ISSN: 1220-3173; E-ISSN: 2457-7618

NECROPSY TECHNIQUE IN REPTILES: REVIEW

TEHNICA DE NECROPSIE LA REPTILE: REVIEW

Raluca Ioana RIZAC[1),*)],
Emilia CIOBOTARU-PÎRVU[1)],
Manuella MILITARU[1)]

ABSTRACT | REZUMAT

Reptiles are increasingly being used as pets worldwide. The most encountered species are turtles, snakes or lizards, and their popularity leads to the need for specialization of clinicians and pathologists alike in order to solve the exotic animal cases.

Necropsy examination is a vital tool in establishing the post-mortem diagnosis. In order to obtain a higher quantity and quality of information following a necropsy examination, it is necessary to know the technique, as well as to implement a well-developed protocol. Reptiles, wild or pets, are exotic species that, by their anatomical particularities, require a different necropsy protocol than mammals.

This paper aims to present various techniques for necropsy and gross examination of organs in reptiles.

Keywords: necropsy, gross examination, reptiles

Reptilele sunt din ce în ce mai folosite ca animale de companie în întreaga lume. Sunt întâlnite cel mai frecvent specii de țestoase, șerpi sau șopârle, iar popularitatea lor duce la nevoia de specializare a clinicienilor și a patologilor deopotrivă în vederea rezolvării cazurilor de animale exotice.

Examenul necropsic este un instrument vital în stabilirea diagnosticului post-mortem. Pentru a obține o cantitate și o calitate superioară a informațiilor în urma unui examen necropsic, este necesară cunoașterea tehnicii, precum și implementarea unui protocol bine pus la punct. Reptilele, sălbatice sau animale de companie, constituie specii exotice ce prin particularitățile anatomice necesită un protocol de necropsie diferit față de mamifere. Această lucrare are ca scop prezentarea diverselor tehnici necropsice și de examinare macroscopică a organelor la reptile.

Cuvinte cheie: necropsie, examinare macroscopică, reptile

Reptiles have become popular not only in zoological or private collections, but also as pets (3, 4). The most common ones are small, non-venomous reptiles, but plenty of other species are also seen in the private area. Since many of these reptiles are imported sometimes even from other continents, and their source is diverse, the knowledge about the pathology of these animals might be limited for now (5). Although not a primary source of diagnosis cases, wild reptiles may also be of concern for post-mortem diagnosis. Even the most discrete environmental changes may cause pathological transformations, therefore a thorough gross and microscopic evaluation, followed by other paraclinical investigations are of great importance (1). Reptiles have certain anatomical particularities that require a different necropsy protocol than mammals. For a higher quantity and quality of information following a necropsy examination, it is necessary to know the technique, as well as to implement a well-developed protocol.

1) University of Agronomic Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania
*) *Corresponding author: raluca.rizac@yahoo.com*

EXTERNAL EXAMINATION

The necropsy technique in reptiles pursues the same stages and principles as in mammals and birds. A particular aspect is the estimating of the time of death in the individual to be examined. In some reptiles after the onset of death, involuntary movements of the skeletal muscles and contractions of the heart can be perceived for a long time (several hours) (13, 18). There are also situations of animals still being alive, but vital signs are very poor or imperceptible (especially in turtles and large reptiles). In this case, animals are euthanized by chemical or physical methods (bleeding or decapitation) (13, 18).

External examination is the first step of the necropsy. It starts with measurements that are useful for determining age or body condition. The most common are weighing the body (Fig. 1), as well as measuring trunk length (from the snout to the vent) and the entire body (from the snout to the tip of the tail) (Fig. 2). In chelonians (turtles/tortoises), measurements of the carapace and of the plastron (length, width) as well as the height of the carapace will be made (13). There are other types of less common measurements

Fig. 1. Order *Testudinata*. Weighing the animal

Fig. 2. Order *Sqamata*. External examination and measuring

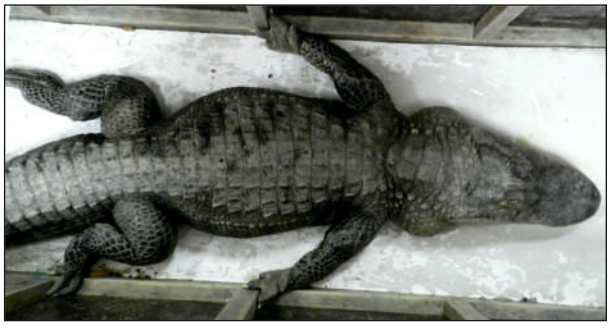

Fig. 3. Order *Crocodilia*. External examination of the dorsal integument

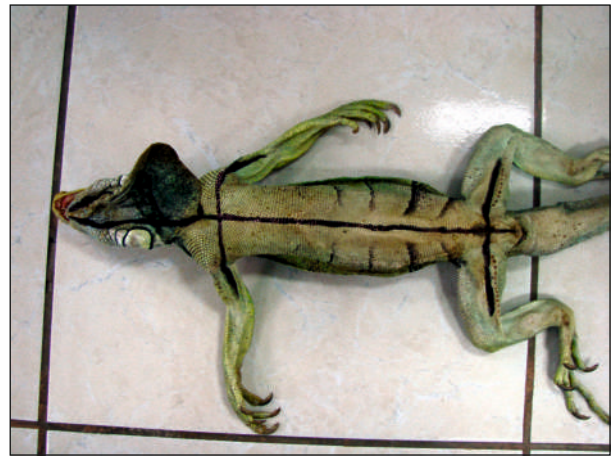

Fig. 4. Order *Squamata*. Dorsal recumbency, marked incision on the median line

used by researchers such as head length and width, trunk circumference at the limit of the median third to the caudal third of the animal - in snakes (11) or the determination of the body mass index, the latter providing information to be compared with databases specific to each species examined (13). The actual examination of the exterior refers to the integument (skin,carapace), eyes, nostrils, ears, mouth, vent, tail, fingers and claws, bone protuberances, muscles (8, 11, 13, 18). Any gross change observed at this level should be noted in the necropsy report with localization as accurate as possible. Photographing is an extremely useful tool and sampling is mandatory (scraping or fragment) before opening the body cavity.

Concerning the **skin** or the **carapace**, their integrity, symmetry (if applicable), irregular pigmentation, the presence of lesions (erosions, ulcers, lack of scales, scars, shedding defects, nodules, trauma, burns) are evaluated (Fig. 3) (8, 10, 13). These lesions can help reaching the diagnosis by providing valuable information on the body condition of the animal under examination, the degree of dehydration, the husbandry conditions. The skin will be examined grossly or with a magnifier for observing ectoparasites (ticks, lice), especially in areas such as corners of the mouth, ears, limb joints, vent (8). Particularly for aquatic reptiles, epibionts such as crustaceans are very important. This category contains species adhering to the skin or shell of aquatic reptiles. The importance of their presence is given by the number of individuals and localization. An increased number of crustaceans on the skin surface may result in functional restrictions, poor shedding or dermatitis. In addition, the fact that the host cannot remove them suggests a pre-existing pathology (11, 13). The examination of the **eyeballs** refers to shape and integrity, provides information on dehydration or body condition (8, 13). Eye trauma or opacification of the spectacles are known as having consequences on the health status of the animal, making feeding difficult or even impossible (11).

Examination of the **nostrils** may reveal the presence of trauma or inflammation. Pathological secretions guide post-mortem diagnosis to a respiratory pathology and are sampled for additional examinations.

The **oral cavity** should be carefully examined in venomous animals (snakes, lizards) because venomous glands store neurotoxic secretion even after the animal´s death (Fig. 9). It is advisable to use instru-

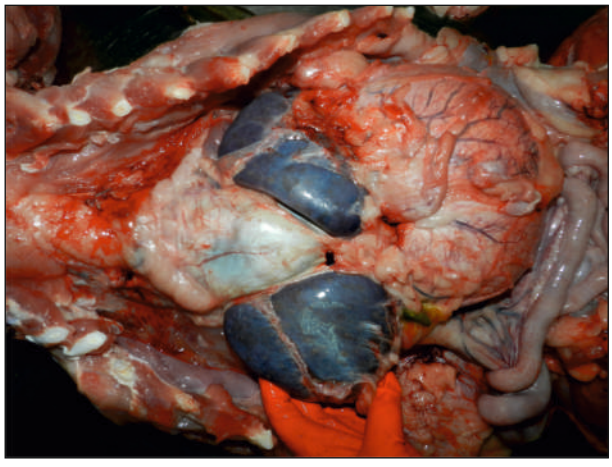

Fig. 5. Order *Crocodilia*. Opening the major cavities

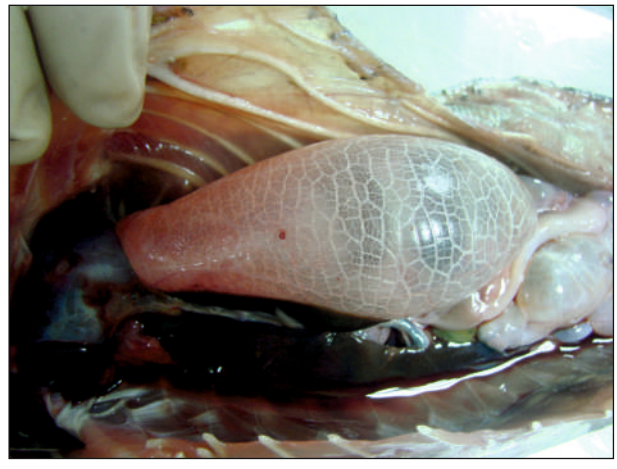

Fig. 6. Order *Squamata*. Saclike lung

Fig 7. Order *Squamata*. Lateral recumbency with detachment of upper body wall

Fig. 8. Order *Crocodilia* Opening the pericardial sac and examination of the heart

ments in order to avoid placing the hands in the animal´s mouth. During the necropsy, it is advisable to cover the head with duct tape, to place the head in a cylindrical container or to remove the head completely (11). If the last option is chosen, the head may be sampled in a formalin container for subsequent histopathological examination of the central nervous system, this method inactivating certain venoms (9, 19, 21). For the oral cavity, the presence of pathological secretions as well as haemorrhages, ulcers, necroses, stomatitis, foreign bodies, glossitis, and pathological growths is sought (11).

The **vent** must be clean, without traces of faeces or other secretions. The unmodified mucosa is pale pink. Changes that can be observed are accumulations of faeces, pathological secretions, haemorrhages, necroses, ulcerations, prolapse or foreign bodies (11).

The **tail** is both an indicator of the body condition and a provider of information on inter- and intra-specific aggressions. In alligators and lizards, tail circumference is an indicator of the body condition (13).

In addition to the skin lesions, the tail may indicate signs of possible aggressions that have resulted in amputation and later tail regeneration (8, 17).

Fingers and claws are carefully examined. At this level lesions such as missing fingers or claws, fractures, erosions, ulcers, hyperkeratosis, swollen joints may occur, suggesting husbandry, metabolic or infectious pathologies.

Palpation of **bone prominences** of the animal undergoing post-mortem examination goes hand in hand with muscular evaluation and provides clues about body condition. Normally, spinal processes are evaluated, which in a healthy animal are hard to palpate and not visible (13). In addition, palpation of the carcass may reveal the presence of fractures or other bone lesions (8).

OPENING OF THE CELOMIC CAVITY AND GROSS EXAMINATION OF THE ORGANS

Lizards and crocodiles

These species, although belonging to different orders, have a similar body shape and therefore use a si-

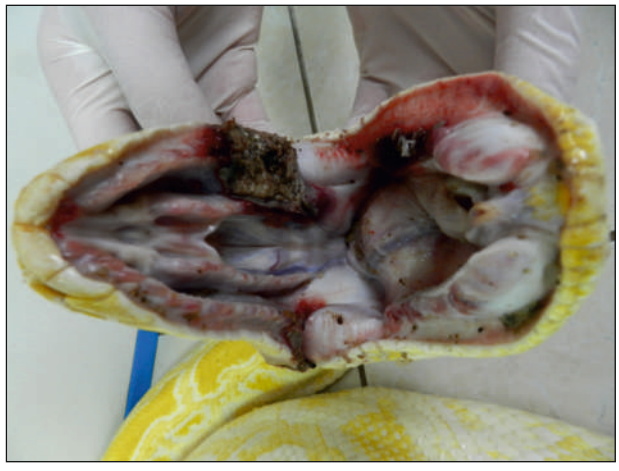

Fig. 9. Order *Squamata*. Examination of the oral cavity

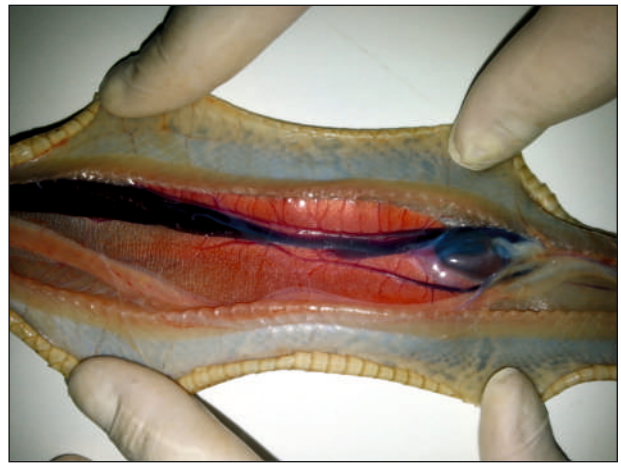

Fig. 10. Order *Squamata*. Opening the celomic cavity

Fig 11. Order *Squamata*. En bloc evisceration

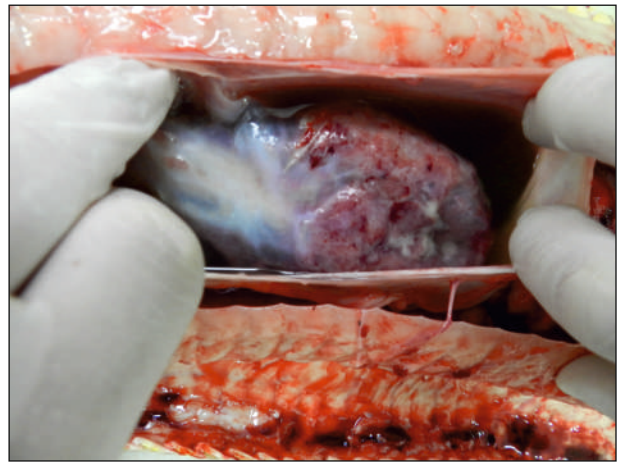

Fig. 12. Order *Squamata*. Opening the pericardial sac

milar necropsy technique. The body is positioned in dorsal recumbency. An incision is made on the median line or a few millimetres left or right (to avoid sectioning the abdominal ventricular vein and blood contamination), from the intermandibular area to the vent (Fig. 4), or two ventral incisions can be made starting from the corners of the mouth to the vent, following the lateral edges of the ventral scales. This secondary technique allows the removal of a skin and muscle flap, facilitating access to organs in the celomic cavity (8, 13, 18). In both versions, in order to gain easier access to the anterior celomic cavity, cutting and detaching the ribs is necessary. A particular case is represented by chameleons, because of their latero-lateral body compression. These species are positioned in lateral recumbency and the entire upper wall (ribs and celomic wall) is detached together with the skin and the muscle layer, while the limbs are partially removed by cutting the muscles and tendons and positioning them laterally (8, 17, 18). At this time of necropsy, muscle fragments can be sampled (13).

Once the celomic cavity is opened (Fig. 5–8), it is examined in order to notice the presence of any pathological effusions (which will be described, photographed, and sampled in sterile containers, weighed and subjected to additional examinations), possible alteration in organ topography, or other lesions (7).

Some species of lizards (more commonly chameleons) may exhibit a physiological melanin pigmentation of the organs´ serosa and celomic cavity walls (17). Reptiles have no muscular diaphragm. Only crocodiles and monitor species exhibit a pseudo-diaphragm or a post-pulmonary septum that separates the thoracic cavity from the abdominal cavity (12, 22).

The opening of the celomic cavity is followed by evisceration, a stage in which the individual weighing of the organs can be performed (18). The tongue and the pharynx will be exposed between the two mandible branches. At this stage, the thymus (single or double), the thyroid (single, bilobate or double) and parathyroids (one or two pairs) are examined. The latter are localized differently depending on the species (in the segment between the mandible and the heart) (13, 18).

Fig. 13. Order *Testudinata*. Detachment of the plastron

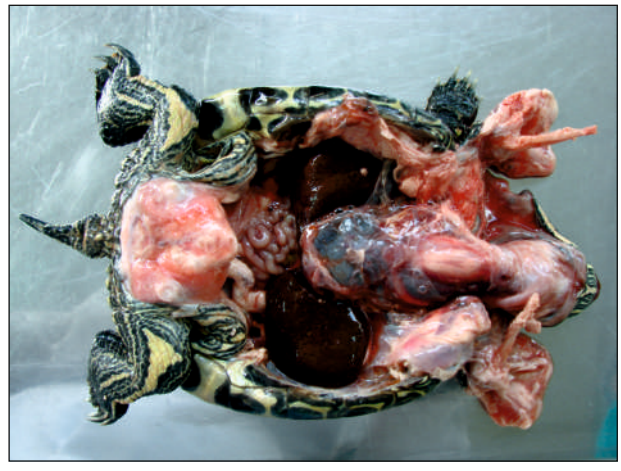

Fig. 14. Order *Testudinata*. Opening the celomic cavity

Fig 15. Order *Testudinata*. En bloc evisceration

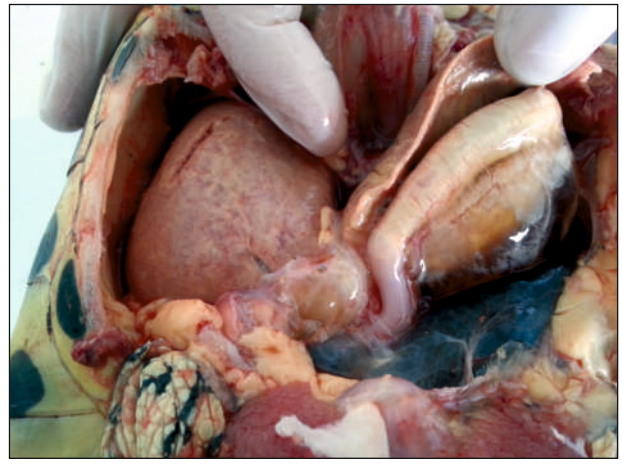

Fig. 16. Order *Testudinata*. Particular aspect of the liver and esophagus

The next step is the examination of the cardiovascular system. In lizards, the heart is positioned in the thoracic cavity, except for the monitors, species in which the heart is located caudally to the sternum (15). Thus, the presence of pathological effusions in the pericardial sac is checked, and then it is sectioned to examine the heart. The heart is eviscerated by sectioning the blood vessels at their origin, with the additional comment that in many species of lizards and crocodiles (not *Varanidae*) there is a fibrous structure, *gubernaculum cordis*, which connects the pericardium to the apex, and should not be confused with an area of adhesion (27). The lizards have a tricameral heart (two atria and a ventricle with an incomplete interventricular septum), and crocodiles represent the only order of reptiles with a tetracameral heart (two atria and two ventricles separated by a complete interventricular septum or a muscular ridge). For examination, all heart compartments and the origin of the large vessels are opened (13, 14, 18).

For the evisceration of the other organs, either the en bloc detachment by cranio-caudal traction of the tongue and sectioning the connective tissue can be chosen, or a section of the esophagus cranially to the liver is executed, keeping the respiratory tract with the esophagus, and eviscerating the rest of the organs. The second technique is used in large species. Caudally, the section is made between the colon and the cloaca, or near the vent (13, 18). It is noteworthy to mention the presence of fat pads in the ventral caudal region of the celomic cavity. In crocodiles, there is only one fat pad, located in the caudo-lateral right side of the celomic cavity (13). These yellowish structures can be detached prior to evisceration of the organ block and are an additional indicator for assessing the body condition of the animal.

The liver is the next organ to be grossly examined, detaching it from the organ block. For all crocodile species and most lizard species the gall bladder is attached to the liver. For the other lizard species, the gall bladder is caudally to the liver. The pancreas, globular, triangular or elongated and flattened, pale pink, is located within the duodenum serosa (12).

The spleen, usually ovoid, is located in the close vi-

cinity of the pancreas, so that in its histological structure of certain species, such as monitors, areas of the endocrine pancreas can be spotted (8, 13).

The digestive tract consists of a short esophagus, an elongated stomach, a small and a large intestine, the colon ending with the cloaca.

The lung is often saclike, and may be unicameral (eg *Gekkonidae*), paucicameral - unicameral but partitioned by a septum (eg *Chamaeleotidae*) or multicameral (eg *Varanidae*, *Helodermatidae*, *Alligatoridae*), continuing in some species with airsacs (22, 24).

The kidneys are often placed in a retrocelomic space in the pelvic canal, and for the evisceration it is necessary to cut the pelvis. In monitors, they are located in the caudal end of the celomic cavity. Occasionally, in the males of some species during the mating season, the terminal portion of the kidneys can be increased in volume due to the presence of the sexual segment (13, 17, 22). The ureters connect the kidneys with the cloaca (urodeum). For the species that present a urinary bladder, the urine enters the bladder through the cloaca. For the species that lack a urinary bladder (crocodiles and some lizards), the urine passes upward into the distal colon for water absorption (22, 25).

The ovaries, depending on the hormonal status, vary in appearance between a granular, oval structure and a saclike, lobular organ with numerous spherical follicles at various stages of development. The oviducts open independently in the cloaca through the genital papillae. Testicles are oval or elongated and can easily be mistaken for the kidneys. The right testicle is located cranially to the left one. Male lizards have two hemipenes and crocodiles have a single penis (16, 25).

Snakes

In snakes, the animal will be positioned in dorsal recumbency. There are two techniques for cutting the skin. The first uses a single section, starting from between the mandibular rami and ending in the vent area. This section can be made on the median or lateral (left or right) edges of the ventral scales, for ease of sectioning in larger species (11, 19).

The second technique involves two ventrolateral parallel incisions, starting from the mandibular rami and ending at the vent. This allows the removal of a cutaneous flap and obtaining a wide examination area for the celomic cavity (19) (Fig. 10).

For the snake species with a latero-lateral compressed body (especially the aquatic species), a lateral recumbency can be used, and after the main incisions are made, the dorsally positioned wall can be turned outward to obtain a larger examination area (11).

Regardless of the species examined, the animal can be positioned on a wax, polystyrene or similar material so that after the medial incision, the animal's lateral walls can be pinned, thus providing better stability and improved visibility on the celomic cavity (11, 19). In these species, the organs can also be weighed individually.

The organs are eviscerated en bloc (Fig. 11) after the trachea and esophagus are cut right behind the pharynx, by slight caudal pulling toward the cloaca, sectioning the dorsal connective tissue (19).

The thymus (paired), the thyroid, and the two pairs of parathyroids are located anterior to the heart.

As in other species, the next step is to open the pericardium and detach the heart (Fig. 12). The heart is tricameral (two atria and one ventricle), usually positioned between the first and second quarter to the limit of the first to second third of the length of the animal. In aquatic species the heart is positioned more cranially (15).

The left lung is, in most snake species, less developed than the right one, except for members of the *Boidae* family. The trachea - with incomplete rings - continues with the axial chamber of the lung, which reaches the caudal area of the celomic cavity through an air sac (13).

The liver - elongated and generally bilobate - presents an extrahepatic gall bladder located caudally to the stomach, in the vicinity of the pancreas (pale pink organ with multilobular structure) and spleen (ovoid, reddish) (13).

The digestive tract consists of the esophagus, stomach, small intestine and large intestine.

The snakes' kidneys are multilobular structures, usually of brick color and much more lighter in the case of active reproductive males, due to the formation of the sexual segment. The ureters open individually in the urodeum and these species have no urinary bladder (6, 13).

Gonads, usually paired, are positioned similar to the kidneys - asymmetric (the right organ is positioned cranially). In species such as *Tantilla spp*., the left oviduct is vestigial (6). Testicles are positioned cranially to the kidneys and females have the ovaries in the vicinity of the pancreas. Males have two hemipenes at the base of the tail (6, 12).

Turtles and tortoises

Regardless of the examined species, the animal is positioned in dorsal recumbency. Due to the convex shape of the carapace, for a better immobilization of the body, a concave wax block to place the carapace can be used (8). The next step is the detachment of the plastron (Fig. 13). Initially, the skin that is inserted on the edges of the plastron is cut, and then, with a scalpel or scissors in small animals, or using a saw or rib shears, the plastron is detached, cutting the two marginal bridges between it and the carapace (8, 13).

Sectioning the bridges is done by tilting the body at an angle of 45° so that the organs gravitationally slide into the lower part of the cavity to prevent from accidentally cutting them (10). The plastron is carefully detached, by cutting the insertions of the pectoral and pelvic muscles as close as possible to the plastron. For an easier approach to the celomic cavity (Fig. 14), the anterior and posterior limbs will be detached together with the associated muscles (13, 20, 26).

For the examination of the oral cavity, along with the tongue, glottis and anterior tracheal segment, two incisions are made on the medial side of the mandibular rami so that the organs can be eviscerated. The esophagus and trachea can be detached from the body, or examined in situ (13, 20). As in other reptile species, localization and en bloc evisceration (Fig. 15) can be followed by weighing the organs.

Before performing other maneuvers, organs such as thymus or thyroid are eviscerated. The chelonian thymus is often bilobate and located near the carotid arteries. The thyroid is located at the base of the aorta. Parathyroids are located very close or even within the structure of the thymic lobules (12, 13).

The next step is to open the pericardium and detach the heart. The heart is located in the sagittal plane, slightly caudal to the scapular belt. It is tricameral (two atria and a ventricle with an incomplete interventricular septum) and in the apex region it is attached to the pericardium by *gubernaculum cordis* - as in lizard species (13, 22, 27).

The liver is the next eviscerated organ. It is bilobate and, compared to other reptile species, its right lobe is attached to the gall bladder, while the stomach is near the left lobe and covered by a thin portion of it (7) (Fig. 16). The digestive tract is eviscerated en bloc, starting from the stomach and ending with the cloaca, by cutting the connective tissue attached to the carapace. Of note is the position of the caudal segment of the esophagus, which suddenly changes orientation from the median plane to the left. The pancreas is located in the duodenal serosa, near the pyloric opening. The spleen - ovoid and dark red - is also near the pancreas, at the distal extremity (12, 13, 20, 22).

The trachea in chelonians is short, consisting of complete cartilage rings and bifurcates into intrapulmonary bronchi (24). The multicameral lung is eviscerated or not, because of being attached to the ventral part of the carapace and the spine (2). Its caudal portion has a saclike appearance (23).

The kidneys are located caudally to the lung, retroperitoneally. They are reddish, have a lobular appearance, and join the cloaca through two separate ureters. The chelonians present a urinary bladder ventrally to the colon, and it opens in the cloaca (26).

The gonads are located in the caudo-lateral part of the celomic cavity.

A successful necropsy should consider the aforementioned particularities, as well as the clinical context. Nevertheless, the protocol will be adapted according to the particularities of the investigated species and the pathological findings.

REFERENCES

1. *Aleksić-Kovačević S., Özvegy J., Krstić N., Rusvai M., Jakab C., Stanimirović Z., Becskei Z.,* (2014), Skin and skeletal system lesions of european pond turtles (*Emys orbicularis*) from natural habitats, Acta Vet Hung, 62(2):180-193
2. *Bennett T.*, (2011), The Chelonian Respiratory System. Vet Clin Exot Anim, 14:225-239
3. *Bošnjak I., Zdravković N., Čolović S., Ranđelović S., Galić N., Radojiĉić M., Šekler M., Aleksić-Kovaĉević S., Krnjaić D.*, (2016), Neglected zoonosis: The prevalence of *Salmonella* spp. in pet reptiles in Serbia. Vojnosanit Pregl, 73(10):980-982
4. *Cristina R.T., Kocsis R*., (2018), About reptiles - A small guide for the passionate [in Romanian], (Ed.) Waldpress, Timisoara, Romania, pp. 1-194
5. *Dervas E., Hepojoki J., Laimbacher A., Romero-Palomo F., Jelinek C., Keller S., Smura T., Hepojoki S., Kipar A., Hetzel U., (2017),* Nidovirus-associated proliferative pneumonia in the green tree python (*Morelia viridis*), J Virol, 91(21)pii:e00718-17
6. *Di Girolamo N., Selleri P.,* (2017), Reproductive disorders in snakes. Vet Clin Exot Anim, 20:391-409
7. *Duffus A.L.J., Fenton H.M.A., Gray M.J., Miller D. L.,* (2017), Investigating amphibian and reptile mortalities: a practical guide for wildlife professionals. Herpetol Rev, 48:550-557
8. *Farris S.C., Squires M.A., Ridgely F., Lavergne E., Serota M., Mazzotti F.J.,* (2013), Necropsies of reptiles: recommendations and techniques for examining invasive species. Available online: https://edis.ifas.ufl.edu/pdffiles/UW/UW38200.pdf [Accessed: 01/05/2019]
9. *Fry B.G.,* (2015), Venomous reptiles and their toxins: evolution, pathophysiology and biodiscovery, (Ed.) Oxford University Press, NY, USA, pp. 1-576
10. *Garner M.M.,* (2005), The reptile necropsy: collection and submission of pathologic samples. North American Veterinary Conference, Jan 2005, Orlando, Florida, USA, Proceeding of the NAVC, 1275-1277
11. *Gillett A.K., Ploeg R., Flint M., Mills P.C.,* (2017), Postmortem examination of Australian sea snakes (*Hydrophiinae*): Anatomy and common pathologic conditions. J Vet Diagn Invest, 29(5):593-611
12. *Hanley C.S., Hernandez-Divers S.*, (2003), Practical gross pathology of reptiles. Semin Avian Exot Pet Med, 12(2):71-80

13. *Jacobson E.R.,* (2007), Infectious diseases and pathology of reptiles: color atlas and text, (Ed.) CRC Press, Boca Raton, FL, USA, pp. 1-736
14. *Jensen B., Moorman A.F., Wang T.,* (2014), Structure and function of the hearts of lizards and snakes. Biol Rev Camb Philos Soc, 89(2):302-336
15. *Kik M.J.L., Mitchell M.A.,* (2005), Reptile cardiology: a review of anatomy and physiology, diagnostic approaches, and clinical disease. Semin Avian Exot Pet Med, 14(1):52-60
16. *Knotek Z., Cermakova E., Oliveri M.,* (2017), Reproductive medicine in lizards. Vet Clin North Am Exot Anim Pract, 20(2):411-438
17. *Mader D.R.,* (2006), Reptile medicine and surgery, 2nd Edition, (Ed.) Saunders Elsevier, St. Louis, MO, USA, pp. 1-1264
18. *Melidone R., Gibson C.J.,* (2010), Necropsy techniques in reptiles, Part 1: General introduction and lizards and crocodilians. UK Vet Companion Animal, 15(5):1-6
19. *Melidone R., Gibson C.J.,* (2010), Necropsy techniques in reptiles, Part 2: Snakes. UK Vet Companion Animal, 15(6):71-74
20. *Melidone R., Gibson C.J.,* (2010), Necropsy techniques in reptiles, Part 3: Terrestrial chelonians, miscellaneous points and conclusions. UK Vet Companion Animal, 15(7):87-90
21. *Moroz-Perlmutter C., Goldblum N., Vries A.D., Gitter S.,* (1963), Detoxification of snake venoms and venom fractions by formaldehyde. Proceedings of the Society for Experimental Biology and Medicine, 112(3):595-598
22. *O'Malley B.,* (2005), Clinical anatomy and physiology of exotic species. Structure and function of mammals, birds, reptiles and amphibians, (Ed.) Saunders Elsevier, Germany, pp. 1-272
23. *Origgi F.C., Jacobson E.R.,* (2000), Diseases of the respiratory tract of chelonians. Vet Clin North Am Exot Anim Pract, 3(2):537-549
24. *Schumacher J.,* (2011), Respiratory medicine of reptiles. Vet Clin Exot Anim, 14:207-224
25. *Vitt L.J., Caldwell J.P.,* (2013), Herpetology. An introductory biology of amphibians and reptiles. 4th edition, (Ed.) Acad Press Elsevier, San Diego, CA, USA, 1-776
26. *Wyneken J.,* (2001), The anatomy of sea turtles. U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470
27. *Wyneken J.,* (2009), Normal reptile heart morphology and function. Vet Clin Exot Anim, 12:51-63.