor efficiency in both aquatic and terrestrial environments. All metatarsals maintained precise articulations with their respective tarsal bones proximally and phalanges distally, as clearly visualized in Figure 6.

Digital Morphology

All *Neurergus kaiseri* specimens exhibited five hindlimb digits (I-V) with a consistent phalangeal formula of 2-2-3-3-2 (lateral to medial), where digits III and IV contained three phalanges while digits I, II, and V each possessed two phalanges (Figure 6). Digit III was the longest in the series, contrasting with the shortest digit I, reflecting an adaptive pattern that balances propulsion efficiency with structural economy in this semi-aquatic species. This configuration is clearly demonstrated in Figure 6, showing both the phalangeal count and proportional length relationships among the digits.

Discussion

In *Batrachuperus*, unlike most salamanders (except sirenids), the scapulocoracoid remains unfused, with the scapula and coracoid maintaining separate ossifications—a condition contrasting with the fused scapulocoracoid typical of *Neurergus kaiseri* and other salamandrids. This genus-specific morphology reflects divergent evolutionary adaptations in pectoral girdle structure among urodeles, where fusion patterns correlate with locomotor ecology. The unfused state in *Batrachuperus* may facilitate enhanced forelimb mobility in its specialized stream habitat.[6]

The Japanese *Hynobius setouchi* exhibits a fused scapulocoracoid structure, analogous to the condition observed in *Neurergus kaiseri* but contrasting with the separate scapula and coracoid of *Batrachuperus*. This morphological convergence between distantly related genera (*Hynobius* and *Neurergus*) suggests adaptive significance in scapulocoracoid fusion for terrestrial locomotion, despite their distinct phylogenetic positions within Urodela. The fused state likely enhances structural stability during weight-bearing movements in these semi-terrestrial species.[7]

The scapulocoracoid in *Batrachuperus londongensis* exhibits a characteristically shortened morphology, consistent across the *Batrachuperus* genus and paralleled in *Neurergus kaiseri*, while *Hynobius setouchi* displays a distinct scapular process at a position anatomically comparable to the radiolucent area observed in *N. kaiseri*'s scapulocoracoid, with its dorsal aspect attaching to suprascapular cartilage. These structural differences reflect genus-specific adaptations: the shortened scapulocoracoid in *Batrachuperus* and *Neurergus* likely enhances hydrodynamic efficiency in aquatic environments, whereas *Hynobius*'s scapular process may facilitate terrestrial locomotion through expanded muscle

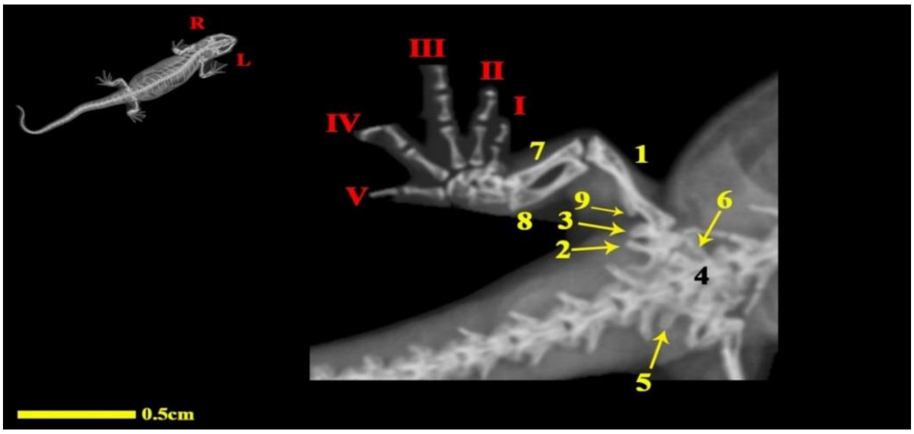

Figure 5. Dorsoventral radiograph of *Neurergus kaiseri* (left panel), with the right panel showing an enlarged view of the hindlimb skeletal structures. Roman numerals label the digits, though the phalangeal joints are not clearly discernible due to non-standard limb positioning. 1. Femur, 2. Sacral rib, 3. Ilium, 4. Sacrum, 5. Ischium (ischial spine), 6. Pubis, 7. Tibia, 8. Fibula, 9. Femoral trochanter.

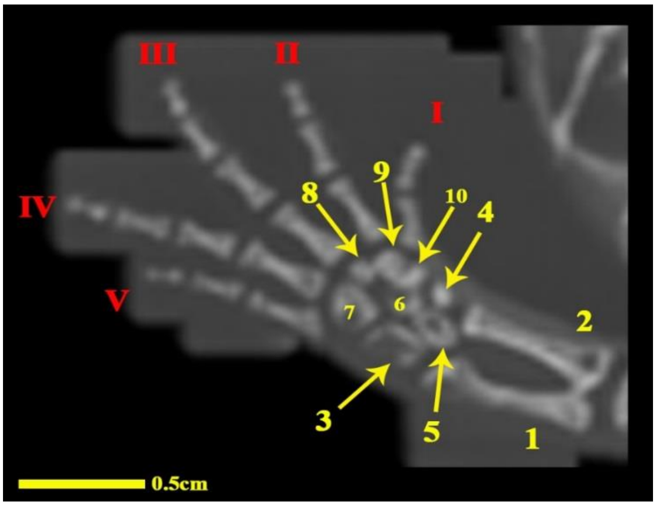

Figure 6. A dorsoplantar radiograph of the pes region in the *Neurergus kaiseri*. The digits are labeled with Roman numerals (I, II, III, ...). 1. Fibula, 2. Tibia, 3. Fibulare, 4. Tibiale, 5. Intermedium, 6. Centrale, 7. Basale commune, 8. Distal tarsal 3. 9. Distal tarsal 2, 10. Distal tarsal 1.

attachment surfaces, demonstrating how divergent evolutionary pressures have shaped pectoral girdle architecture in these urodeles.[8]

The humerus of *Hynobius setouchi* differs notably from *Neurergus kaiseri* in several aspects: (1) it lacks a distinct anatomical neck, instead exhibiting a hemispherical proximal articular surface; (2) features two crests - the *crista dorsalis* (homologous to *N. kaiseri*'s crista caudalis) and *crista ventralis* (corresponding to crista cranialis), demonstrating alternative anatomical terminology for functionally similar structures. These morphological differences reflect distinct biomechanical adaptations between these semi-aquatic species, where *H. setouchi*'s simpler humeral geometry may facilitate different locomotor demands in its Japanese wetland habitats compared to *N. kaiseri*'s Zagros Mountains stream environment.[9]

The humerus of *Batrachuperus londongensis* exhibits two functionally specialized crests: (1) the triangular *crista dorsalis* (homologous to *Neurergus kaiseri*'s crista cranialis) serving as the insertion site for the *subscapularis* muscle, and (2) the *crista ventralis* (equivalent to *N. kaiseri*'s crista caudalis) providing attachment for both *pectoralis* and *supracoracoideus* muscles. These distinct musculoskeletal associations reveal how subtle morphological differences in crest shape and orientation (triangular vs. linear) between *Batrachuperus* and *Neurergus* reflect micro-adaptations in force transmission mechanisms, likely tied to their respective ecological specializations - torrent-dwelling versus semi-aquatic stream habitats.[6]

In the Setouchi salamander (*Hynobius setouchi*), similar to the Kaiser's Mountain newt (*Neurergus kaiseri*), the radius and ulna bones were approximately equal in length. These two bones were separate, with no fused segments between them. The distal end of the radius was wider than its proximal end, while the opposite pattern was observed in the ulna. In *H. setouchi*, the carpal region contains eight bones. The proximal row consists of: one ulnare bone, one radiale bone, two centrale bones, and one prepollicis bone. The distal row includes: one distal carpal 4 bone, one distal carpal 3 bone, and one basale commune bone. As mentioned earlier, the Kaiser's Mountain newt has seven carpal bones, with the difference in count compared to *H. setouchi* being due to the presence of a single centrale bone in *N. kaiseri* instead of two.[7]

In *Hynobius setouchi*, it has been mentioned that the *ulnare* and *intermedium* bones are fused together, forming a large bone, and it appears that the same process has occurred in the Kaiser's Mountain newt (*Neurergus kaiseri*). It has been noted that in *Batrachuperus londongensis*, the smallest carpal bone is the *prepollicis*, and this condition also exists in the Kaiser's Mountain newt. Regarding the distal row carpal bones of *Batrachuperus londongensis*, it has been stated that the *basale commune* results from the fusion of *distal carpal 1* and *distal carpal 2* bones, which appears to be the same case in the Kaiser's Mountain newt. This bone in both species is located adjacent to metacarpal bones 1 and 2 and articulates with them. Additionally, in both species, the *distal* carpal 1 and distal carpal 2 bones articulate with metacarpals 1 and 2, respectively.[6,7]

In *Hynobius setouchi*, metacarpal I exhibits a distinctive proximal enlargement resulting in significant proximal broadening compared to its distal end, making it morphologically distinct from other metacarpals, whereas *Neurergus kaiseri* shows uniform dumbbell-shaped morphology across all metacarpals with metacarpal I being smaller but similarly shaped to others, reflecting species-specific adaptations - the specialized structure in *H. setouchi* potentially enhancing grasping capability, while *N. kaiseri*'s homogeneous morphology likely facilitates balanced load distribution during locomotion.[7]

As noted by Jiang *et al.*, most salamanders exhibit four forelimb digits, a pattern consistently observed in *Neurergus kaiseri*. However, interspecific variation exists in digit proportions: while *Hynobius setouchi* displays digit II as the longest, both *N. kaiseri* and *Batrachuperus londongensis* demonstrate digit III elongation, suggesting genus-specific adaptations in manual structure that may correlate with differential locomotor demands in their respective ecological niches (arboreal vs. semi-aquatic habitats).[6,7]

The phalangeal formula (2-2-3-2, medial-to-lateral) is conserved in *Hynobius setouchi*, *Batrachuperus londongensis*, and *Neurergus kaiseri*, though *H. setouchi* additionally exhibits variant formulae (1-2-2-2, 1-2-2-1, 2-2-2-1). All three species possess a tripartite pelvic girdle (ilium, ischium, pubis), with documented cases in *H. setouchi* and *B. londongensis* of completely cartilaginous pubis - a condition not observed in the examined *N. kaiseri* specimens, suggesting interspecific differences in ossification patterns potentially linked to locomotor adaptations.[6,7]

In both *Hynobius setouchi* and *Batrachuperus londongensis*, the ischium and pubis fuse to form a *pubo-ischium plate*, a feature also observed in *Neurergus kaiseri* given the indistinct boundary between these bones. These species share a short, broad *ischial spine* (rudimentary and occasionally absent in *B. londongensis*), which in *B. londongensis* serves as the origin for the *ischiocaudalis* (tail flexor) muscle, highlighting conserved pelvic adaptations among semi-aquatic urodeles.[6,7]

The absence of an *obturator foramen* in both *Hynobius setouchi* and *Neurergus kaiseri* contrasts with its presence in some *Batrachuperus* species (anterior pubis region). Notably, *Batrachuperus londongensis* exhibits a ligamentous connection between the sacral rib and ilium - an anatomical relationship also observed in *N. kaiseri*, suggesting homologous ligamentous attachments in these semi-aquatic salamanders despite their phylogenetic divergence.[6,7]

While *Hynobius setouchi* and *Batrachuperus londongensis* exhibit near-equal length of the femur and humerus, *Neurergus kaiseri* demonstrates a distinct morphological pattern with the femur being significantly longer than the humerus, suggesting species-specific adaptations in limb biomechanics correlated with their respective locomotor ecologies (semi-aquatic vs. rheophilic habitats).[7]

All three species - *Hynobius setouchi*, *Batrachuperus londongensis*, and *Neurergus kaiseri* - exhibit a *femoral trochanter* where the *pubo-ischio-femoralis externus* muscle attaches, along with a characteristically broadened distal femoral end, confirming conserved osteological adaptations for limb motion across these semi-aquatic urodeles despite differences in their femoral-humeral length ratios.[6,7]

In both the *Setouchi salamander* (*Hynobius setouchi*) and *Batrachuperus londongensis*, the tibia and fibula are approximately equal in length, and both are notably shorter than the femur. Additionally, in these species, the fibula exhibits a thinner morphology compared to the tibia. In the *Setouchi salamander*, as in the Kaiser's Mountain newt (*Neurergus kaiseri*), the proximal end of the tibia is broader than its distal end, while the fibula displays the opposite pattern—its distal end is wider than the proximal end.[6,7]

Comparative anatomical studies reveal that the Setouchi salamander (*Hynobius setouchi*) possesses two central tarsal bones, while the Kaiser's Mountain newt (*Neurergus kaiseri*) exhibits only one. In *H. setouchi*, the distal tarsals corresponding to digits III-V remain completely separate, whereas the distal tarsals of digits I and II are fully fused, forming a unified structure known as the basal commune bone. This pattern differs in *N. kaiseri*, where the basal commune results from the fusion of distal tarsals from digits IV and V. Additionally, *H. setouchi* displays two accessory skeletal elements -the postminimus and element y- which are entirely absent in *N. kaiseri*. Both species maintain the ancestral pentadactyl condition in their hindlimbs.[7]

Batrachuperus londongensis demonstrates a distinct pattern of limb morphology, exhibiting only four digits in its hindlimbs. Tarsal examination in this species confirms the presence of a single central bone along with two accessory elements (postminimus and element y). Notably, similar to *H. setouchi*, this species shows complete fusion of the distal tarsals from digits I and II, forming the basal commune bone. These comparative observations reveal significant differences in tarsal organization among the three species, likely reflecting their distinct evolutionary adaptations within amphibian lineages. The consistent fusion pattern of distal tarsals I-II in both *H. setouchi* and *B. londongensis*, contrasting with the IV-V fusion in *N. kaiseri*, suggests potential phylogenetic significance in tarsal developmental pathways among these salamanders.[6]

Both the Setouchi salamander (*Hynobius setouchi*) and Kaiser's Mountain newt (*Neurergus kaiseri*) exhibit pentadactyl hindlimbs, with each digit possessing a single metatarsal. In these species, metatarsals II-IV are notably longer than the others. However, while *N. kaiseri* displays uniformly hourglass-shaped metatarsals (including metatarsal I), *H. setouchi* shows distinct morphology - its metatarsal I lacks the hourglass shape and demonstrates a proximal width exceeding its distal width. Both species share the characteristic of digit III being the longest in the hindlimb. The digital arrangement differs in relative lengths: in *H. setouchi*, digit V is the shortest, contrasting with *N. kaiseri* where digit I holds this distinction. Both species maintain an identical phalangeal formula (2-2-3-3-2). *Batrachuperus londongensis* presents a tetradactyl condition with similar relative digit lengths to *N. kaiseri*, despite having one fewer digit. This species exhibits remarkable bilateral asymmetry in its phalangeal formula: the left side typically shows 2-2-3-3 patterning while the right side varies (2-2-3-2). Importantly, this asymmetrical pattern demonstrates intraspecific variability across different subspecies. The observed lateral asymmetry in phalangeal count, combined with the conserved relative digit length proportions (mirroring the Kaiser's Mountain newt despite reduced digit number), suggests complex developmental pathways in limb patterning within this genus. These morphological variations may reflect either phylogenetic constraints or ecological adaptations specific to each species' microhabitat requirements.[6,7]

In conclusion, this study provides the first detailed radiographic characterization of the appendicular skeleton in *Neurergus kaiseri*, a critically endangered salamander endemic to Iran. The findings reveal distinct osteological adaptations, including a fused scapulocoracoid, elongated femur, and specialized carpal/tarsal arrangements, which likely reflect the species' semi-aquatic locomotion in mountainous stream habitats. The use of digital mammography proved highly effective for non-invasive skeletal imaging, offering a practical alternative to traditional dissection methods, particularly for delicate and endangered species.

Comparative analysis with related salamanders (*Hynobius setouchi*, *Batrachuperus londongensis*) highlighted key interspecific variations in limb morphology, such as differences in bone fusion patterns and digit proportions. These differences may correlate with ecological adaptations, providing insights into evolutionary divergence within the Urodela order.

The data presented here serve as a crucial anatomical reference for future research in herpetology, evolutionary biology, and conservation. Given the vulnerable status of *N. kaiseri*, non-invasive techniques like mammography should be prioritized in further studies to minimize ecological impact while advancing scientific understanding. This work underscores the importance of integrating imaging technologies into wildlife research to support both anatomical discovery and species preservation efforts.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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