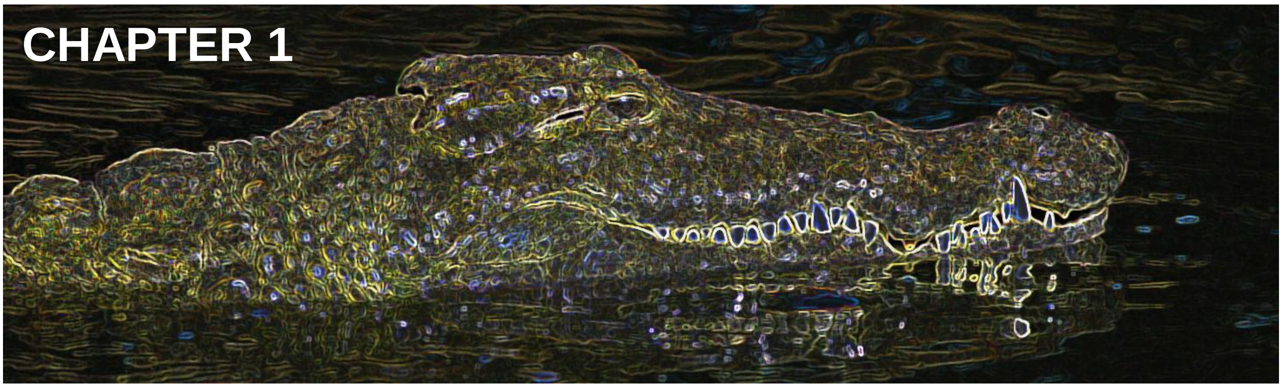

GENERAL INTRODUCTION

Modern crocodiles descended from ancestors about 225 million years ago in the late Triassic period in Europe, South America and South Africa (Seymore *et al.* 2004; Summers 2005). According to Trutnau & Sommerlad fossil records of the Nile crocodile found in North and East Africa date back 2.5 to 3.5 million years (2006, cited in http://library.sandiegozoo.org/factsheets/nile_crocodile/crocodile.htlm).

The Nile crocodile belongs to the class Reptilia, the order Crocodylia, the suborder Eusuchia (modern crocodiles), the family Crocodylidae and the subfamily Crocodylinae. (http://library.sandiegozoo.org/factsheets/nile_crocodile/crocodile.htlm). The Crocodylinae includes the genera *Osteolaemus* and *Crocodylus.* Crocodiles are diapsid ("two arched") reptiles (Benton 1985) and the diapsids are subdivided into Lepidosauria (lizards & snakes) and Archosauria that include modern birds and crocodiles.

The scientific name *Crocodylus niloticus* is derived from the Greek *kroko* (meaning "pebble") referring to the rough texture of the skin, and *deilos* (meaning "worm"). (http://library.sandiegozoo.org/factsheets/nile_crocodile/crocodile.htlm). *Niloticus* means "of the Nile River". The Nile crocodile is the largest crocodile in Africa with total body lengths of between 2 and 5 m and a mass of up to 1000 kg having been recorded (Pooley & Gans 1976).

Crocodile farming is a niche farming industry with approximately 40 farms still viable in South Africa and Zimbabwe. The commercial importance of crocodiles lie in their skin and meat and the Chinese population use certain organs for medicines (Putterill 2002). It has been reported that the Nile crocodile has been one of the top commercially utilized species of crocodiles in the world (Ross 1998).

The liver is the largest internal organ in the reptilian body and has many complex functions that are vital for metabolism and homeostasis. Major metabolic functions include carbohydrate (energy storage), protein (plasma proteins, peptide hormones) and lipid metabolism (bile salts) (McClellan-Green, Celander & Oberdörster 2006). As such the liver also stores vitamins and iron and is involved in glucose, lipid and cholesterol metabolism, the synthesis of amino acids and the degradation of toxins. The hepatic enzymes are responsible for the metabolism of xenobiotic substances, for example pollutants. The antioxidant defense system in the liver through its enzymes manages the elimination of the reactive oxygen species, namely hydroxyl radicals, superoxide radicals and hydrogen peroxide. The melanin in reptilian livers acts additionally as a free radical trap for superoxide.

The morphology of the mammalian liver has been studied extensively (Elias & Bengelsdorf 1952; Elias 1955; Schaffner 1998). Elias & Bengelsdorf (1952) also noted the liver morphology of alligators, birds and lizards. Beresford & Henninger (1986) tabulated the microscopical variations in livers of mammals, birds, fishes and reptiles. Crocodiles were not included in these comparative studies. Ultrastructural features of the livers of various animals were described by Kalashnikova (1996), but crocodiles were excluded.

An overview of the anatomy and histology of the reptilian liver was given by Jacobson (2007), with McClellan-Green *et al.* (2006) covering the basic anatomy and fine structure of the same. The liver morphology of the freshwater turtle was described by Moura *et al.* (2009) and that of the Newt by Goldblatt *et al.* (1987), while Marycz & Rogowska (2007) discussed the general liver topography of terrestrial tortoises. Many articles concentrate only on specific constituents of the liver, for example, Ito cells, Kupffer cells, sinusoids or the perisinusoidal space, and not on the liver architecture as a whole (Purton 1976; Aterman 1986; De Brito Gitirana 1988; Kalashnikova 1992; Geerts, Bouwens & Wisse 1998; Ghoddusi & Kelly 2004). The liver of reptiles differs in size, shape and appearance among species (Schaffner 1998) and although liver cells in the different classes of animals share common organelles, the cell structure varies both between and within classes (Kalashnikova 1996). Divers & Cooper (2000) stated that there was a lack of knowledge of hepatic metabolism and the pathogenesis of hepatic disease in the reptilian liver. McClellan-Green *et al.* (2006) commented on the lack of documented neoplastic diseases in reptiles and the need to form a link between liver disease and exposure to specific chemicals. Ganser, Hopkins, O'Neil, Hasse, Roe & Sever (2003) found a definite connection between liver tissue damage and dietary contamination in their study on the liver of the Southern Watersnake. Schaffner (1998) mentioned that regarding the reptilian liver the two main routes of investigation have been its metabolic function and its place in the structural evolution of the organ in vertebrates. Hopkins (2000) stated that "Reptiles are the least studied group of vertebrates with regard to environmental contaminants".

General descriptions of the crocodilian liver can be found in Huchzermeyer (2003) and Schaffner (1998). The macroscopic anatomy of the abdominal organs of the Nile crocodile was documented by Van der Merwe & Kotzé (1993) and the serous cavities by Mushonga & Horowitz (1996). Light microscopical and ultrastructural findings on the liver of immature West African crocodiles *Osteolaemus tetraspis* were published by Storch, Braunbeck & Waitkuwait (1989). The general anatomy of the liver of the saltwater crocodile *Crocodylus porosus* was discussed by Richardson, Webb & Manolis (2002) and the occurrence of fibrous trabeculae in the American alligator *Alligator mississipiensis* liver was described by Beresford (1993).

The gallbladder functions as an auxiliary organ of the digestive tract that stores diluted bile received from the hepatic duct via the cystic duct (Oldham-Ott & Gilloteaux 1997; Ross, Kaye & Pawlina 2003; Fried 2008). It concentrates the bile by reabsorbing water and replacing electrolytes through an active sodium chloride pump. The presence of fat in the proximal duodenum triggers hormones that effect smooth muscle contraction of the gall-bladder with resulting secretion of the concentrated bile into the duodenum. The gallbladder therefore plays a significant role in controlling the flow of bile to the intestine when needed.

The anatomy of the reptilian and crocodilian gallbladder is briefly mentioned by Richardson *et al.* (2002), Huchzermeyer (2003) and Jacobson (2007). Xu, Elsey, Lance, Javors, Chen, Salen, & Tint (1997) studied the biliary ductal system and the bile fistula in the American alligator. A comparative morphological study of the gallbladder and biliary tract of vertebrates was done by Oldham-Ott & Gilloteaux (1997). There was no specific mention of the Nile crocodile gallbladder.

The aims of the study are to describe:

- the topography of the liver and gallbladder
- the macroscopic appearance of the liver and gallbladder.
- the histology of the liver and gallbladder.
- the ultrastructural features of the liver and gallbladder.

Benefits

Ecologists have considered the Nile crocodile as an important keystone species for aquatic biodiversity in Africa (Pooley, 1969; Tinley, 1976, cited in Ashton 2010) and also specifically for the Olifants River in South Africa (Joubert 2007, cited in Ashton 2010). Nile crocodile mortalities along the Olifants River between 2008 and 2009 amounted to almost 200 carcasses with the histolopathogical examination finding pansteatitis as the cause of death (Ashton 2010). Polluted freshwater ecosystems were possibly responsible for the demise of the animals.

The literature review clearly shows that data on the normal liver and gallbladder morphology of the Nile crocodile are virtually non-existent, which means that there is no reference baseline information to compare with diseased livers.

An in-depth study of the topography, anatomy, histology and ultrastructure of the Nile crocodile liver and gallbladder will enhance our understanding of the normal morphology of these organs. Knowledge of the normal micro-morphology of the liver is essential for the pathological evaluation of disease. Baseline data will be established for comparative liver and gallbladder studies.

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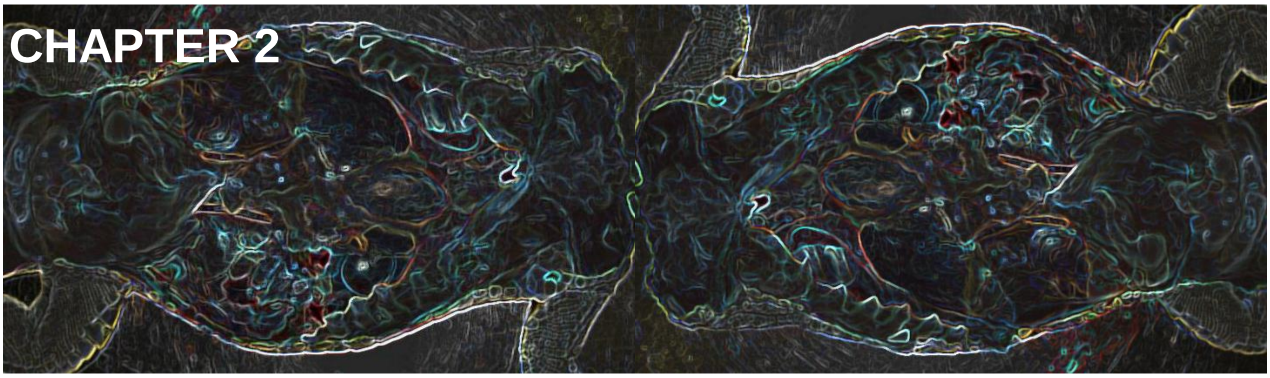

TOPOGRAPHY OF THE LIVER AND GALLBLADDER

2.1 INTRODUCTION

The variety of forms of the reptilian liver have long been recognized (Owen 1866). The topography of this organ has received moderate attention in the literature with authors giving consideration mostly to testudines, lizards, alligators, snakes and crocodiles. The liver of reptiles have unusual shapes because they conform to the shape of the coelomic cavity of the species in which it is found, for example, it is elongated and slim in snakes (Schaffner 1998). Goldblatt *et al.* (1987) described the gross morphology of the Newt liver, Marycz and Rogowska (2007) explained the liver topography of Horsfield's and Hermann's tortoises and Moura *et al.* (2009) described the coelomic cavity and identified four liver lobes in the freshwater turtle. A short discussion of the topography of the liver of three species of the family Alligatoridae was given by Romão, Santos, Lima, De Simone, Silova, Hirano, Vieira & Pinto (2011). Richardson *et al.* (2002) reported on the positioning of the liver and its adjacent structures in the Saltwater crocodile *Crocodylus porosus*. The anatomical relationship of the crocodilian liver with other organs and the location of the crocodilian liver in the coelomic cavity was noted briefly by Schaffner (1998), Huchzermeyer (2003) and Jacobson (2007).

Two articles have referred specifically to the topography of the Nile crocodile liver. One described the hepatic coelom and provided a schematic drawing depicting the position of the liver in relation to heart, stomach and duodenum (Van der Merwe & Kotzé 1993). The other article described the right and left hepatopulmonary bursae which included detailed topography of the liver (Mushonga & Horowitz 1996).

The topography of the gallbladder is briefly mentioned in most of the referenced literature above, as well as by Owen (1866). In addition Oldham-Ott & Gilloteaux (1997) reviewed the morphology of the gallbladder and biliary tract in fish, reptiles, amphibians, birds and mammals. Xu *et al.* (1997) demonstrated the potential anatomical variations of the biliary ductal system in the American alligator *Alligator mississippiensis*.

2.2 MATERIALS AND METHODS

The carcasses of three 1-year old juvenile Nile crocodiles that were slaughtered for their skins at the Le Croc Crocodile Breeding Farm near Brits in the North West Province were used for the topographical description of the liver and gallbladder. The ventral body wall, the ribs and fat layer were removed to expose the abdominal organs *in situ.* The organs were photographed with a Canon 5D Mark II camera using a 28-135mm zoom lens.

2.3 RESULTS

The liver was located in a cavity between the thoracic and abdominal organs. It was surrounded cranially by the post-pulmonary membrane and caudally by the post-hepatic membrane (Fig. 2.1). The liver, in relation to the ribcage, was located between the third and seventh intercostal spaces with its margins spreading to the ninth intercostal space. The liver was bi-lobed with the right lobe being larger than the left lobe (Fig. 2.2). The right lobe was associated caudally with the double-looped duodenum via the hepatoduodenal ligament and the left lobe was attached to the cranial surface of the stomach by the gastrohepatic ligament. A narrow isthmus, situated dorso-medially, connected the two liver lobes. The heart, in its pericardial sac, was connected to both liver lobes by the coronary ligament with the apex lodged ventrally to the isthmus between the two liver lobes.

The empty gallbladder had a flattened sac-like shape and was attached caudally to the dorso-medial aspect of the right liver lobe by the hepatocystic ligament. When fully distended the gallbladder had the form of a blind-ending pouch with its body lodged ventromedially between the duodenum on the right and the stomach on the left (Fig. 2.3).

2.4 DISCUSSION

This study confirms the description by Van der Merwe & Kotzé (1993) that the Nile crocodile liver is located in its own coelomic cavity with the post-pulmonary and the post-hepatic membranes being intimately associated with the cranial and caudal surfaces of the bi-lobed liver respectively. The findings also endorse the topographical description of the Nile crocodile liver given by Mushonga & Horowitz (1996) who note that the right lobe is larger than the left lobe and that they are found at the level the third to the seventh intercostal spaces with their extremities extending to the ninth intercostal space.

Schaffner (1998) concurred that the crocodilian liver occupies a large portion of the coelomic cavity and is situated under the posthepatic septum. Richardson *et al.* (2002) also stated that the large bilobed liver of crocodilians lies transversely across the junction of the thoracic and abdominal regions and that the visceral surface is associated with the stomach on the left and the duodenum on the right. He also found that the Saltwater crocodile was almost unlobed with just an indentation indication the beginning of a division. Huchzermeyer (2003) described the crocodilian bi-lobed liver as occupying the hepatic coelom between two transverse membranes with the lobes being of almost equal size, in contrast to the findings of this study where the right lobe is larger than the left lobe. Romão *et al.* (2011) commented on a direct connection of the liver to the pancreas in one alligator specie (*Paleosuchus palpebrosus*), but a similar connection was not found in the Nile crocodile.

This study also confirms that the Nile crocodile liver lobes are joined in the middle by a narrow isthmus (Mushonga & Horowitz 1996). Richardson (2002) described the hepatic isthmus as being dorsally located between the two lobes in crocodiles. Some crocodile species have two separate liver lobes with other species' lobes linked by a dorsal bridge (Huchzermeyer 2003). Chiasson (1962) noted briefly that the bi-lobed liver of alligators were connected by a "middle constricted portion". Van der Merwe & Kotze (1993) and Romão *et al.* (2011) did not mention the existence of a liver isthmus in the Nile crocodile or in three alligator species respectively.

The liver topography of other reptiles differs significantly from crocodilians, for example, Goldblatt *et al.* (1987) and Moura *et al.* (2009) described four liver lobes in *Notophthalmus viridescens* (Newt) and *Phrynops geoffroanus* (freshwater turtle) respectively. Machado

Júnior, Sousa, Carvalho *et al.* (2005, cited by Moura *et al.* 2009) found five lobes in *Kinosternon scorpioides* (scorpion mud turtle) and Ells (1954) discovered three lobes in the lizard *Sceloporus occidentalis biseriatus.* As already mentioned, the livers in snakes are elongated, but are also unlobed and in some lizards only a deep indentation defines a form of lobation (Guibe 1970, cited by Oldham-Ott & Gilloteaux 1997). In Chelonians the liver is a large, saddle-shaped organ filling the abdominal region under the lungs (McClellan-Green *et al.* 2006) with the left lobe being considerably smaller than the right lobe (Jacobson 2007). In 2009 Kassab *et al.* stated that the left lobe of the Desert tortoise (*Testudo graeca*) liver was larger than the right lobe which opposes what was found in the Nile crocodile. Lizards lack a posthepatic septum (Schaffner 1998) which contrasts with the caudal presence of this membrane around the liver of the Nile crocodile.

Regarding the Nile crocodile gallbladder, Van der Merwe & Kotzé (1993) stated only that it is located to the right of the midline within the hepatic coelom. Mushonga & Horowitz (1996) found that the gallbladder was situated at the caudal surface of the right liver lobe at its ventromedial angle. The current study also describes the gallbladder as being located caudal to the right liver lobe in the ventromedial position, but the neck area is attached to the right liver lobe through the hepatocystic ligament in the dorso-medial position.

In crocodilians in general, the gallbladder is ‘held close’ to the right liver lobe by a fibrous ligament (Richardson *et al.* 2002) or lies between the two lobes (Huchzermeyer 2003). Romão *et al.* (2011) in their study of the digestive system of three alligator species placed the gallbladder’s location dorso-medially and caudal to the right liver lobe. Richardson *et al.* (2002) in their illustration of the Saltwater crocodile showed the gallbladder to be attached caudally to the liver by a fibrous ligament in the dorso-medial region.

The position of the gallbladder varies among reptilian species. In testudines, chelonians and some lizards the gall bladder is embedded in the right lateral lobe, with the scorpion mud turtle being the exception by having its gall bladder located in the middle of the right lateral and medial lobes (Moura *et al.* 2009; Jacobson 2007). The gallbladder of the lizard *Sceloporus* is located in the middle liver lobe (Ells 1954). Snakes have their gall- bladders separate but related caudally to the liver (Owen 1866; Moura *et al.* 2009). In the newt with its four liver lobes the gallbladder lies dorsally on the liver surface between the right upper and lower lobes (Goldblatt *et al.* 1987).

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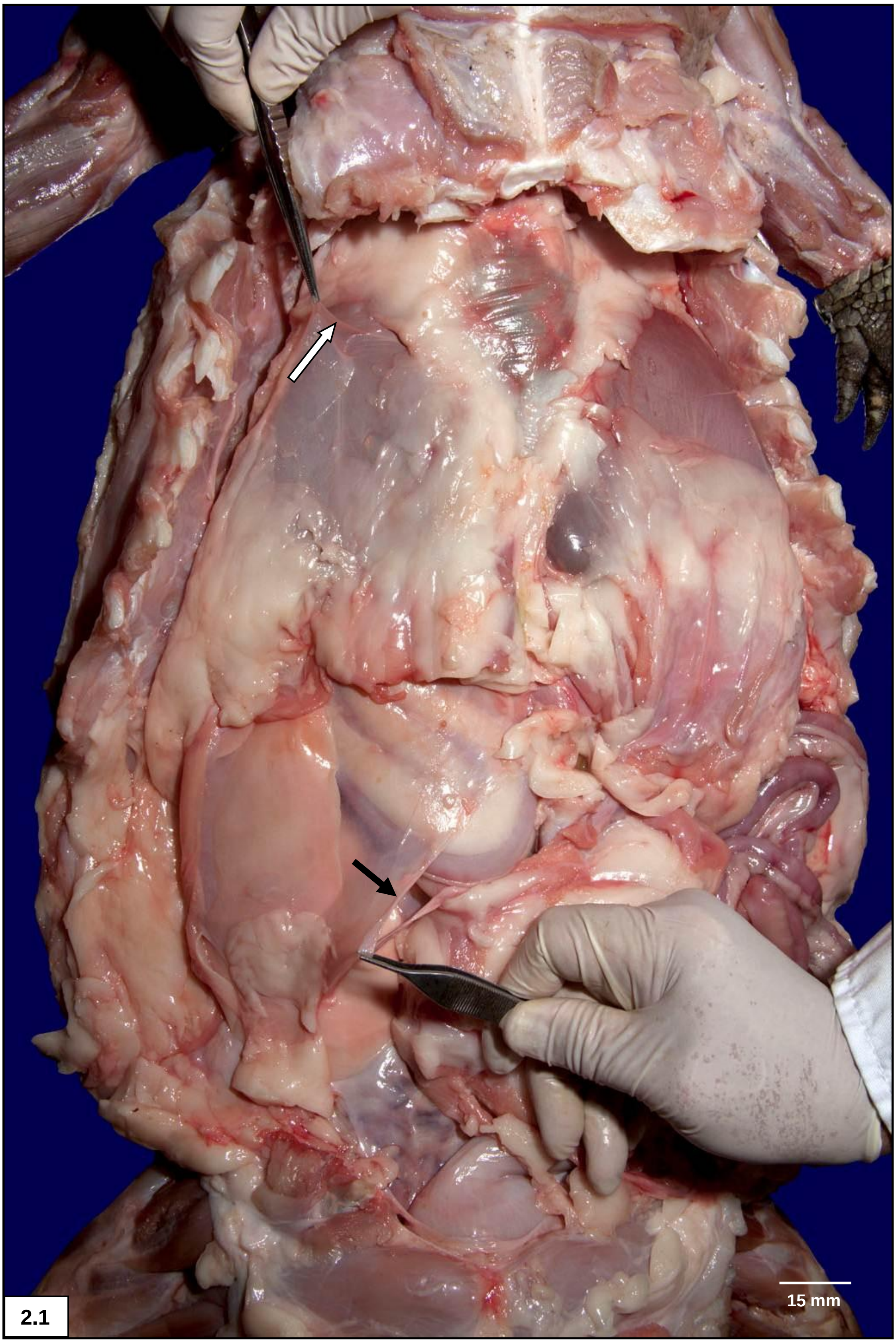


Figure 2.1: Ventral view of the liver *in situ* showing the post-pulmonary membrane (white arrow) and the post-hepatic membrane (↑).

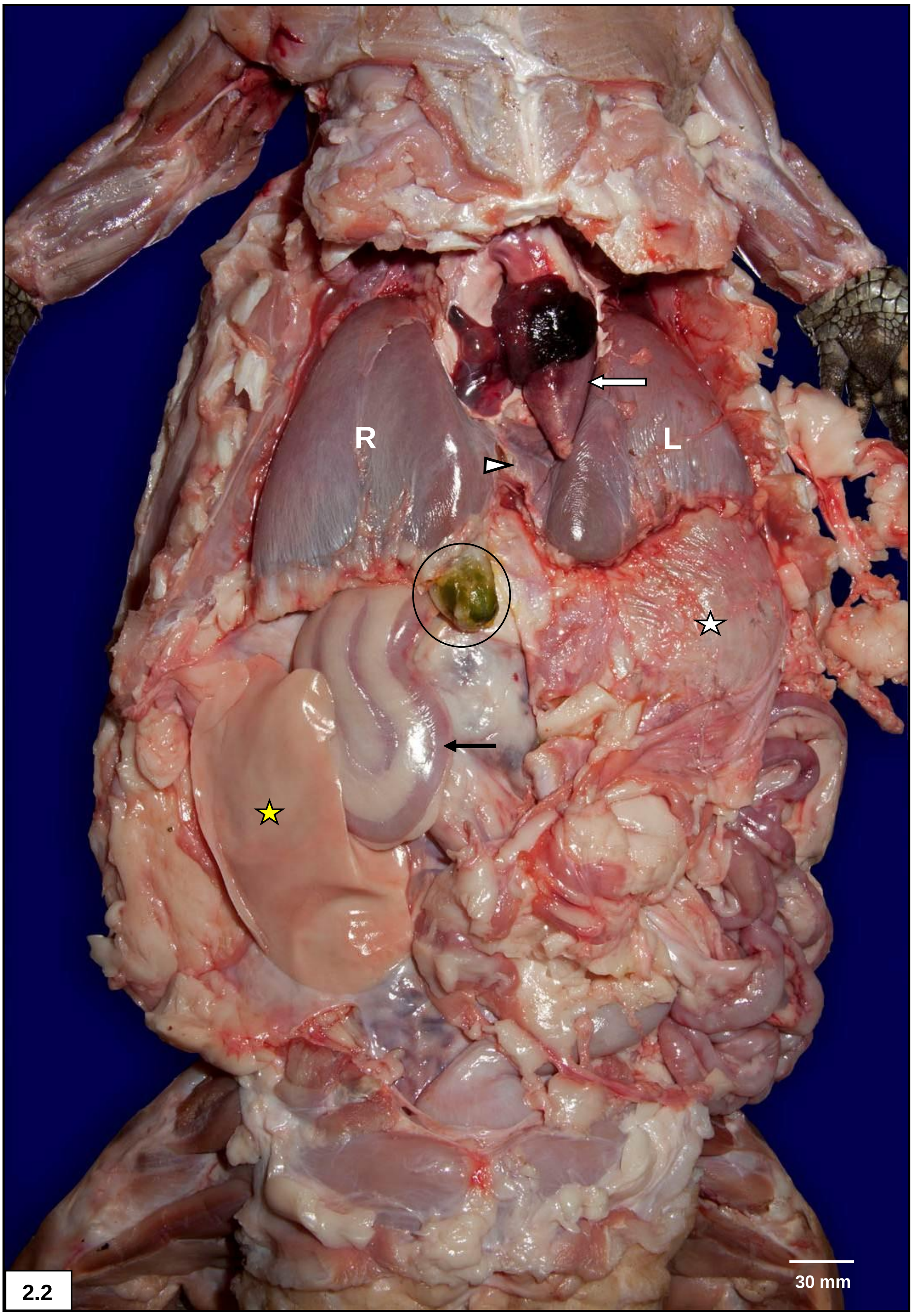


Figure 2.2: Ventral view of the liver showing the right (**R**) and left (**L**) liver lobes with the heart (white arrow) nestled in between, ventral to the isthmus (white arrowhead), and their association with the stomach (white star), the duodenum (black arrow) and the gallbladder (circled). Note the fat body (yellow star).

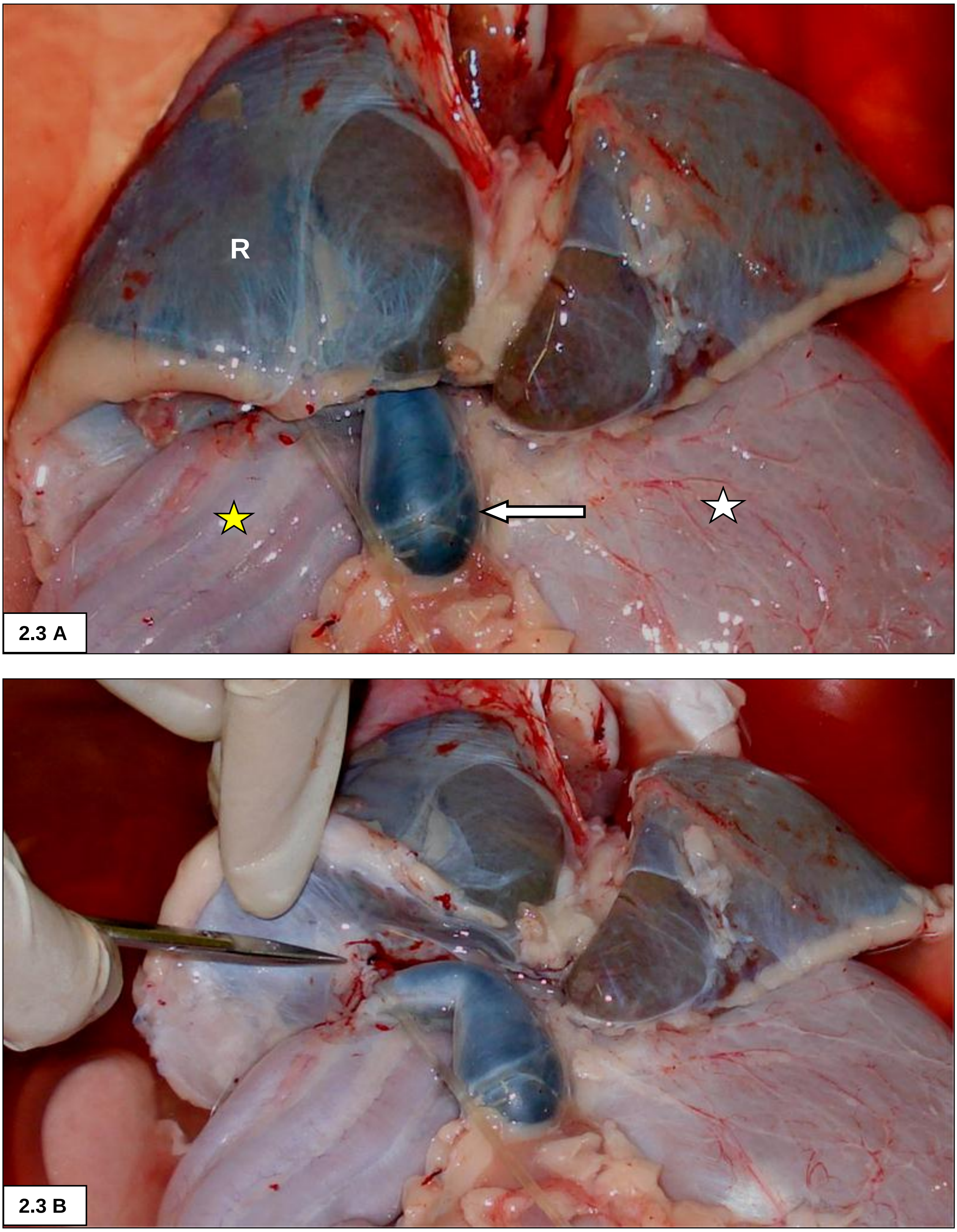
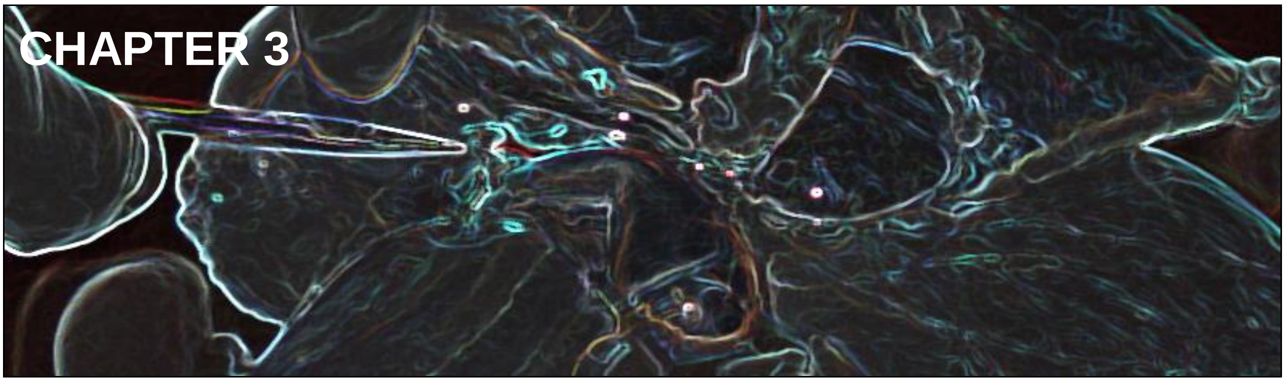


Figure 2.3: A - Ventral view of the pouch-like gallbladder (white arrow) positioned caudally to the right liver lobe (**R**) at the ventromedial aspect. Duodenum (yellow star); stomach (white star).

Figure 2.3: B - Partial reflection of the right liver lobe showing the dorso-medial attachment of the gallbladder to liver.



GROSS ANATOMY OF THE LIVER AND GALLBLADDER

3.1 INTRODUCTION

There is a shortage of information regarding the macroscopic features of the liver and gallbladder of crocodilians and particularly that of the Nile crocodile. Two publications gave anatomical descriptions of the liver and gallbladder of *Crocodylus niloticus* (Van der Merwe & Kotzé 1993; Mushonga & Horowitz 1996). Richardson *et al.* (2002) illustrated the liver and gall- bladder of the Saltwater crocodile (*Crocodylus porosus*). Romão *et al.* (2011) included a brief anatomical explanation of the liver and gallbladder in their discussion of the digestive system of alligators. Divers and Cooper (2000) stated, “We remain ignorant of the specific features of liver anatomy of many reptiles”.

3.2 MATERIALS & METHODS

After the topographical description was completed, the intact liver with the gallbladder attached was removed from the body cavity and the ventral and dorsal aspects photographed with a Canon 5D Mark II camera using a 28-135mm zoom lens.

3.3 RESULTS

The livers were a dark reddish-brown colour, were covered by capsules and weighed 98 g, 165 g and 215 g. It consisted of two lobes with the right lobe being the largest. Both lobes had the shape of a triangular prism with a caudal base and a blunt cranial apex (Fig. 3.1). Each liver lobe exhibited dorsal, ventral and lateral borders with the caudal, cranial and lateral surfaces being rounded and not flattened. The two lobes were joined by a narrow, flattened isthmus, measuring approximately 7 x 2.5 x 1 cm and apparently composed of liver tissue. The isthmus was located in the medial dorsal region between the two lobes (Fig. 3.2).

The distended pouch-like gallbladder measured approximately 5 x 2 x 2 cm and was enclosed by a thin wall. Three anatomical zones could be identified, namely, a long narrow neck closest to the hepatocystic ligament, a middle area constituting the body of the organ, and the blind end or fundus. The distended gallbladder was filled with around 5 ml of green mucoid bile contents and in one crocodile the gallbladder displayed a curved appearance.

3.4 DISCUSSION

The present study agrees with the findings of Mushonga & Horowitz (1996) that the right liver lobe of the Nile crocodile is larger than the left lobe, that the two lobes are joined by an isthmus and that the gall bladder is attached caudally to the right liver lobe. Van der Merwe & Kotze (1993) concurred with the previous statement, but did not mention an isthmus. The Caiman liver and gall bladder descriptions were identical to that of the Nile crocodile, but again an isthmus was not referred to (Romão *et al.* 2011). *Crocodylus porosus* differs in that this Saltwater crocodile displayed an unlobed liver with only a short indentation in the medial region (Richardson *et al.* 2002). The macroscopic appearance of the isthmus in the current study gave the impression that it was composed of liver tissue. In other reptiles, for example tortoises, the isthmus was described as a “narrow band of connective tissue” (Marycz & Rogowska 2007; Kassab *et al.* 2009). Some reptiles differ in respect of liver lobe sizes, for example, Kassab *et al.* (2009) found the left liver lobe of the Desert tortoise to be larger than the right lobe.

Many publications fail to comment on the form of the gallbladder. The shape of the gallbladder however varies between reptile species: oval in the desert tortoise (Penninck *et al.* 1991, cited in Oldham-Ott & Gilloteaux 1997); oblong or piriform in alligators (Xu *et al.* 1997; Romão *et al.* 2011) and pouch-like when full in the Nile crocodile (current study). The illustration of the Saltwater crocodile liver and gallbladder given by Richardson *et al.* (2002) also depicted the gall bladder’s shape as pouch-like. As in the present study the gallbladder of alligators were also described as having a thin wall (Xu *et al.* 1997). The division of the Nile crocodile gallbladder into three anatomical zones has also been noted in the snake *Bothrops jararaca* gallbladder (Silveira & Mimura 1999).

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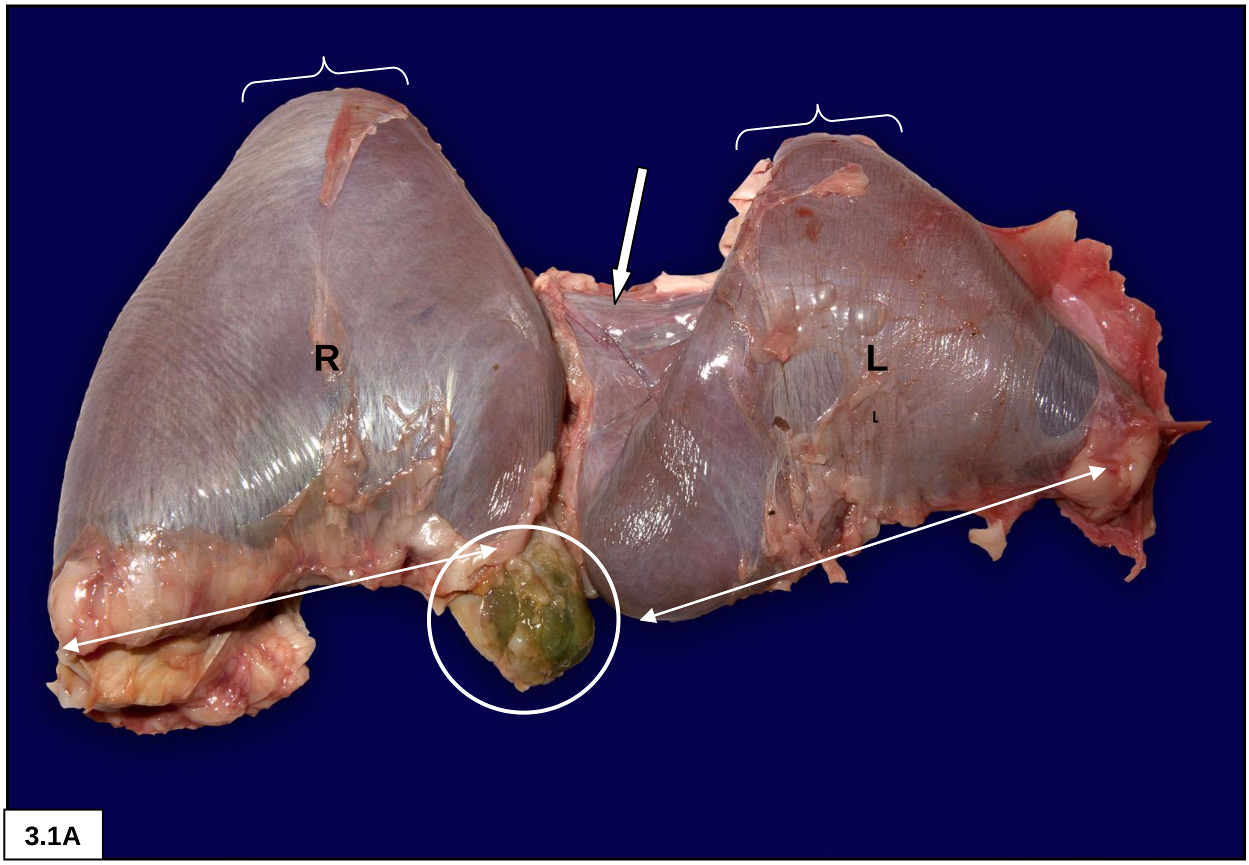
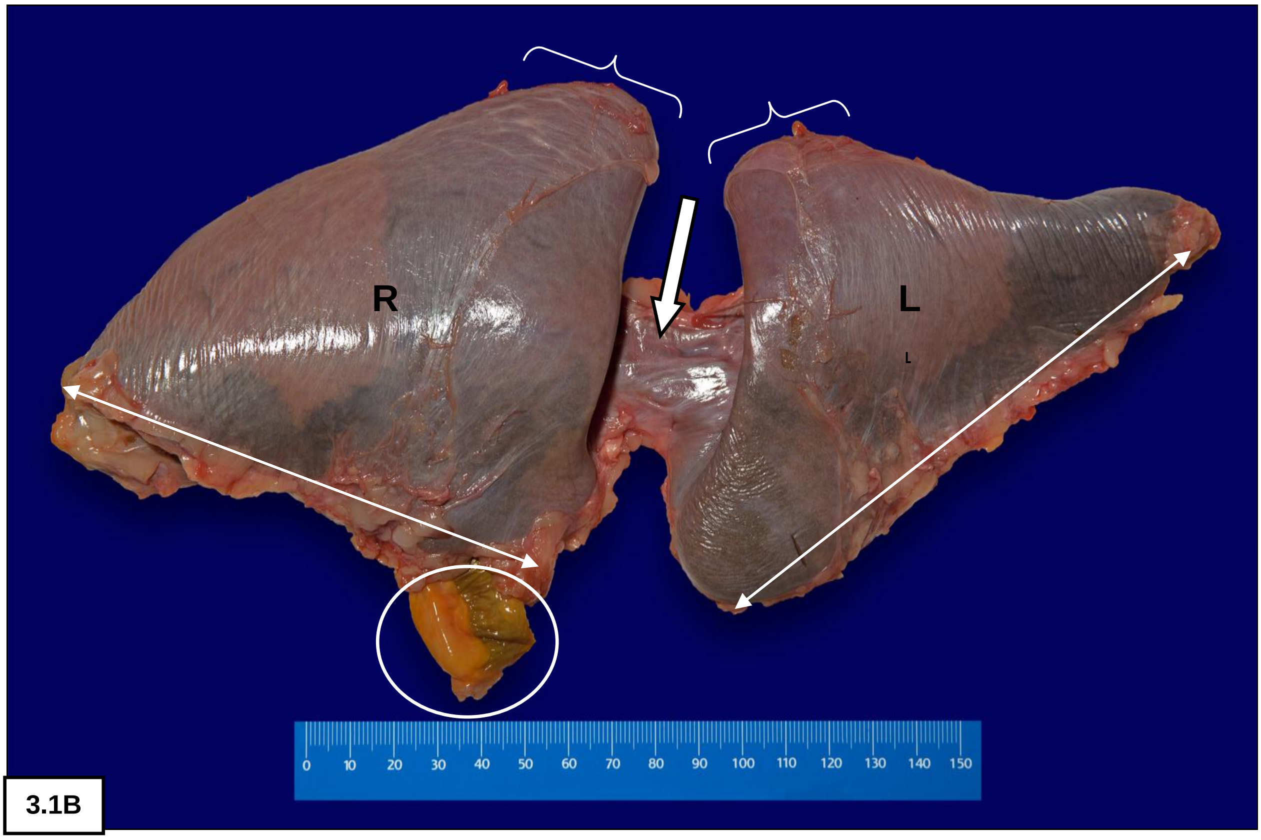


Figure 3.1: A & B- Ventral view showing the difference in size and the shape of the two liver lobes, the position of the gallbladder (encircled) and the isthmus (white arrow) between the right (**R**) and left (**L**) lobes. Base (double arrows), apex (brackets)



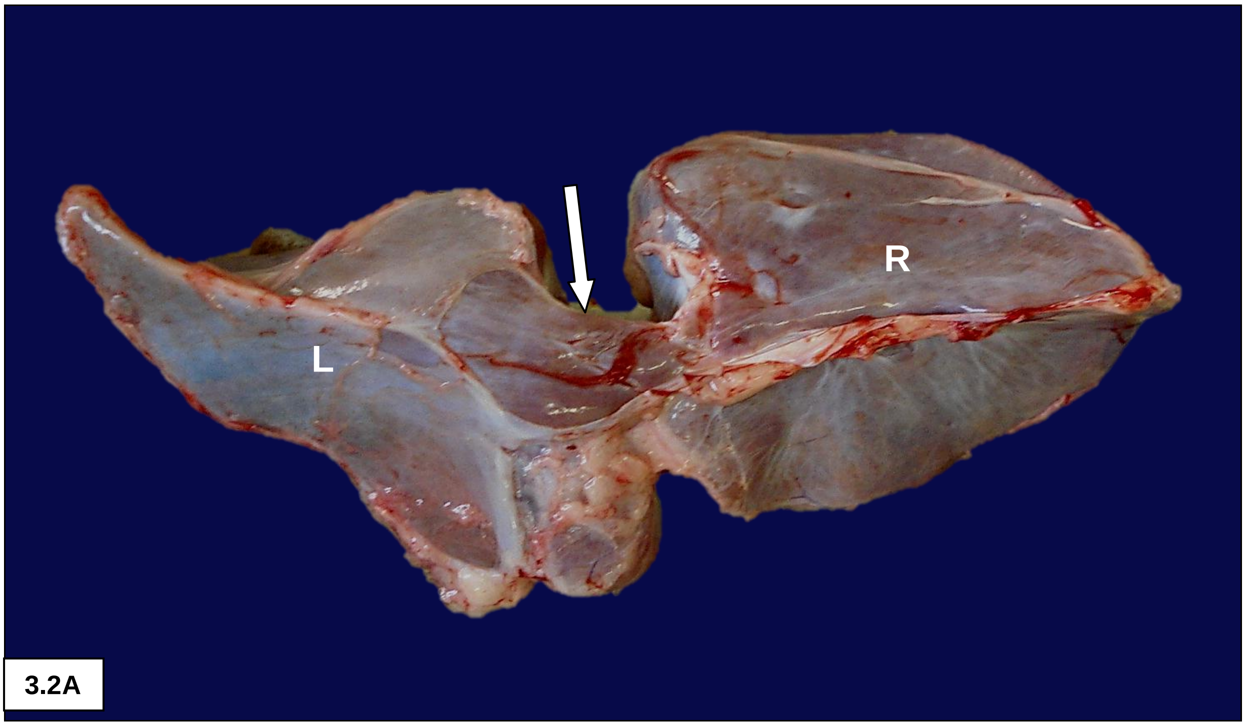
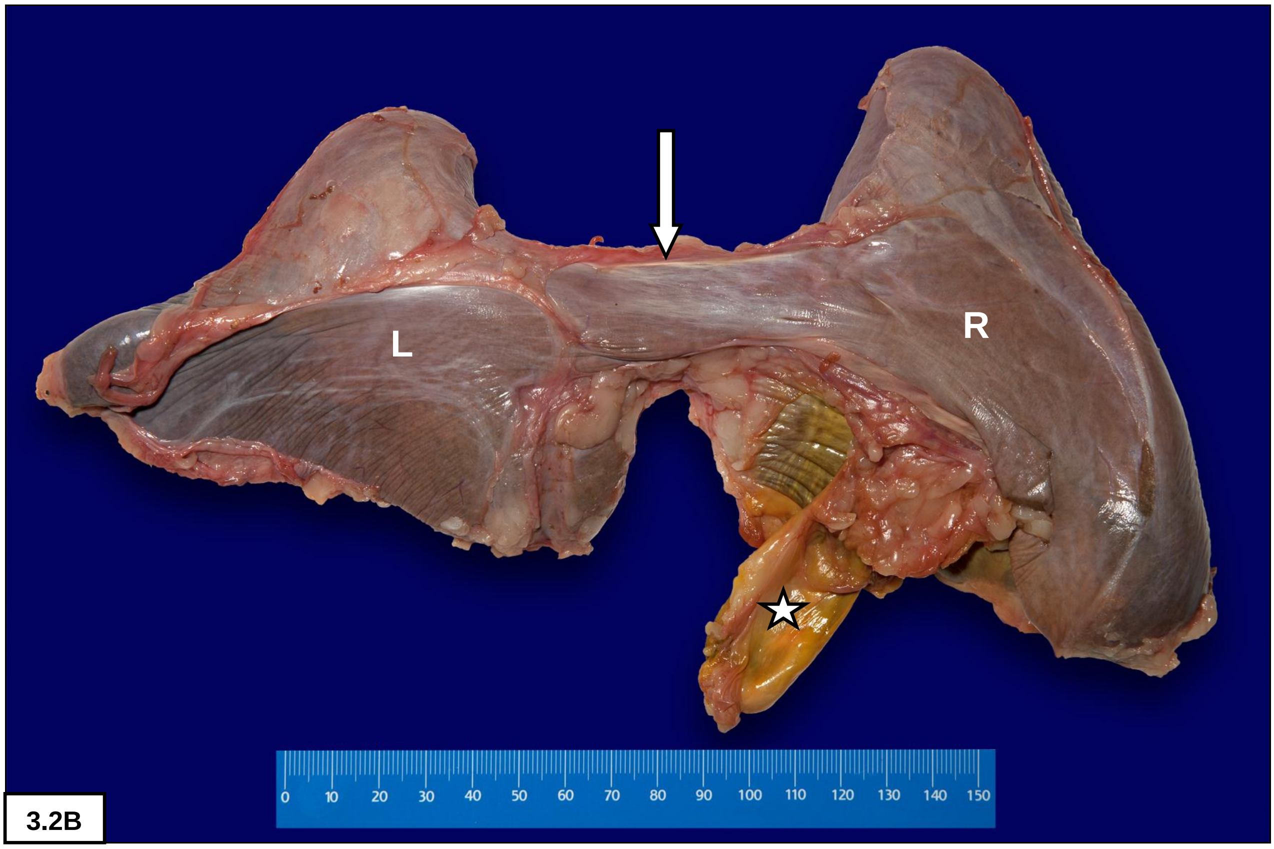
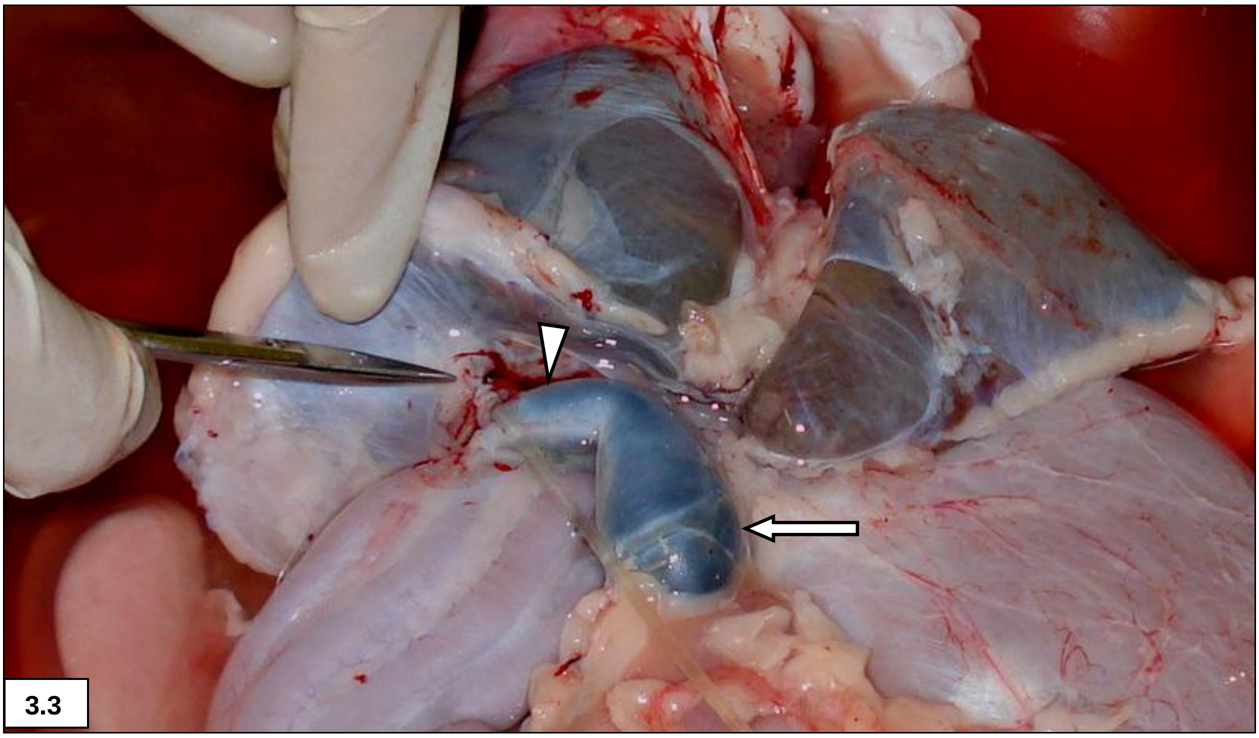


Figure 3.2: **A & B-** Dorsal view of the right (R) and left (L) liver lobes showing their prismatic appearance, the isthmus (white arrow) and the position of the gallbladder (star).





Figures 3.3: Dorso-medial view of the blind-ending pouch-like gallbladder (arrow), curving at the body-neck juncture, and a narrowed neck area (arrowhead).