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# **Evaluation of the middle ear in water buffalos (*Bubalus bubalis*) by gross anatomy and cone beam computed tomography**

**Running title:** Morphology of the middle ear in water buffalos

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## **Abstract**

**Background:** The purpose of this study was to provide a description of gross middle ear morphology in water buffalos, augmented with additional data on the osseous structures of middle ear derived from cone-beam computed tomography (CBCT).

**Materials and methods:** Skulls of 10 young adult male water buffalos were used to examine their middle ear.

**Results:** Anatomical features noted included the presence of tympanic cells in the tympanic bulla, the location of malleus head and neck, and all of incus in the dorsal epitympanic recess, the oval tympanic membrane (TM), absence of a prominent notch on the articular surface of malleus, positional variations of the lateral process of malleus relative to the muscular process and muscular process relative to the rostral process of malleus, absence of complete coverage of the articular facet



of malleus head by incus body, and presence of the lenticular process of incus. In CBCT images, the osseous part of external acoustic meatus, the petrous part of temporal bone and the details of the ossicles were seen, except for stapes.

**Conclusions:** Although TM, malleus and stapes of water buffalos are similar to those of ox, the incus of water buffalos is more similar to that of goats. The heaviest ossicles among the ruminants studied belonged to water buffalos; the mean length of malleus head and neck, total length and width of incus body as well as length of stapes head were greatest in water buffalos too. The auditory ossicles of water buffalos show ‘transitional type’ morphological characteristics. These features suggest a relatively wide frequency range of hearing, but not one biased towards especially low or especially high frequencies.

**Key words:** anatomy, auditory ossicles, cone beam computed tomography, middle ear, morphometry, water buffalo

## **INTRODUCTION**

The middle ear cavity is an irregular space within the temporal bone that is filled with air. It contains the malleus, incus, and stapes, which form a chain and serve to convey vibrations from the tympanic membrane across the cavity to the internal ear [12]. Many studies on the middle ear have been conducted from morphological, functional, and surgical viewpoints, with physiological experiments typically having been performed on relatively small laboratory animals such as gerbils, guinea pigs and cats. There is considerable variation in middle ear architecture among the many species studied [7, 16, 20, 21]. Body size, phylogeny, style of life, ecology, and acoustic environment can all be reflected in aspects of ear morphology [29].

Recently, there has been more interest in the middle ear of larger animals such as sheep and pig, which potentially represent better models of the human middle ear [21, 30]. However, the gross morphology of middle ear of ox, sheep, and goat is not described or illustrated in any detail in most veterinary anatomical textbooks, atlases and dissection guides [25, 8, 5, 14, 32]. Only Getty [1975], in his veterinary anatomical textbook, included detailed comparative descriptions of this area, but did not present any schematic pictures and photographs. There are a few descriptive anatomical accounts of sheep and ox ears [34, 35, 4], but there remains much to be clarified regarding the comparative anatomy of ruminant auditory systems.

CBCT acquires data volumetrically, providing accurate, three-dimensional (3D) radiographic imaging which is ideal for the assessment of osseous structures of the maxillofacial region at sub-millimeter resolution [18]. This has proved to be an excellent technology for the identification and description of the normal anatomy of middle ear structures in veterinary research [31]. CT studies on the normal anatomy of middle ear structures have been conducted in sheep [33], and horses [3].

Water buffalos (*Bubalus bubalis*) are placed in the family Bovidae, within the order Artiodactyla. Some archeological evidence suggests that water buffalos were first domesticated in Iran and migrated to southern Europe from this region [24]. The Khuzestan ecotype of Iranian water buffalos are likely to be the biggest buffalo breed in the world [27], the morphological appearance of which differs from that of Mediterranean water buffalos [24]. No description of the middle ear of water buffalos seems to have been published. The goals of this study were, therefore, (1) to provide a detailed description of the middle ear of water buffalos, (2) to determine the similarities and differences between water buffalos ears and those of other ruminants, and (3) to describe the osseous structures of the middle ear of water buffalos using CBCT.

## **MATERIALS AND METHODS**

Skulls of 10 young adult male water buffalos, without any external abnormality or pathology, were collected from a local slaughterhouse in Ahvaz, southwest Iran. The age of animals, as estimated from the eruption of teeth [9], ranged between 2 to 3 years. The animals' live weight (316–383 kg) was estimated based on carcass weight (190–230 kg). There was no need of approval from the Local Ethical Committee as the animal skulls were collected from the slaughterhouse. The maceration of skull and the dissection of tympanic cavity were performed according to the procedures of Nummela [1995], and Martonons et al. [2019], respectively. Afterwards, the following measurements for separated auditory ossicles were taken according to Martonons et al. [2019]. Malleus: total length (TL), length of the manubrium (LM), head diameter (HD), and head and neck length (HNL). Incus: total length (TL), body width (BW), length of short crus (LSC), and length of long crus (LLC). Stapes: total length (TL), head length (HL), and thickness of crura at the middle (TCM). All variables were measured three times by a single researcher using a digital caliper (150 mm, Mitutoyo, Japan) and presented as means and standard deviations. The masses of ossicles were measured to an accuracy of 0.001 g on laboratory scales (A & D Company, MA 3000, Japan). Photographs of the ossicles were taken under a stereomicroscope (Nikon, SMZ800, Japan) using a Canon digital camera (G9, Tokyo, Japan).

CBCT images were acquired using New Tom VGi scanner (New Tom GRsrl; Verona, Italy) with a field of view 12cm × 8cm at a 0.15 mm voxel resolution with the scanning parameter of 110 kVp, 9.18 mA (for left side) and 7.33 mA (for right side), and 5.4 seconds exposure time. CBCT data were evaluated in the dorsal and sagittal planes by an expert maxillofacial radiologist.

## RESULTS

### **Tympanic membrane (TM)**

The mean and standard deviation of rostrocaudal and dorsoventral dimensions of TM were  $11.66 \pm 0.63$  mm and  $9.12 \pm 0.12$  mm, respectively. The TM was made up of two major parts, the pars flaccida (PF) and pars tensa (PT). The PF, an irregular triangle, was small and extended from the surrounding parts of the tympanic incisures of squamous temporal bone to the lateral process of malleus (Figure 1B). The shape of PT was a regular oval. The medial surface of TM appeared to be irregularly concavo-convex. The entire length of manubrium was embedded in PT. The manubrium almost in its middle was very strongly attached to the medial surface of PT (Figure 1 A, B).

The epitympanic recess housed the head and neck of malleus, and all of the incus (Figure 1A). The ventral part of middle ear cavity was divided into tympanic cells (Figure 5A).

### **Auditory ossicles**

The morphometric data obtained from the ossicles are presented in Tables 1 and 2.

**Malleus Head.** Caudally, the head was slightly convex and its edges rounded and somewhat pressed (Figures 2A, C) and there was a small, shallow fossa just rostral to the root of the lamina on the lateral surface of head (Figure 2A, C). The head had a concave facet on its caudomedial aspect for articulation with incus. The facet was divided by a shallow notch into dorsal and ventral parts (Figure 2B).

**Malleus Neck.** The curved neck was not very distinct below the head (Figure 2A, B). The well-developed lamina was thin and irregularly triangular in shape: it originated from the dorsolateral surface of neck and extended rostrally, dorsally and laterally. The rostral process was a delicate projection in continuity with lamina, and was lateral to the large muscular process (Figure 2A). The rostral process was attached by a ligament to the wall of tympanic cavity (not shown). There was a very distinct shallow fossa caudal to the muscular process on the mediodorsal side (Figure 2B). The medial surface of neck, just rostral to the muscular process, was crossed by chorda tympani branch

of facial nerve (Figure 1A). The muscular process was a very well-developed and prominent spur which arose medially from the distal part of the neck (Figure 1A, 2B).

**Malleus Manubrium.** The manubrium had four-sided. The lateral edge of manubrium was convex. It was attached to TM, whereas the medial edge of the manubrium was free. The rostradorsal surface of the manubrium was slightly concave longitudinally from body to tip, whereas the caudoventral surface was convex. The lateral process was a right-angled triangular protuberance which arose from the base of the manubrium at the same level as the muscular process on the opposite side (Figure 2A, B).

The imaginary line extending between the anterior process of malleus and the short process of the incus, two points where the ossicles are tethered to skull, has been called the ‘anatomical axis’ (Lavender et al. 2011). The manubrium took an intermediate orientation relative to the anatomical axis, i.e. between perpendicular and parallel (Figure 1A).

**Incus.** The medial surface of body was convex with a distinct oval facet (Figure 3A), while the lateral surface was almost smooth (not shown). The shape of the articular facet was slightly saddle-shaped and occupied virtually the whole width of incus, and was divided by a ridge into two facets (Figure 3B). The long process extended in a rostroventral direction. The lenticular process seemed to ossify with the extremity of long process. It was directed mainly caudally and formed a small, nodular projection. The pyramidal short process was oriented in a caudoventral direction. The incuomalleolar joint was easily separable (Figure 1A).

**Stapes.** The head of stapes was convex. The stapes was quadrilateral in shape. The rostral crus of stapes was slightly inclined rostrally, while the caudal crus was almost straight. Below the level of junction of caudal crus and neck at its caudomedial aspect, there was a small but prominent muscular process. The intercrural foramen was a regular oval in shape (Figure 4A). Cross-section of the rostral crus was as a narrow semicircle near footplate, but caudal crus was somewhat appeared as an irregular c-shaped (Figure 4C). Footplate was an irregular oval in shape (Figure 4B), its extremities extending considerably beyond the limits of insertion of crura. However, it extended farther beyond the insertion of caudal crus than beyond the rostral crus. The well-developed labrum was thick at the extremities, especially at the caudal extremity. The caudal extremity was relatively pointed, whereas the rostral one was rounded when viewed from its vestibular aspect. The center of footplate was relatively thin and was slightly concave towards the vestibule (Figure 4A). The footplate was placed in fossa ovalis leading to the oval window at its bottom (Figure 4C).

**CBCT anatomy:** Osseous structures of water buffalos middle ear were very well visualised with CBCT. The sagittal plane images provided excellent depictions of anatomical structures. No significant anatomic variations were noted between right and left sides of temporal bone. In the sagittal plane tomogram shown in Figure 5A, the body, short and long processes of incus, the head, neck, rostral process, and manubrium of malleus, incudomalleolar joint, tympanic bulla, tympanic cells and epitympanic recess were readily identified. In the dorsal plane tomogram shown in Figure 5B, the osseous part of external acoustic meatus, the bony rim supporting the tympanic membrane, part of the malleus, and the petrous part of temporal bone were seen clearly.

## DISCUSSION

**Tympanic membrane (TM):** Although the typical mammalian TM has a PT and smaller PF, the outline of PT differs among the species, from nearly circular to an approximately elongated oval [6]. Like that of the ox, the PT of TM in water buffalos was a regular oval, whereas the shape of PT in sheep and goats takes the form of a regular circle [12]. The shape of PF is more or less rectangular [12] or roughly triangular [35] in the ox and irregularly triangular in sheep and goats [12], as it was in water buffalos. The tympanic membrane is circular in one-humped camels [22].

Like in the ox, sheep, dog, cat, and pig [6] the manubrium divides PT into asymmetrical dorsal and ventral sections in water buffalos. Puria and Steele [2010] hypothesize that a similar asymmetry in cats and humans ears could lead to a force differential on the manubrium, resulting in a rotation of malleus about its long axis. Because such a change in rotatory axis would reduce the moment of inertia, they suggest that this change in ossicular vibratory mode could improve the efficiency of sound transmission at higher frequencies.

As in the goat and ox [12], the entire length of manubrium in water buffalos was embedded in PT, whereas in the sheep part of manubrium was superficially placed over PT [12]. The medial surface of PT on its central part was strongly convex in the ox or regularly convex in goats and sheep [12]. In water buffalos, it was not convex; it appears to be irregularly concavo-convex.

The size of PF is very variable among mammals; out of humans, cats, dogs, the ox, sheep and rodents, the biggest PF belongs to the sheep [6]. Although we did not measure PF separately in water buffalos, it is likely very similar in size to that of the ox as illustrated by Decraemer and Funnell [2005]. In short, the TM of water buffalos resembled that of the ox rather than those of goats and sheep.

**Tympanic cavity:** The epitympanic recess in water buffalos was occupied by head and neck of the malleus, as well as all of the incus, whereas in small ruminants the head, neck and rostral process of malleus, along with its articulation with incus, are all situated in the recess [12]. In the ox only the head of malleus and part of the incus [12] or the head of malleus and incus [4] are housed in the recess. In Bactrian camels, the malleus head and main part of incus are located in the recess [2].

The cellular divisions of the bullar cavity in water buffalos resemble what has been described in the ox [1, 35]. This is not the case in other domestic ruminant species in veterinary anatomical textbooks [12, 26] nor in Bactrian camels [2]. According to Fleischer [1978] and Plestilova et al. [2016], similar bony septa which divide the middle ear cavities of several types of mammals are expected to change the resonance properties of such cavities, but experimental data are lacking.

**Morphometry of ossicles:** Differences in ossicle size in animals correlate with variations in their auditory range [13, 27]. Nummela [1995] found that although there is a correlation between skull mass and ossicular mass among mammals in general, the ossicular mass cannot be reliably predicated from skull mass in mammals with large skulls. There are very few morphometric studies documented for ossicles of ruminants. Mohammadpour [2005] reported masses and other measurements of the different parts and process of the ossicles in the ox, sheep, goat, and dromedary camel. Bai et al. [2009] weighed all ossicles and measured malleus head and length of stapes in Bactrian camels. Nummela [1995] reported average masses of ossicles in various mammals including Bactrian camels, cattle and sheep.

According to Table 2, the heaviest ossicles belonged to water buffalos although it is not the largest animal among the reported animals. The mean values for HNL of malleus, TL and BW of incus, as well as HL stapes, were all greatest in water buffalos among the other ruminants. However, the mean values of BL and TL of stapes were greatest in dromedary and Bactrian camels, respectively (Table 3). In the ox, the long crus was almost twice the length of short crus, whereas in the sheep the long crus was nearly three times longer than the short crus. The two crura of goats were almost equal in length [12], like in water buffalos, while high frequency hearing is limited by middle ear size and in particular ossicular mass [13].

**Malleus Head:** Unlike in small ruminants [12], caudally the malleus head is smoothly convex in the ox [12], as it was in water buffalos. Wilkie [1936] also reported that malleus head has very pronounced features in the flatness of head caudally.

Unlike in the ox and water buffalo, the notch on the articular surface of head is very prominent in goat and sheep [12], which is the most striking difference among the mallei of these animals.

The articular surface of malleus head was covered by the incus body completely in the ox [12], while it was covered partly in goat and sheep [12], as it was in water buffalos.

**Malleus Neck:** As in the ox [12, 35], the curved neck was not very distinct in water buffalos, whereas in the sheep the neck was the thickest part [12].

**Malleus Processes:** The best-developed muscular process belonged to one-humped camels [12], followed by the ox and small ruminants [12, 22]. The process of water buffalos resembled the ox in this respect.

The rostral process arises from the medial and just rostral to the muscular process in small ruminants, whereas the rostral process arises medially from the neck at the same level as at which the muscular process arises in the ox [12], as it was in water buffalos.

In water buffalos, the rostral process was attached by a ligament to the wall of tympanic cavity. According to Getty [1975], the ossicle in domestic animals are connected with walls of tympanic cavity by ligaments. While in the rodents there is an osseous connection between the process and tympanic cavity [21].

**Manubrium:** In goats [12], the manubrium is three-sided, whereas the manubrium of water buffalos was four-sided, like those of ox and sheep [12].

As in the goat and ox [12], in water buffalos the entire length of manubrium were embedded in PT, whereas in the sheep part of manubrium lies superficially over PT, almost in its middle [12].

The best-developed lateral process belonged to one-humped camels [22], followed by the goat, sheep, and ox [12]. In water buffalos, the process resembled that of the ox.

In the goat, the lateral process arises almost at the same level as the muscular process [12], whereas in the sheep and ox this process arises caudal to the muscular process, on the opposite side [12], similar to that of water buffalos.

One of distinguishing features of ossicle morphology among mammals is the orientation of the manubrium relative to the anatomical axis, but this has not been the focus of attention in the larger domestic animals. The manubrium is roughly parallel to the axis in many ve

ry small mammals, but it is perpendicular in humans, rabbits, guinea pigs, and chinchillas [12]. In water buffalos, the manubrium took an intermediate position (not perpendicular or parallel).

**Incus:** Unlike in the ox [35] and water buffalo, the articular surface of body had a concave depression in sheep and goat because of the presence of a high ridge on the surface [12]. The incus body of the ox [12] and water buffalo were a large, well-developed, unlike those of small ruminants [12].

In water buffalos, the lenticular process was located at the extremity of long crus of incus, as in goats [12], dromedary [22], and Bactrian camels [2]. However, in the sheep and ox the process was absent [2] or present [34, 35]

**Stapes:** Like in the ox [12], this ossicle was nearly rectangular in shape in water buffalos, whereas it is more trapezoidal in sheep [35].

As in the ox [12], the head of stapes in water buffalos was convex, whereas the head was flattened in goats [12].

The footplate of mammalian stapes was oval or bean-shaped, as in man. It is elongated, thus having a long and a short axis [10]. As in the ox [35], the shape of footplate was an irregular oval in water buffalos, whereas it has a squarer shape in sheep [34] and is elliptical in Bactrian camels [2].

## CONCLUSIONS

Although general anatomical characteristics of the middle ear structures and the relationship of the ossicles in the water buffalo was almost similar to those in the ox and small ruminates, several distinctive morphologic and morphometric variations of the middle ear structures are recognized in the water buffalo.

In general, among the mammals, there seems to be correlations between ossicle morphology and the frequency that an animal can hear [27]. Therefore, although some of morphological characteristics of middle ear described here remain of unknown functional significance, the middle ear of water buffalos shows characteristics of the transitional type ear, following the terminology of Fleischer [1978]. These characteristics consist of an enlarged malleus head, heavy ossicles, a ligamentous connection of a reduced rostral process to the skull, reduction of malleus anterior lamina, the possession of a relatively large incus, and oblique manubrial orientation to the anatomical axis [19]. Such a transitional type ear leads to the prediction of quite a wide frequency range: not especially low nor especially high. Water buffalos, like cattle, belong to the Bovidae family and cattle are able to hear a much wider range of sound frequencies (16 to 40,000 Hz) than humans (20 to 20,000 Hz). This should permit them to hear, in principle, both low-frequency rumbles of elephants and the



ultrasonic screams of flying bats [12]. However, intraspecific variations in ossicle morphology among water buffalos and other ruminants remain to be examined.

### **Conflict of interest**

The authors declare that they have no conflict of interest.

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### **REFERENCES**

1. Ardouin P. Anatomie comparée de la chaîne des osselets de l'ouïe dans la série des mammifères. *Rev Laryngol Otol Rhinol.* 1935; 56: 427-489.
2. Bai Z, Wang H, Yuan G, Ye W, He J, Wang J. A functional anatomy of the external and middle ear of the Bactrian camel. *J Camel. Pract Res.* 2009; 16(1): 115-120.
3. Blanke A, Ohlerth S, Hollerrieder J, Schusser G. Computed tomographic features of osseous ear canal, tympanic membrane and tympanic bulla in clinically normal horses. *J Equine Vet Sci.* 2016; 45: 17-21.
4. Botti M, Secci F, Ragionieri L, Dessole A, Acone F. Auditory ossicles in the ruminants: comparative morphological analysis with the analogous formations of horse. *Ann Fac Medic Vet di Parma.* 2006, 26, 91-96.
5. Budras K. D, Habel E, Wunsche A, Buda S. *Anatomy of the bovine: An illustrated text*, Schluetersche GmbH, Hanover 2003.
6. Decraemer WF, Funnel WR. Anatomical and mechanical properties of the tympanic membrane. *In: ARS B. (ed.). Chronic otitis media: pathogenesis-oriented therapeutic management*, The Netherlands, Kugler. 2005; 51-84.
7. Doran AHG. Morphology of the mammalian *ossicula auditûs*. *Trans Linn Soc Lond 2nd Ser.* 1878; 13 (68):185-189.
8. Dyce KM, Sack WO, Wensing CJG. *Textbook of veterinary anatomy*, W.B. Saunders Company, Philadelphia 2018.
9. FAO. *The water buffalos*, Food and agriculture organization of the United Nations, Italy 1977.

10. Fleischer G. Evolutionary principles of the mammalian middle ear. *Adv Anat Embryol Cell Biol.* 1978; 55(5): 1-70, doi: 10.1007/978-3-642-67143-2, indexed in Pubmed: 735912.
11. George Noussios, Pantelis Chouridis, Lazaros Kostretzis, Konstantinos Natsis. *J Clin Med Res.* 2016 Feb; 8(2): 76–83. doi: 10.14740/jocmr2369w. PMID: PMC4701061.
12. Getty R. Sisson and Grossman's the anatomy of the domestic animals. W.B. Saunders Company, Philadelphia 1975.
12. Hemila S, Nummel S, Reuter T. What middle ear parameters tell about impedance matching and high frequency hearing? *Hearing Res.* 1995; 85(1-2): 31-44, doi: 0.1016/0378-5955(95)00031-x, indexed in Pubmed: 7559177.
13. Konig HE, Liebich HG. *Veterinary anatomy of domestic mammals, text book and colour atlas*, Schattauer, New York 2004.
14. Kumar DV, Chaitanya DK, Singh V, Sridhar DR. A morphometric study of human middle ear ossicles in cadaveric temporal bones of Indian population and a comparative analysis. *J Anat Soc India.* 2018. 67(1): 12-17, doi.org/10.1016/j.jasi.2018.01.001
15. Kurtul I, Cevik A, Bozkurte EU, Dursun N. A detailed subgross morphometric study on the auditory ossicles of the New Zealand rabbit. *Anat Histol Embryol.* 2003; 32(4): 249-52, doi: 10.1046/j.1439-0264.2003.00483.x, indexed in Pubmed: 12919078.
16. Lavender D, Taraskin SN, Mason MJ. Mass distribution and rotational inertia of "microtype" and "freely mobile" middle ear ossicles in rodents. *Hearing Res.* 2011; 282(1-2): 97-107, doi: 10.1016/j.heares.2011.09.003, indexed in Pubmed: 21951489.
17. Mallya S, Lam E. *White and Pharoah's oral radiology: principles and interpretation.* Mosby, USA 2018.
18. Mantokoudis G, Huth ME, Weisstaneer C, Friedrich HM, Nauer C, Candreia C, Caversaccio MD, Senn P. Lamb temporal bone as a surgical training model of round window cochlear implant electrode insertion. *Otol Neurotol.* 2016; 37(1): 52-56, doi: 10.1097, indexed in Pubmed: 26649606.
19. Martonos C, Damian A, Gudea A, Bud LT, Stan F. Morphological and morphometrical study of the auditory ossicles in chinchilla. *Anat Histol Embryol.* 2019; 48(4), doi: 10.1111/ahe.12446, indexed in PubMed: 31041818.
20. Mason MJ. Of mice, moles and guinea pigs: functional morphology of the middle ear in living mammals. *Hearing Res.* 2013; 301: 4-18, doi:10.1016/j.heares.2012.10.004, indexed in Pubmed: 23099208.
21. Mohammadpour AA. Comparative anatomical and morphological study of the middle ear bones between camel and other ruminants. *Pajouhesh and Sazandegi.* 2005; 64: 70-75.
22. Moiola B, Borghese A. Buffalo breeds and management systems. In: Borghese A (ed.). *Buffalo Production and research.* Food and Agriculture Organization of the United Nations, Rome 2005; 51-108.
23. Naserian AA, Saremi B. Water buffalo industry in Iran. *Ital. J Anim Sci.* 2007; 6 (2): 1404 -1405.

24. Nickel R, Schummer A, Seiferle E. Lehrbuch der Anatomie der Haustiere: Nervensystem, Sinnesorgan, Endokrine Drüsen. Verlag Paul Parey, Berlin 1975.
25. Nickel R, Schummer A, Seiferle E. The Anatomy of the domestic animals, the locomotor system of the domestic mammals. Verlag Paul Parey, Berlin 1986.
26. Nummela S. Scaling of the mammalian middle ear. *Hearing Res.* 1995; 85(1-2): 18-30, doi: 10.1016/0378-5955(95)00030-8, indexed in Pubmed: 7559173.
27. Peus D, Dobrey I, Prochazka L, Thoele K, Dalbert A, Boss A, Newcomb N, Probst R, Rossli C, Sim JH, Huber A, Pfifner F. Sheep as a large animal ear model: middle-ear ossicular velocities and intracochlear sound pressure. *Hearing Res.* 2017;351: 88-97, doi: 10.1016/j.heares.2017.06.002, indexed in Pubmed: 28601531.
28. Plestilova L, Hrouzkova E, Burda H, Šumbera R. Does the morphology of the ear of the Chinese bamboo rat (*Rhizomys sinensis*) show "Subterranean" characteristics? *J Morphol.* 2016; 277(5): 575-584, doi: 10.1002/jmor.20519, indexed in Pubmed: 26880690.
29. Puria S, Steele C. Tympanic-membrane and malleus-incus-complex co-adaptations for high-frequency hearing in mammals. *Hearing Res.* 2010; 263(1-2):183-90, doi: 10.1016/j.heares.2009, indexed in Pubmed: 20430077.
30. Russo M, Covelli E, Memertino M, Lamb C., Brunetti R. Computed tomographic anatomy of the canine inner and middle ear. *Vet Radiol Ultrasound.* 2002; 43(1): 22-26, doi: 10.1111/j.1740-8261.2002.tb00437.x, indexed in Pubmed: 11866039.
31. Schaller O, Ghsrghe M, Constaninescu G. Illustrated veterinary anatomical nomenclature. Enke Verlag, Germany 2007.
32. Seibel VA, Lavinslky L, Olivera JA. Morphometric study of the external and middle ear anatomy in sheep: a possible model ear examination. *Clin Anat.* 2006; 19(6): 503-509, doi: 10.1002/ca.20218, indexed in Pubmed: 16287111.
33. Wilkie HC. The ossicula auditus of the sheep (*Ovis aries*). *J Comp Pathol. Ther.* 1925; 38: 298-301.
34. Wilkie HC. The Auditory organ of the ox (*Bos Taurus*). *Proc Zool Soc Lond.* 1936; 106 (4): 985-1009.

**Table 1.** Means (mm)  $\pm$  standard deviations of the ossicles (water buffalos)

<b>Malleus</b>	<b>TL</b>	<b>ML</b>	<b>HNL</b>	<b>HD</b>
	11.07 $\pm$ 0.46	6.54 $\pm$ 0.6	5.73 $\pm$ 0.29	3.17 $\pm$ 0.4
<b>Stapes</b>	<b>TL</b>	<b>BL</b>	<b>HL</b>	<b>TMB</b>
	3.6 $\pm$ 0.57	2.7 $\pm$ 1.27	1.44 $\pm$ 0.33	0.7 $\pm$ 0.16
<b>Incus</b>	<b>TL</b>	<b>LCL</b>	<b>SCL</b>	<b>BW</b>
	5.74 $\pm$ 0.88	2.78 $\pm$ 0.16	2.74 $\pm$ 0.18	3.3 $\pm$ 0.22

TL: Total Length; ML: Malleus Length; HNL: Head and Neck Length; HD: Head Diameter; BW: Body Width; HL: Head Length; BL: Body Length; TMB: Thickness in the Middle of Bone; LCL: Long crus Length; SCL: Short Crus Length.

**Table 2.** Comparison of mean masses (mg)  $\pm$  standard deviations of the ossicles (water buffalos) with those obtained in various ruminates.

	<b>Malleus</b>	<b>Incus</b>	<b>Stapes</b>
Present study	0.050 $\pm$ 0.030	0.048 $\pm$ 0.008	0.006 $\pm$ 0.001
Ox (Getty, 1975)	0.032 $\pm$ 0.006	0.030 $\pm$ 0.005	0.005 $\pm$ 0.0005
(Nummela, 1995)	0.022	0.026	0.002
Sheep (Getty, 1975)	0.008 $\pm$ 0.001	0.008 $\pm$ 0.001	0.001 $\pm$ 0.0003
(Nummela, 1995)	0.007	0.055	
Goat (Getty, 1975)	0.010 $\pm$ 0.001	0.008 $\pm$ 0.001	0.001 $\pm$ 0.0004
Dromedary camel (Mohammadpour, 2005)	0.039 $\pm$ 0.080	0.030 $\pm$ 0.003	0.004 $\pm$ 0.0004
Bactrian camel (Bai et al., 2009)	0.037 $\pm$ 0.02	0.032 $\pm$ 0.008	0.005 $\pm$ 0.0005
Bactrian camel (Nummela, 1995)	0.037	0.038	0.004
Human ( Harneja et al,1973)	0.023 $\pm$ 0.002	0.025 $\pm$ 0.002	0.003 $\pm$ 0.0006
Human (Nummela, 1995)	0.028	0.033	0.002

**Table 3.** Comparison of means (mm)  $\pm$  standard deviations of the ossicles (water buffalos) with those obtained in various ruminates.

		Present study	Ox	Sheep	Goat	Dromedary camel	Bactrian camel (Bai et al., 2009)	Human (Noussios et al., 2016)
Malleus	TL	11.07 $\pm$ 0.46	11.26 $\pm$ 0.61	8.28 $\pm$ 0.39	8.40 $\pm$ 0.41	10.25 $\pm$ 0.54	-----	7.15 $\pm$ 0.31
	ML	6.54 $\pm$ 0.60	7.32 $\pm$ 0.13	5.56 $\pm$ 0.31	5.10 $\pm$ 0.55	7.60 $\pm$ 0.46	-----	4.22 $\pm$ 0.35
	HNL	5.73 $\pm$ 0.20	3.17 $\pm$ 0.40	2.60 $\pm$ 0.21	2.60 $\pm$ 0.21	2.72 $\pm$ 0.17	-----	4.85 $\pm$ 0.29
	HD	3.17 $\pm$ 0.40	1.76 $\pm$ 0.08	1.26 $\pm$ 0.16	2.18 $\pm$ 0.32	3.25 $\pm$ 0.17	1.60 $\pm$ 0.20	2.36 $\pm$ 0.21
Incus	TL	5.74 $\pm$ 0.88	6.20 $\pm$ 0.38	3.22 $\pm$ 0.13	3.10 $\pm$ 0.10	4.84 $\pm$ 0.20	-----	3.14 $\pm$ 0.19
	BW	3.30 $\pm$ 0.22	2.92 $\pm$ 0.28	2.08 $\pm$ 0.08	2.14 $\pm$ 0.15	3.04 $\pm$ 0.15	-----	-----
Stapes	TL	3.60 $\pm$ 0.57	3.60 $\pm$ 0.57	2.72 $\pm$ 0.20	2.27 $\pm$ 0.20	4.12 $\pm$ 0.35	4.30 $\pm$ 0.60	3.12 $\pm$ 0.21
	HL	1.44 $\pm$ 0.33	1.42 $\pm$ 0.05	1.26 $\pm$ 0.11	1.07 $\pm$ 0.09	1.35 $\pm$ 0.17	-----	-----
	BL	2.70 $\pm$ 1.27	2.57 $\pm$ 0.09	2.08 $\pm$ 0.08	2.02 $\pm$ 0.05	3.25 $\pm$ 0.37	-----	2.68 $\pm$ 0.27

TL: Total Length; ML: Malleus Length; HNL: Head and Neck Length; HD: Head Diameter; BW: Body Width; HL: Head Length; BL: Body Length. The parameters are based on millimeters (mm).

Note: The author did not record the related parameters which are empty in Bactrian camel and human.

**Figure 1.** Medial aspect of right ossicles and tympanic membrane in water buffalos (in situ). A and B: 1. Pars tensa; 2. Chorda tympani nerve; 3. Manubrium; 4. Muscular process; 5. Epitympanic recess; 6. Malleus head; 7. Incus body; 8. Long process; 9. Lenticular process; 10. Short process; 11. Tympanic cell; 12. Tympanic ring; 13. Pars flaccida. \*.Scale = 5 mm.

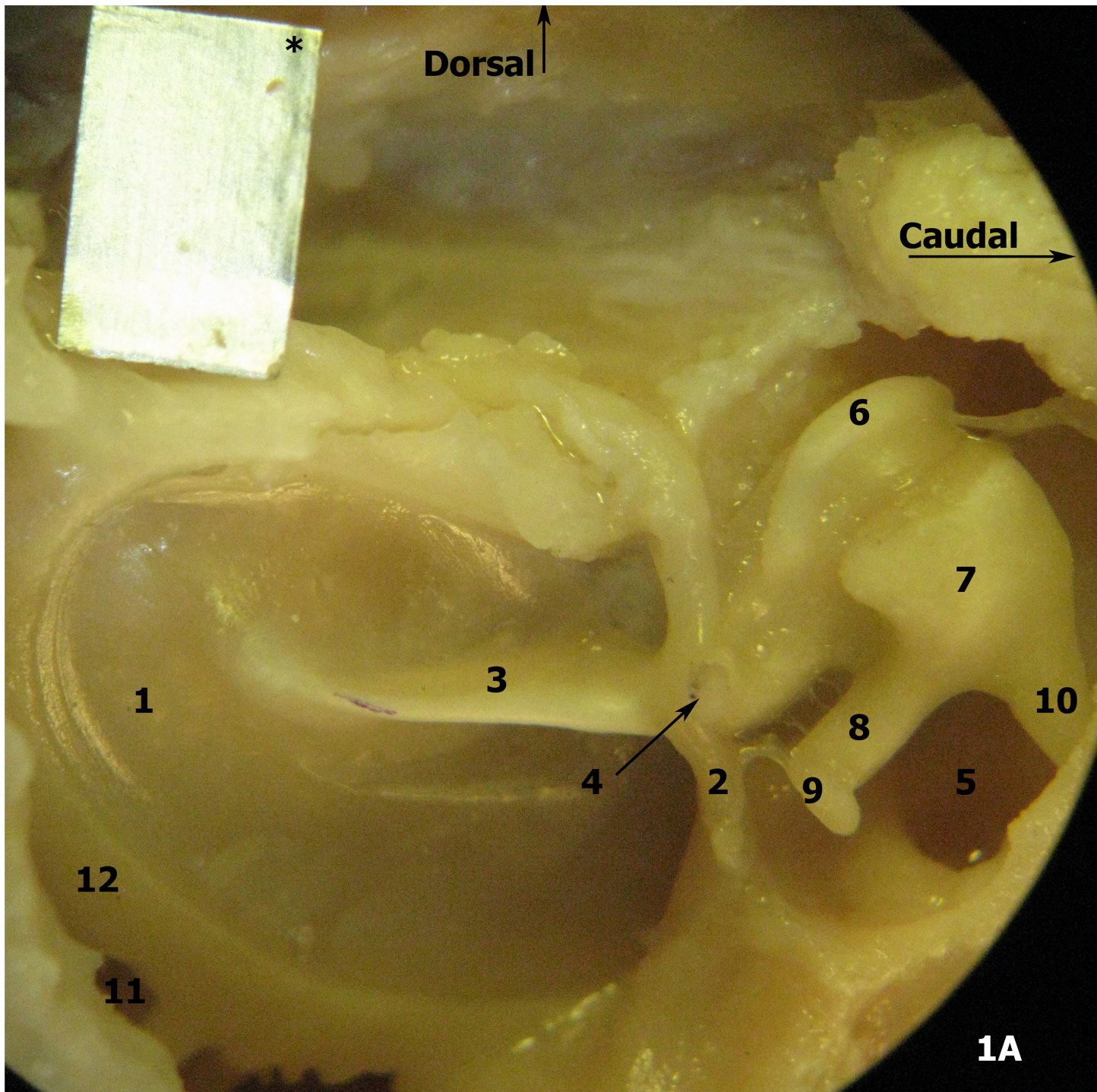
**Figure 2.** The left malleus. A (Lateral view, \*.Scale = 5 mm) and B, C (Medial view, \*.Scale = 1 mm) (water buffalos). 1. Head; 2. Neck; 3. Lamina; 4. Rostral process; 5. Manubrium; 6. Lateral process; 7, 7'. Dorsal and ventral parts of articular facet; 8. Extremity; 9. Fossa, 10. Muscular process.

**Figure 3.** The left incus (water buffalos). A: Lateral view and B: Articular surface. 1. Body; 2. Long process; 3. Short process. 4, 4'. Dorsal and ventral parts of articular facet.\* Scale = 5 mm.

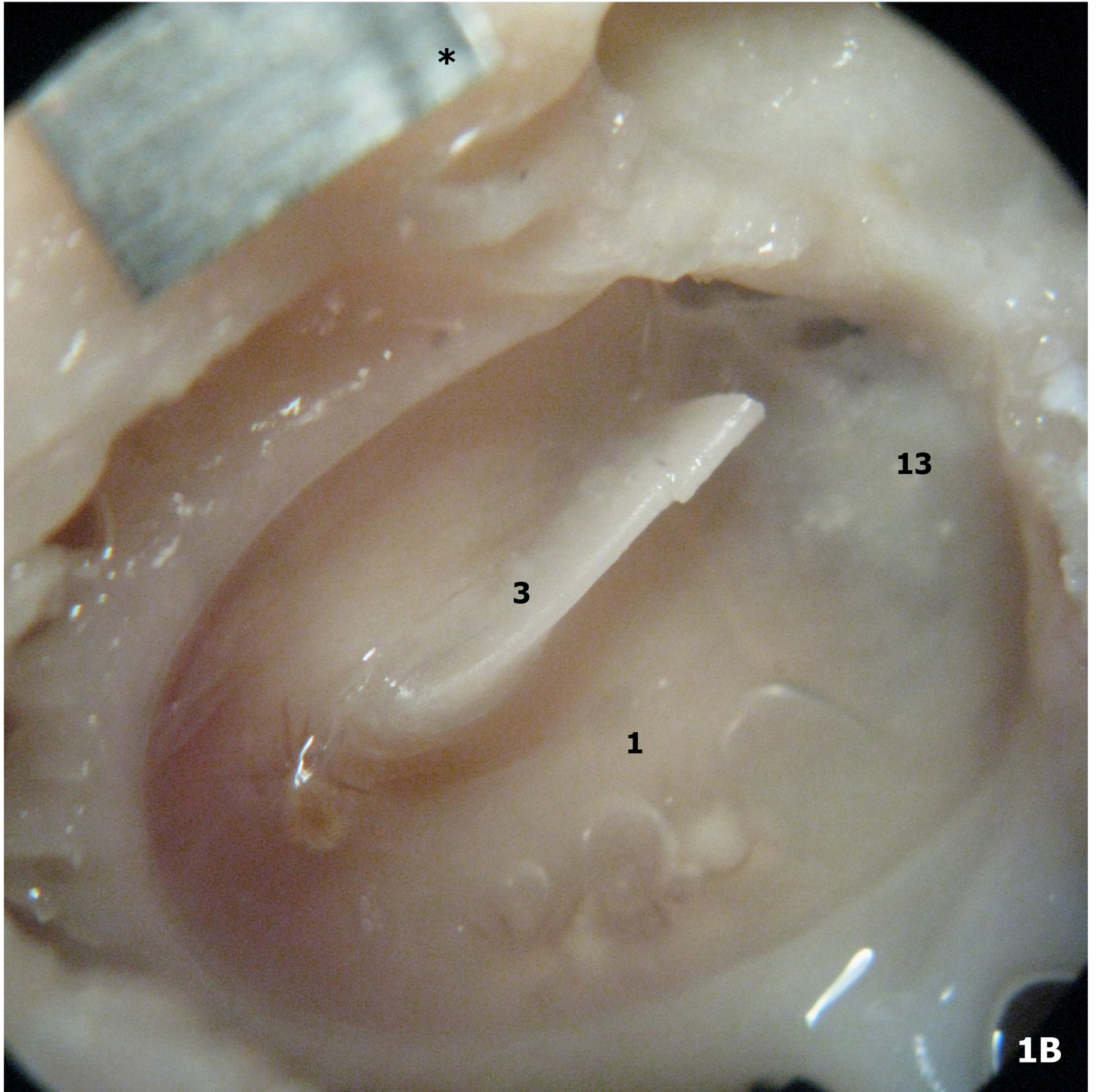
**Figure 4.** The left stapes (water buffalos). A (Dorsal surface; \*.Scale = 5 mm), B (Vestibular surface of footplate, \*.Scale = 1 mm), and C: Cross-section of crura. 1. Muscular process; 2. Footplate; 3. Caudal crus; 4. Rostral crus; 5. Intercrural foramen; 6. Head; 7. Rostral extremity; 8. Caudal extremity.

**Figure 5.** Sagittal (A) and dorsal (B) planes tomogram created by cone-beam computed tomography of right temporal bone (water buffalos). 1. Tympanic cavity; 2. Tympanic cell; 3. Manubrium; 4. Long process of incus; 5. Short process of incus; 6. Incus body; 7. Epitympanic recess; 8. Incus-malleus joint; 9. Malleus head; 10. Malleus neck; 11. Rostral process of malleus; 12. Facial and vestibulocochlear nerves; 13. Petrosal part of temporal bone; 14. External acoustic meatus; 15. Tympanic rim.









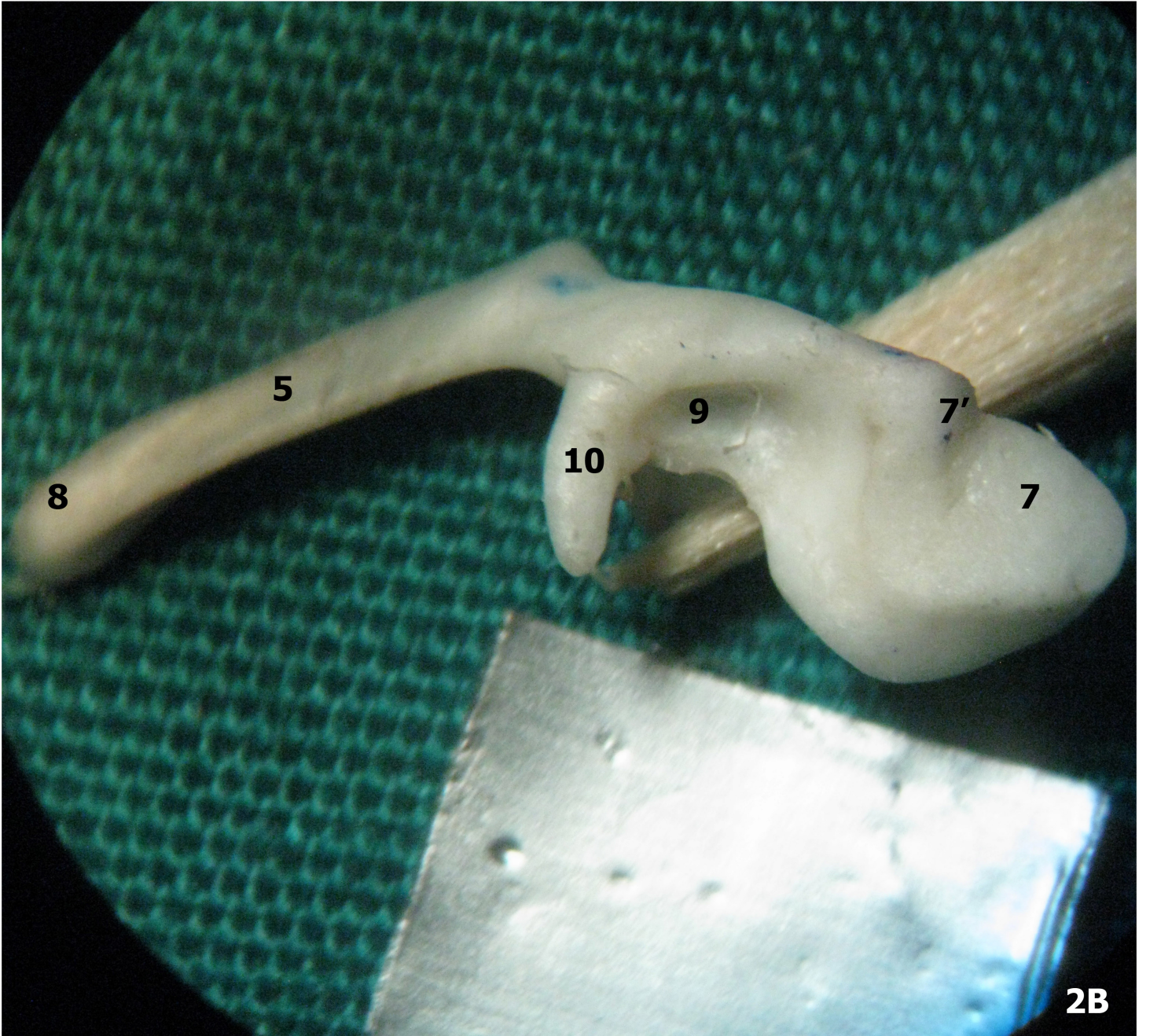
**1B**





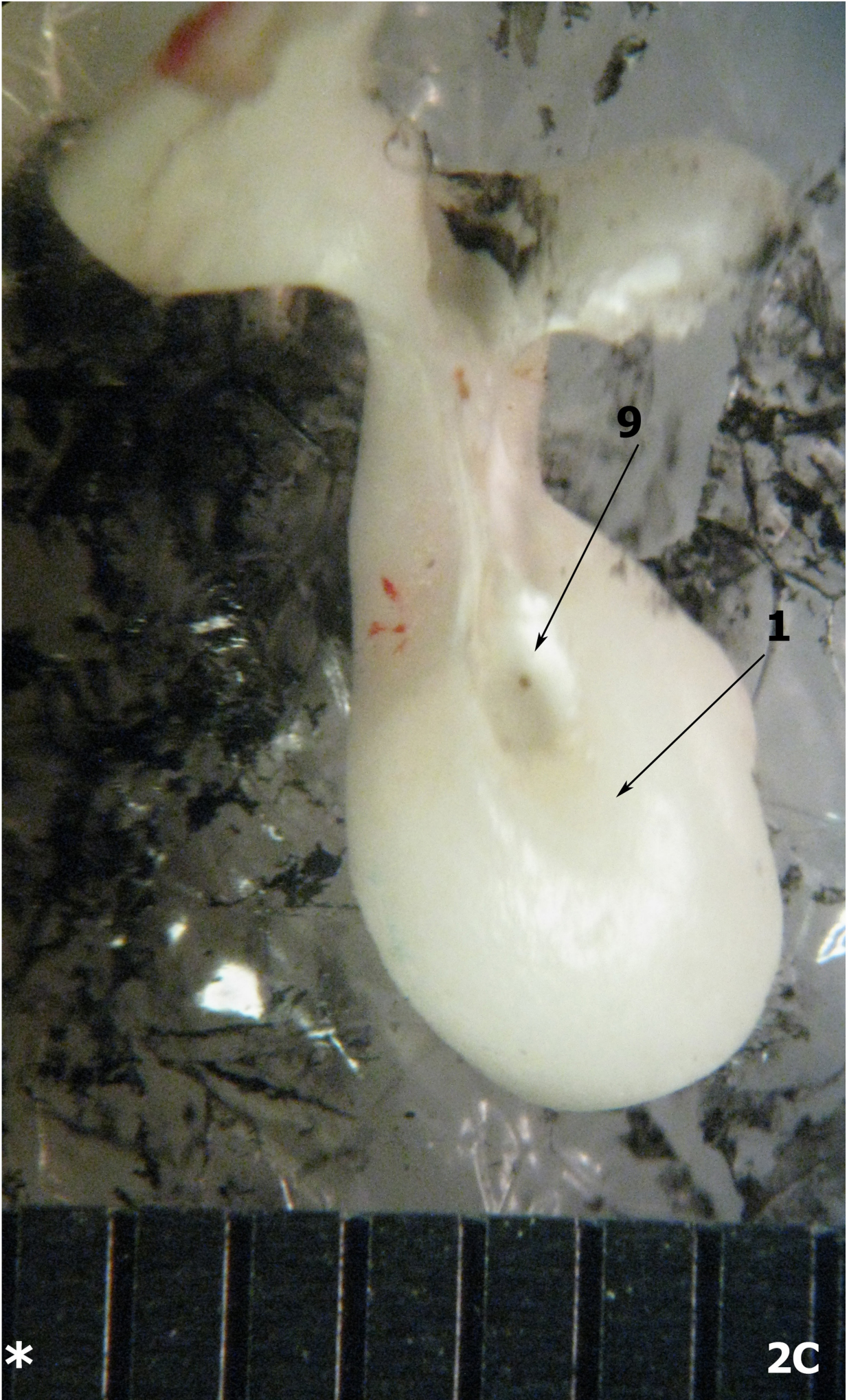
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2B

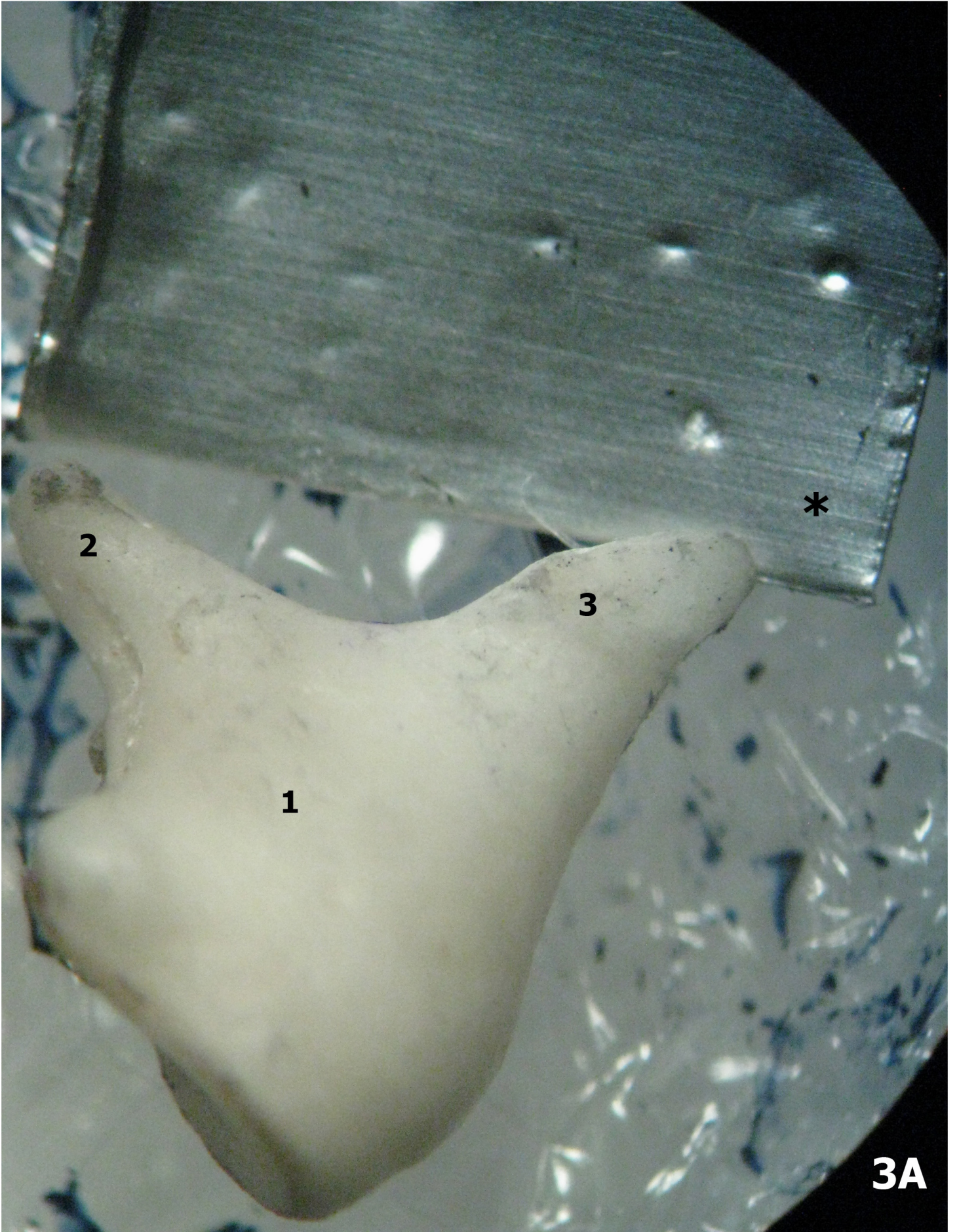




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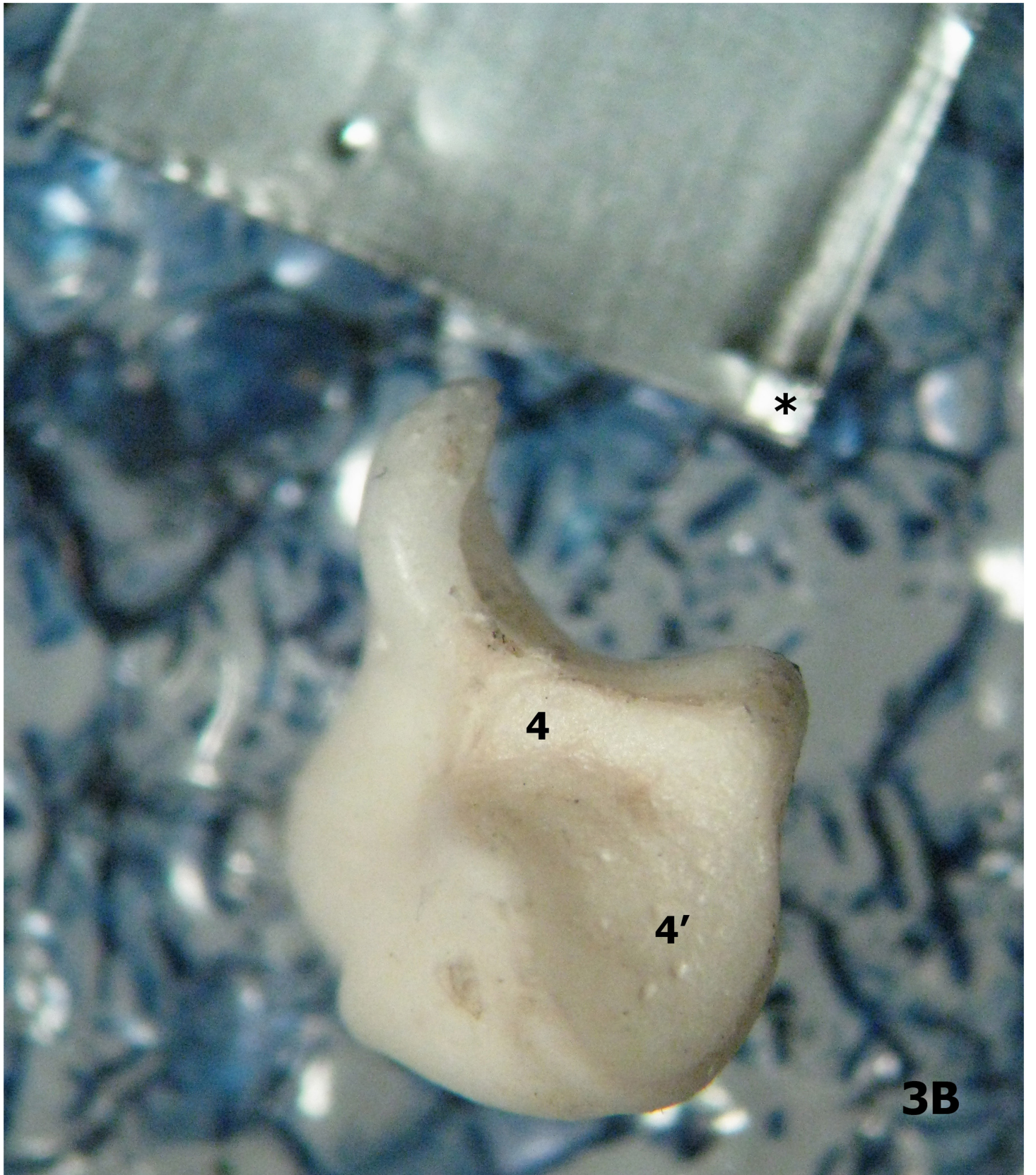
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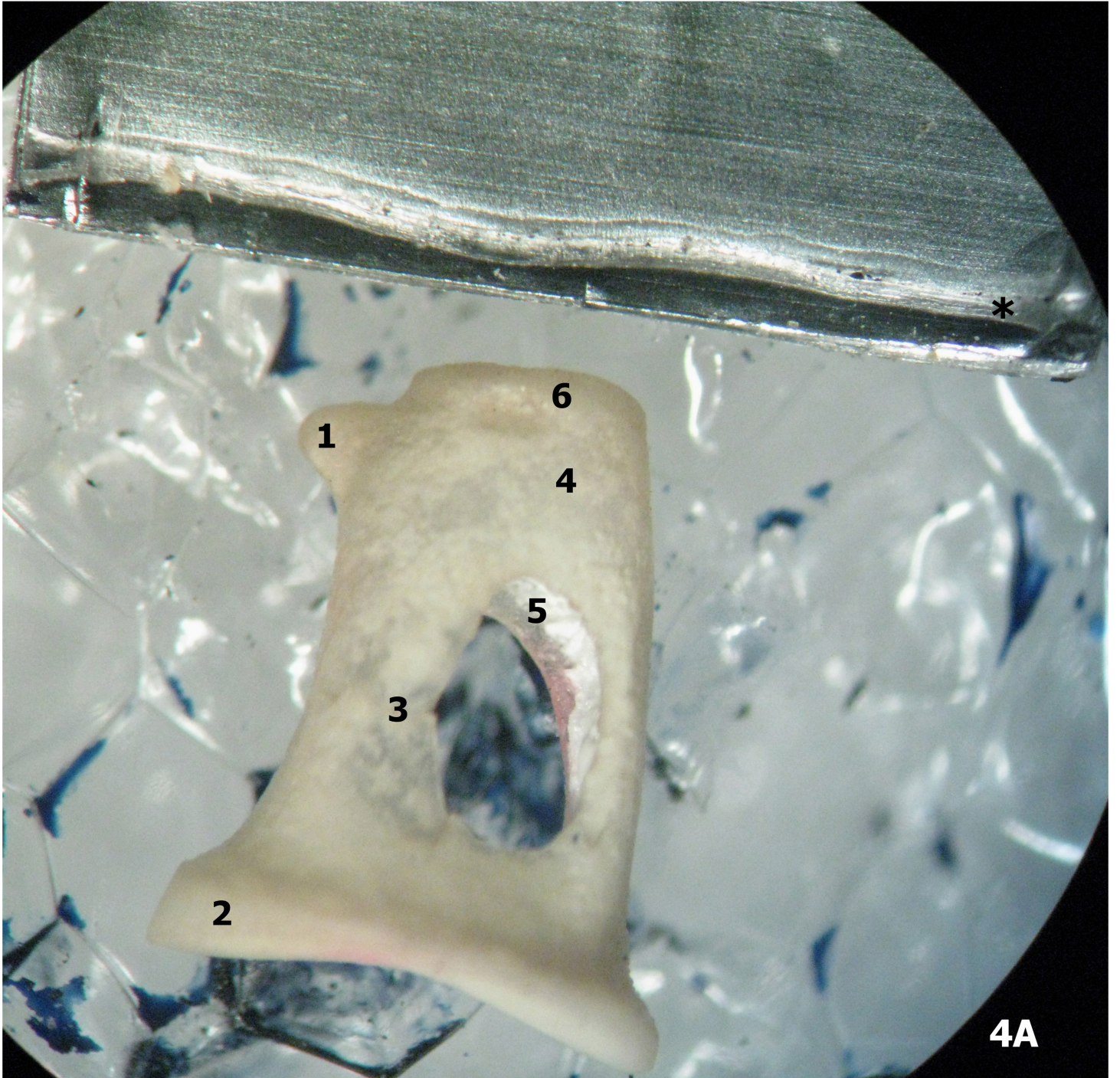


**3A**

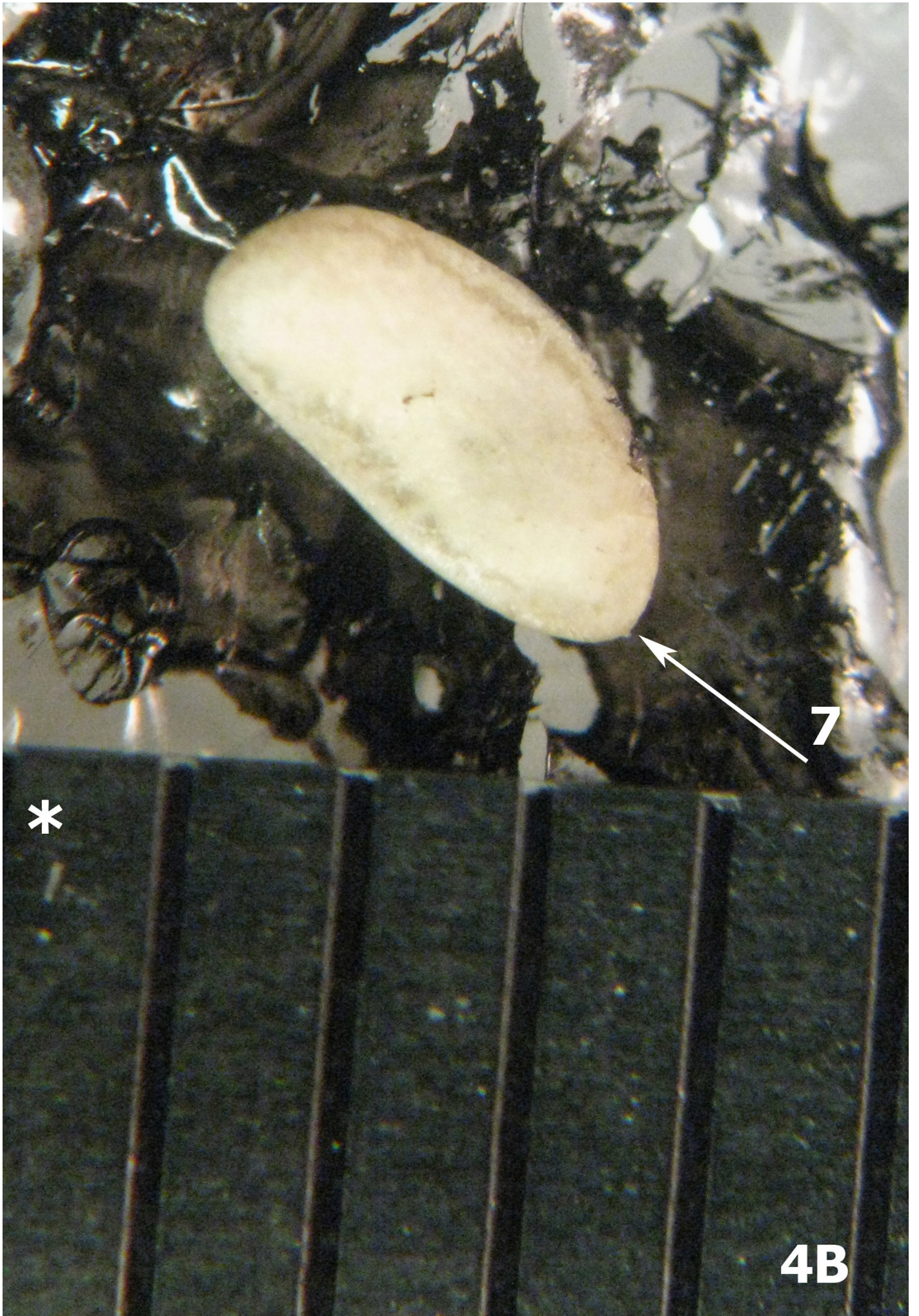




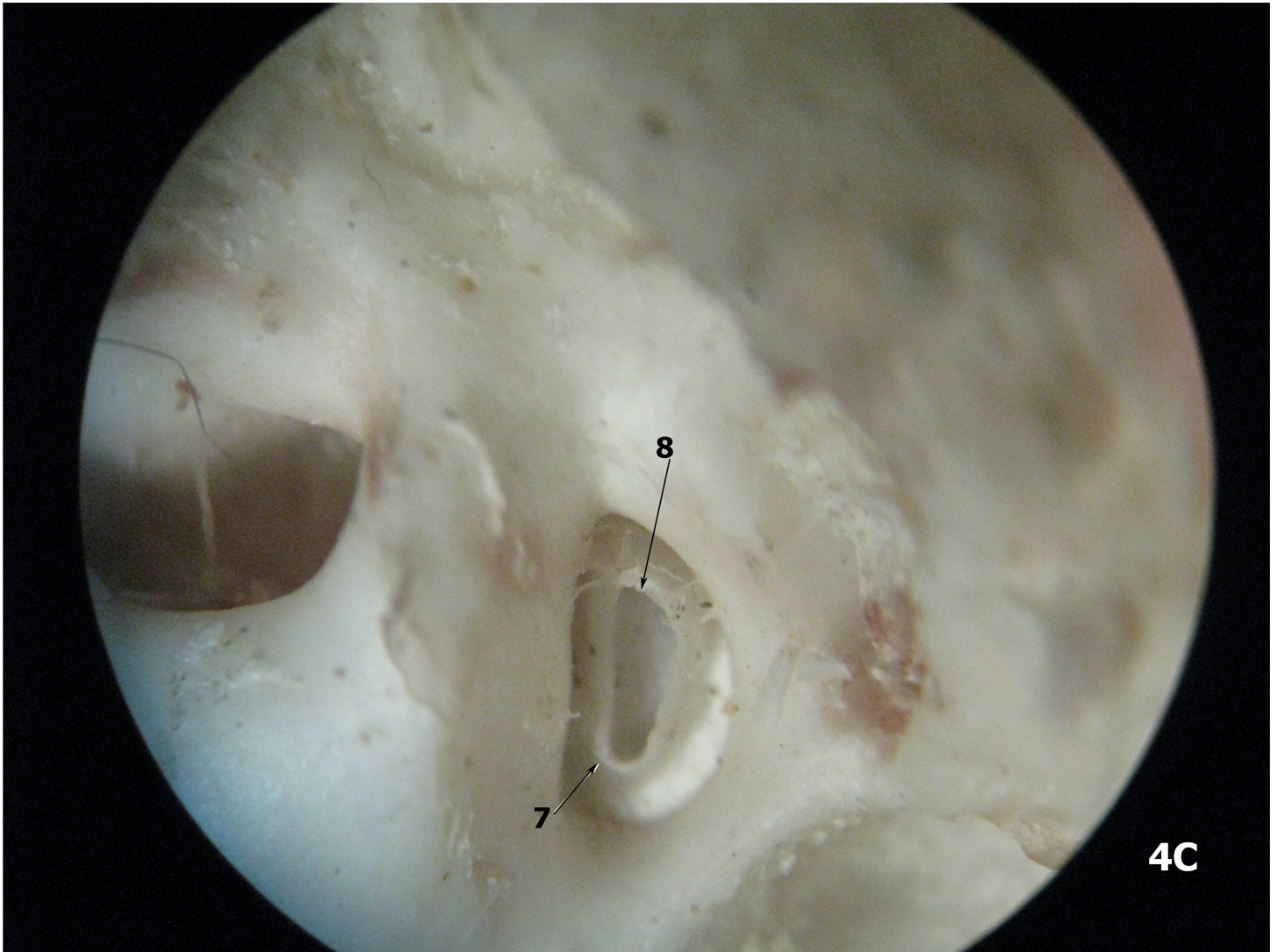












4C



