Proposed Standardization of the Two-Dimensional Echocardiographic Examination in Snakes

Lionel Schilliger[1], DVM, Dominique Tessier[2], DVM, MS, Jean-Louis Pouchelon [2], DVM, PhD, Valérie Chetboul[2], DVM, PhD, DECVIM-CA (Cardiology)

1. Clinique Vétérinaire, 26 route de Massy, 91380 Chilly–Mazarin, France

2. Cardiology Unit of Alfort, National Veterinary School of Alfort, av. du Général De Gaulle, 94704 Maisons-Alfort cedex, France

Abstract: The ultrasound examination is a diagnostic tool of choice for *ante mortem* evaluation of heart diseases. Specific anatomical features of the ophidian heart such as mobility in the coelomic cavity, a single ventricular cavity, a tubular sinus venosus opening into the right atrium, and the presence of three arterial trunks, have direct consequences on the echocardiographic examination. We propose a standardization of the two-dimensional echocardiography in snakes including materials, methods, and transducer positions, approaches and cross-sections based on the approach used for humans or other companion carnivorous mammals. This should improve the veterinarian's knowledge of ophidian heart diseases and thus contribute to the development of cardiology in reptiles.

Key Words: exotic pets, reptiles, snakes, ophidians, echocardiography.

Introduction

Compared to mammals and other reptiles, the ophidian heart exhibits unique anatomical and physiological features and can be subject to various lesions such as endocarditis, myocarditis, infarct, pericarditis, cardiomyopathy, parasitic infestation, and tumors (Barten and Frye, 1981, Frye, 1991a, Frye, 1991b, Jacobson, *et al*, 1991, Hruban, *et al*, 1992 , Frye, 1994b , Rishniw and Carmel, 1999, Schilliger, *et al*, 2003). Few reports of cardiovascular diseases in reptiles exist and only one details the exact correlation between the anatomy of the heart and two-dimensional ultrasound cross-sections in a snake species (Snyder, *et al*, 1999, Sklandsky, *et al*, 2001). In most cases, the diagnosis if cardiac disease is obtained *post mortem*.

Because of its non-invasive and easily reproducible aspect, the ultrasound examination is a choice option for establishing an *ante mortem* diagnosis of heart disease in human and animal medicine. However, cardiac ultrasonography is rarely used in snakes. The first step in the correct interpretation of the echocardiogram is knowledge of the specific features of the ophidian heart and standardization of two-dimensional ultrasound cross-sections.

I. Distinctive anatomic characteristics

In ophidians, the heart is elongated, especially in colubrid snakes, and matches the general appearance of the body (White, 1968, Frye, 1991a, Funk, 1996, Murray, 1996, Williams, 1996, Farrell, *et al*, 1998, Snyder, *et al*, 1999). It is completely covered by a white fibrous pericardial sac (Farrell, *et al*, 1998) (Figure 1). The location of the heart varies, depending on the species and their behavior. It is positioned caudal to the head about 15 to 25% of the total length of the body in terrestrial species (15% in arborial species and 25% in non-tree-dwellers) and located near the middle of the body in marine and freshwater species (White, 1968, Funk, 1996, Murray, 1996, Farrell, *et al*, 1998). The snake's heart is mobile within the coelomic cavity, because of the absence of a diaphragm. This mobility probably facilitates the movement of large whole prey within the esophagus (Farrell, *et al*, 1998). The heart is caudal to the thyroid, at the cranial pole of the lung(s), and slightly in front of the liver facing the most caudal tracheal rings (Funk, 1996). Unlike other reptiles, in which the heart apex is attached to the pericardium by a ligament termed the *gubernaculum cordis*, the extremity of a snake's ventricle remains free within the pericardial space, offset slightly towards the left, thereby positioning the path of the caudal vena cava not far from the median plane (Farrell, *et al*, 1998).

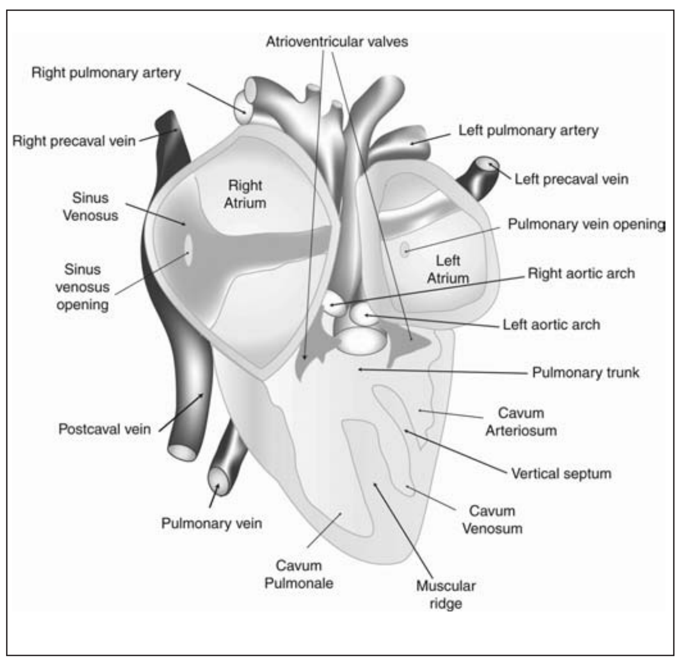

Figure 1: Schematic illustration of the intracardiac structures, the afferent veins and and the efferent arterial trunks of the ophidian's heart.

Seen from the outside, the heart is composed of:
four main cavities: a single ventricle (looks like an inverted cone, often taller than it is wide, with rounded edges), two atrial chambers which can be seen on a ventral view (the right atrium being markedly more developed than the left one, sometimes twice as much) (Figure 2) and a *Sinus Venosus*, which is of tubular shape, only visible from a dorsal approach to the heart, and rests on the dorsal aspect of the right atrium, at the confluence of the three venae cavae (Figure 3): the right precaval cava, the left precaval vein and the postcaval vein (Murray, 1996, Snyder, *et al*, 1999, Chetboul, *et al*, 2004, Schilliger, *et al*, 2005).
three arterial trunks, proceeding from the ventricle, rotating towards the right, and describing an angle of 180º: the left aortic arch, the right aortic arch and the pulmonary trunk (Frye, 1991a, Funk, 1996, Murray, 1996, Williams, 1996, Farrell, *et al*, 1998, Rishniw and Carmel, 1999, Snyder, *et al*, 1999) (Figure 4). The two aortic arches merge together caudally to form the common abdominal aorta. The pulmonary trunk divides up into the left and right pulmonary arteries in ophidians with two lungs, such as most of boid snakes (Funk, 1996, Murray, 1996). In other snakes, such as colubrids, viperids and elapids, the pulmonary trunk develops into one anterior pulmonary artery pointing forwards and a posterior pulmonary artery pointing backwards (Farrell, *et al*, 1998),
four afferent veins: the pulmonary vein, which, in double-lunged snakes, proceeds from the point of confluence between the right pulmonary vein and the left pulmonary vein (Farrell, *et al*, 1998), the two precaval veins and the post-caval vein, draining into the *Sinus Venosus*.

Seen from the inside, the heart is composed of (Figure 1):
three other cavities, recessed within the ventricular cavity and communicating with each other (Figure 5): the *Cavum Venosum* (right dorsal chamber of the ventricle), the *Cavum Arteriosum* (left dorsal chamber of the ventricle) and the *Cavum Pulmonale,* (left chamber or "ventral ventricle") (Murray, 1996, Farrell, *et al*, 1998, Snyder, *et al*, 1999).
several openings and valves: the pulmonary vein drains into the dorsal wall of the left atrium through the pulmonary vein orifice, located close to the interatrial septum. The sinus venosus proceeds into the right atrium at the sinoatrial opening. This orifice has a pair of flap valves derived from the endocardium that are membranous or muscular, depending upon the species, and known as sinoatrial valves (Farrell, *et al*, 1998). The atrial chambers communicate with the single ventricle through the left and right atrioventricular orifices. These orifices are all equipped with a single atrioventricular valve, known as the single septal monocuspid atrioventricular valve (Frye, 1991a, Funk, 1996, Farrell, *et al*, 1998). The left and right aortic arches arise from the *Cavum Venosum* at two orifices with bicuspid valves, rather than tricuspid valves as in mammals. The pulmonary trunk is a continuation of the *Cavum Pulmonale* and its base also contains small semilunar valves (Farrell, *et al*, 1998).
two septa: one muscular ridge, called the vertical septum, is located in the ventricular cavity, between the *Cavum Arteriosum* and the *Cavum Venosum*. Because of its position facing the interatrial septum, it contributes to the separation of pulmonary vein flow from systemic flow during ventricular diastole. A second relatively strong muscular ridge, called the horizontal septum, originates between the pulmonary trunk and the left aortic arch, and twists towards the dorso-apical region of the ventricle. This muscular ridge marks a

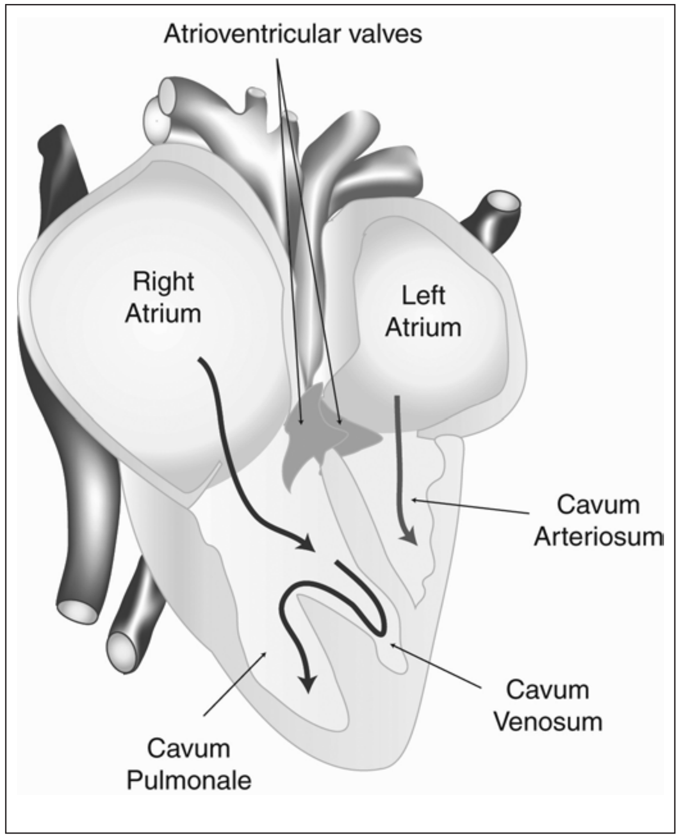

Figure 2a: Intracardiac blood flow during the atrial systole.

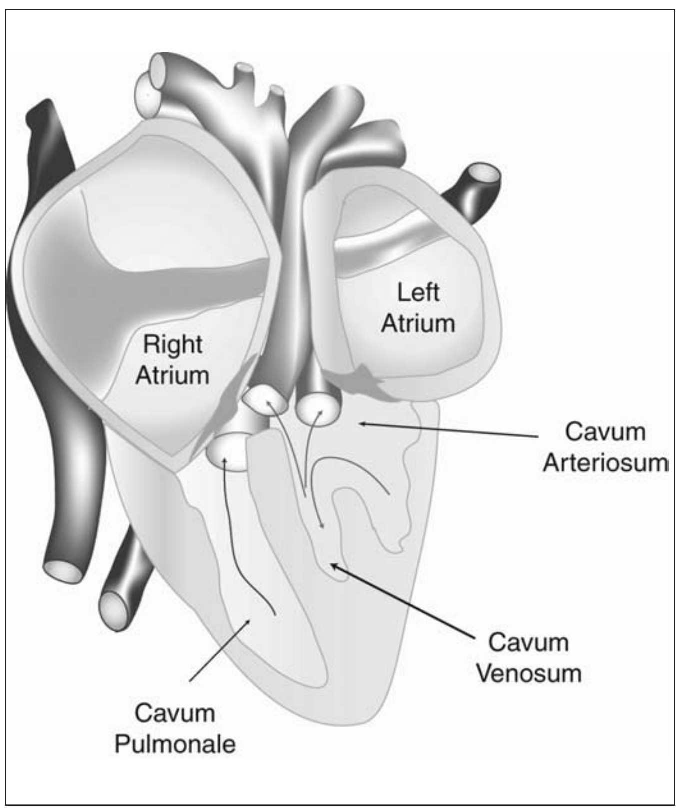

Figure 2b: Intracardiac blood flow during the ventricular systole.

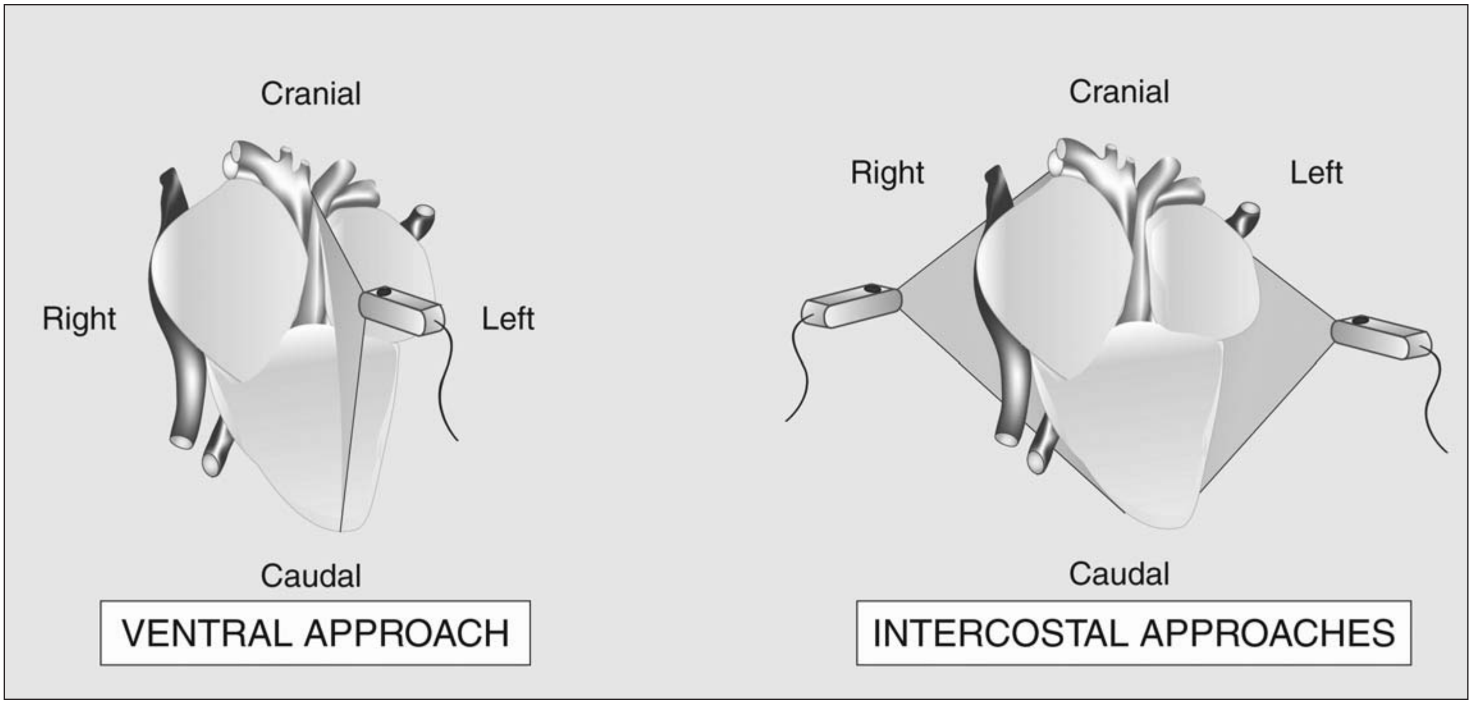

Figure 3. The 3 ultrasound windows for an echocardiographic examination in ophidians: ventral, right and left intercostal approaches.

separation between the *Cavum Venosum* and the *Cavum Pulmonale* (Farrell, *et al*, 1998).

During atrial systole, both atrioventricular valves open, the oxygenated blood in the left atrium flows into the *Cavum Arteriosum* and the deoxygenated blood in the right atrium flows into the *Cavum Pulmonale* via the *Cavum Venosum* (Murray, 1996, Williams, 1996, Farrell, *et al*, 1998, Rishniw and Carmel, 1999, Snyder, *et al*, 1999, Girling and Hynes, 2004) (Figure 2a).

During ventricular systole, both atrioventricular valves close, oxygenated blood is then pumped out of the *Cavum Arteriosum* towards the *Cavum Venosum* and then on to the left and right aortic arches. As with the carbonated blood, it is pumped out from the *Cavum Pulmonale* towards the pulmonary arterial trunk (Murray, 1996, Williams, 1996, Farrell, *et al*, 1998, Rishniw and Carmel, 1999, Snyder, *et al*, 1999, Girling and Hynes, 2004) (Figure 2b). Blood that flows along the two aortic arches is mixed, because the *Cavum Venosum* contains both oxygenated and unoxygenated blood. However, this mixture is incomplete, because the contraction in the ventricle, of the vertical septum during diastole, and of the horizontal septum during systole, ensures almost complete separation of the pulmonary and systemic circulation (White, 1968, Frye, 1991a, Murray, 1996, Williams, 1996, Farrell, *et al*, 1998, Hicks, 1998).

II. Proposed standardization of the two-dimensional echocardiographic examination in snakes.

II.1. Materials and Methods.

Altogether, nine adult captive born boid snakes (three red-tailed boas – *Boa constrictor imperator* – and six Burmese pythons – *Python molurus bivittatus*) were scanned, using an ultrasound unit equipped with a 5.0 – to 7.5-MHz phased-array transducer (Vingmed Vivid 5, General Electric Medical System, Waukesha, WI). Most of these snakes were referred or presented by different breeders for respiratory disorders. The purpose of this study was to define a clear methodology for the two-dimensional echocardiographic examination in snakes, based on a detailed anatomic description already published on this topic (Snyder, *et al*, 1999).

The patients were generally not anesthetized, placed on their back (Figure 6) and preferably held in position by two assistants. When sedation was necessary for examination of aggressive or agitated animals, an intramuscular injection of tiletamine-zolazepam (ZOLETIL®, TELAZOL®) at the dosage of 15 – 30 mg/kg (Stein, 1996, Malley, 1997, Bennet, *et al*, 1999, Snyder, *et al*, 1999) produced sufficient muscular relaxation to diminish, if not completely abolish, the righting reflex. At this time, the cardiovascular effects of the tiletamine-zolazepam are unknown in reptiles. In mammals, the dissociative anesthetics (tiletamine, ketamine) are renowned for their chronotropic and inotropic effects on the myocardium (Kohn, *et al*, 1997) and their cardiovascular effects resemble sympathetic nervous stimulation (increased systemic and pulmonary arterial blood pressure, heart rate, cardiac output, cardiac work and myocardial oxygen consumption). Zolazepam doesn't have any depressive effect on the mammals cardiac function. In our study, the anesthetized pythons showed an expected heart rate within their Preferred Optimal temperature Zone (P.O.T.Z), as predicted by the formula of the heartbeat frequency (hbf) in reptiles: hbf = 33,4 $(W_{kg})^{-0,25}$ (Murray, 2006).

The position of the heart was indicated by visualization of the ventral precordial tap (Snyder, *et al*, 1999). As the snake's heart is mobile inside the coelomic cavity, we had to move the transducer by a few centimeters, either cranially or caudally in relation to the initial position during the ultrasound examination. The patients have not been immersed in a shallow water bath, as recommended in some reports

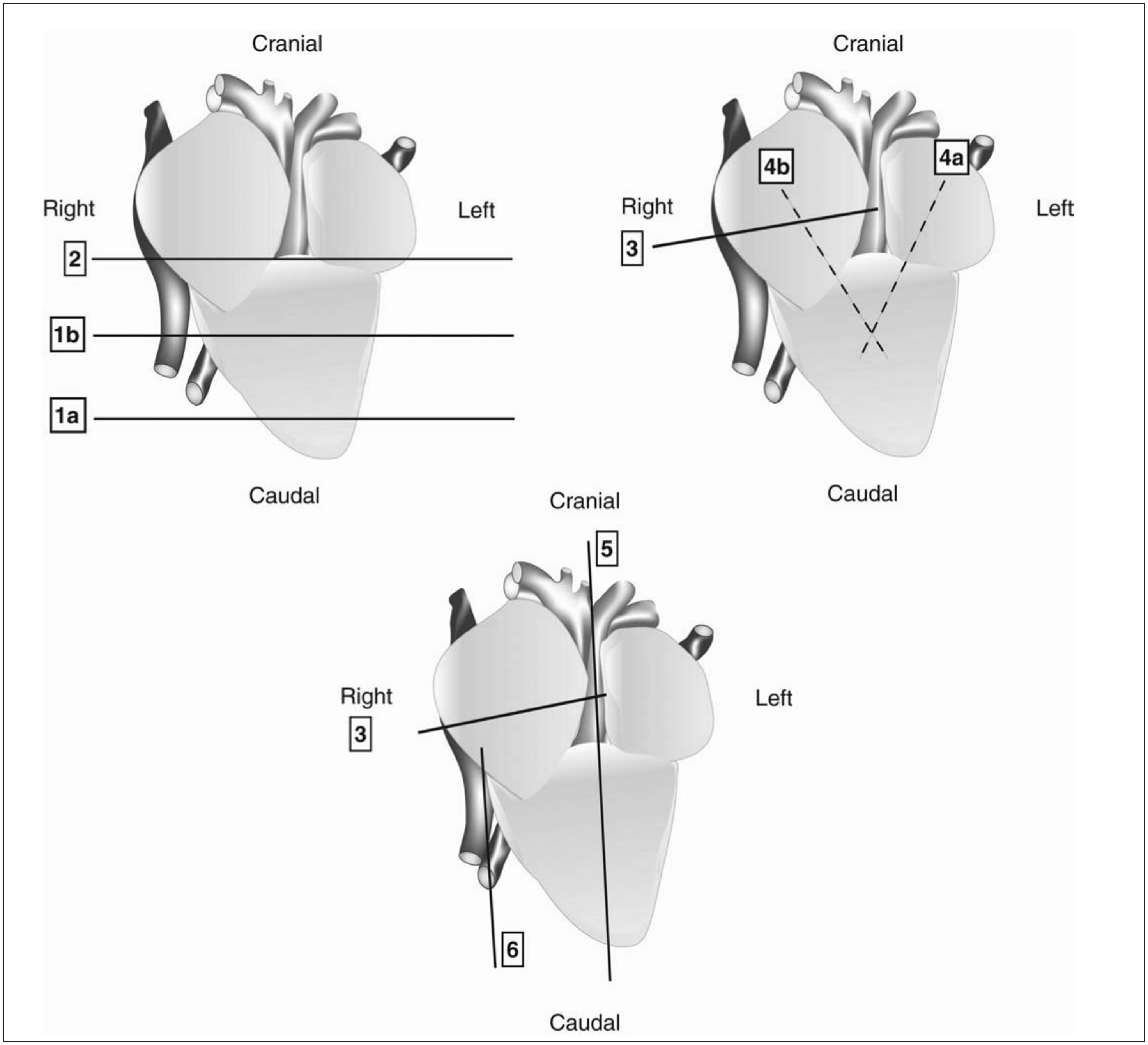

Figure 4: The 6 cross-sections obtained using a ventral approach: short-axis sections (N°1 to 3) and long-axis sections (N°4 to 6).

(Hochleitner and Hochleitner, 2004, Schildger, *et al*, 1994, Schildger, *et al*, 1996, Snyder, *et al*, 1999), but a thick layer of acoustic coupling gel was applied ventrally to ensure perfect cohesion between the probe and the snake's scales (Isaza, *et al*, 1993, Schildger, *et al*, 1994, Schildger, *et al*, 1996, Chetboul, *et al*, 2004, Hochleitner and Hochleitner, 2004, Schilliger, *et al*, 2005). With this method, we obtained consistently good quality echocardiograms.

II.2. Approaches.

Three approaches to define the ultrasound windows were used in succession (Figure 3). Most of the ultrasound examination was carried out by placing the probe ventral to the heart. This ventral approach makes it possible to view the organ from the caudal ventricular apex to the cranial atria, and examine the *sinus venosus*, the atrioventricular junctions and the three arterial trunks. Two other approaches, known as right and left intercostal approaches, were obtained by laterally positioning the probe. These approaches were used to complete the ventral cranial examination and obtain a lateral visualization of the three arterial trunks and both atria that proceed into the single ventricle.

II.3. Designation of imaging planes.

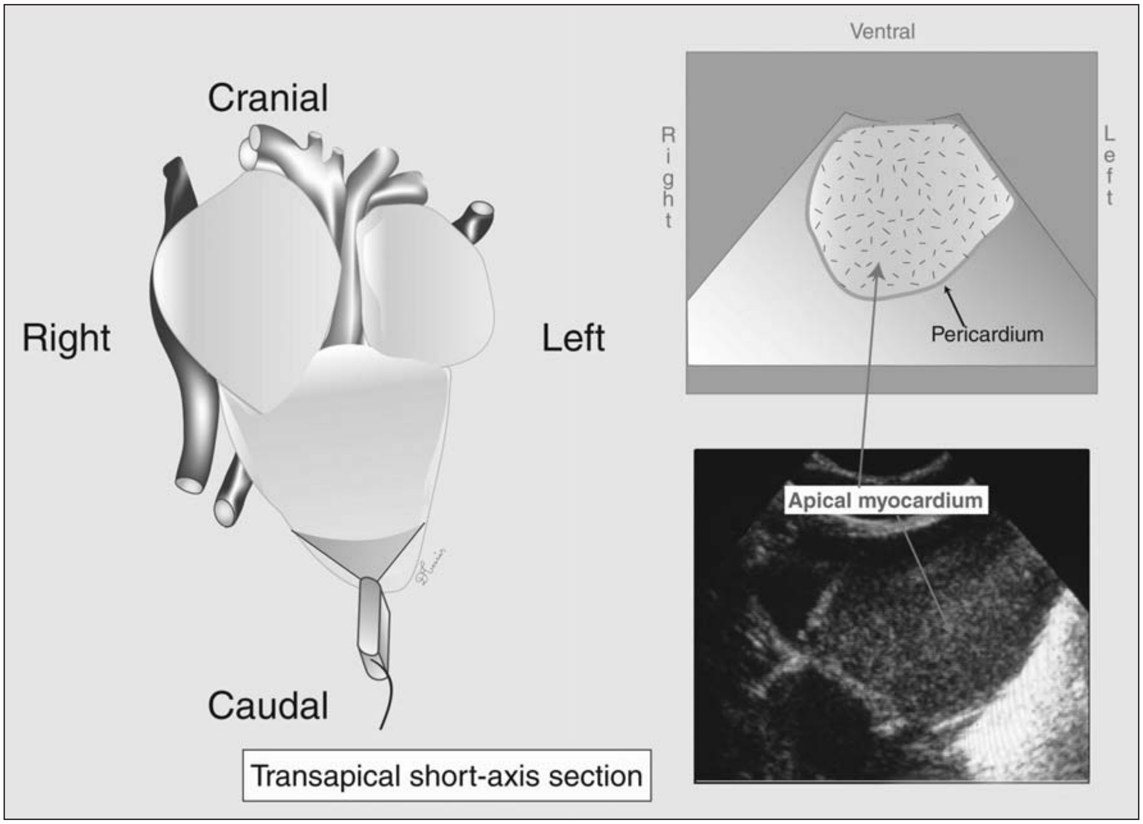

Figure 5. Transventricular apical (or transapical) short-axis section (N°1a). This apical short-axis view shows a transversal section of the apical myocardium.

In both human and animal medicine, "long-axis" (or longitudinal) planes are obtained by taking a cross-section parallel to the long axis of the heart, from the apex to the base, in other words parallel to the body of the animal (Thomas, *et al*, 1993). In contrast, "short-axis" planes are obtained by taking sections perpendicular to the long axis of the heart, that is to say perpendicular to the body of the animal (Figure 4).

II.3.1. Two-dimensional sections obtained by ventral approach (sections N°1 to 6-Figure 4).

Short-axis views (sections N°1 to 3-Figure 4)
By placing the probe ventral to the heart, the operator can scan or "sweep" the organ from the apex to the arterial trunks along its short axis (short-axis views). Thus two transventricular sections (sections 1a, 1b-Figure 4) can be obtained by ventral approach.

On moving the probe caudal to cranial, from the apex towards the base:

the **apical or transapical short-axis section** (section 1a-Figure 4) shows a transverse section of the apical myocardium and the pericardium from behind, in the form of an echogenic line (Figure 5).

the **transventricular subarterial short-axis section** (section 1b-Figure 4) shows a transverse section of the three *Cava* surrounded by the peripheral myocardium (the ventral *Cavum Pulmonale* the right dorsal *Cavum Venosum*, the left dorsal *Cavum Arteriosum*) (Figure 6). The muscular ridge, known as the vertical septum, located in the ventricular cavity between the *Cavum Arteriosum* and the *Cavum Venosum* can be partially visualized, as can the horizontal septum marking the separation between the *Cavum Venosum* and the *Cavum Pulmonale*.

By continuing to move the probe cranially, a **transarterial short-axis section** (section 2-Figure 4) is obtained giving a transverse section visualization of the three large arterial trunks: the two aortic arches of equal diameter and the pulmonary trunk of larger diameter ("Mickey-Mouse-head" form of section) (Figure 7).

By leaving the probe in a ventral position, but by moving it slightly towards the right the **right transatrial short-axis section** (section 3-Figure 4) shows the opening of the sinus venous into the right atrium and enables the aspect of both sinoatrial valves to be assessed (Figure 8).

Long-axis views (sections Nº 4 to 6-Figure 4).

Long-axis views are obtained by turning the probe 90º in relation to the previous projections, referred to as short-axis ones.

Thus, starting from the transventricular or subarterial section, the rotation of the probe enables two long-axis sections of the heart to be obtained. These are called **atrioventricular sections** (sections 4a and 4b-Figure 4) and they show both atrial cavities opening into the single ventricle. The left atrioventricular junction can be observed (section 4a-Figure 4) by orienting the ultrasound plane ventro-dorsally from the right to the left (Figure 9). Similarly, the right atrioventricular junction can be observed (section 4b-Figure 4) by orienting the ultrasound plane ventro-dorsally from the left to the right (Figure 10). The atrioventricular valves, also known as septal monocuspid valves, can thus be observed.

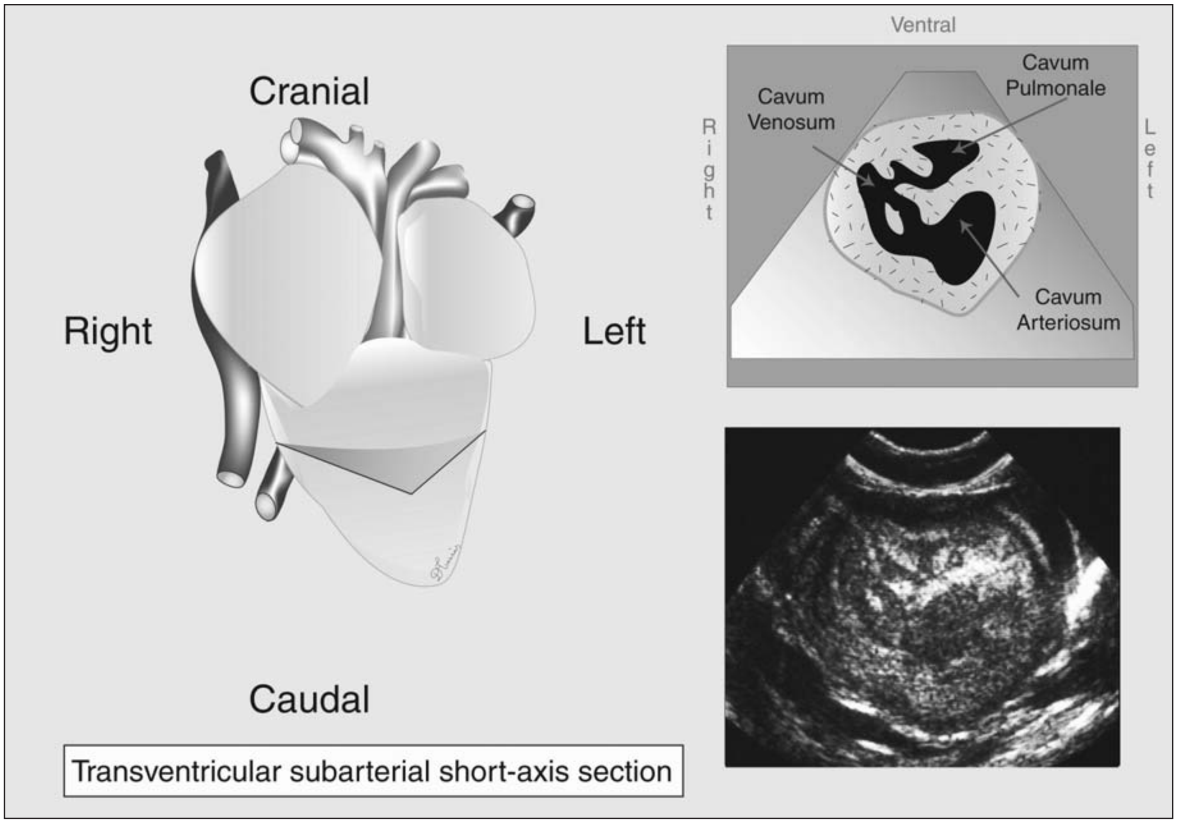

Figure 6. Transventricular subarterial short-axis section (N°1b) showing the 3 cava (*Cavum Pulmonale, Cavum Venosum, Cavum Arteriosum*).

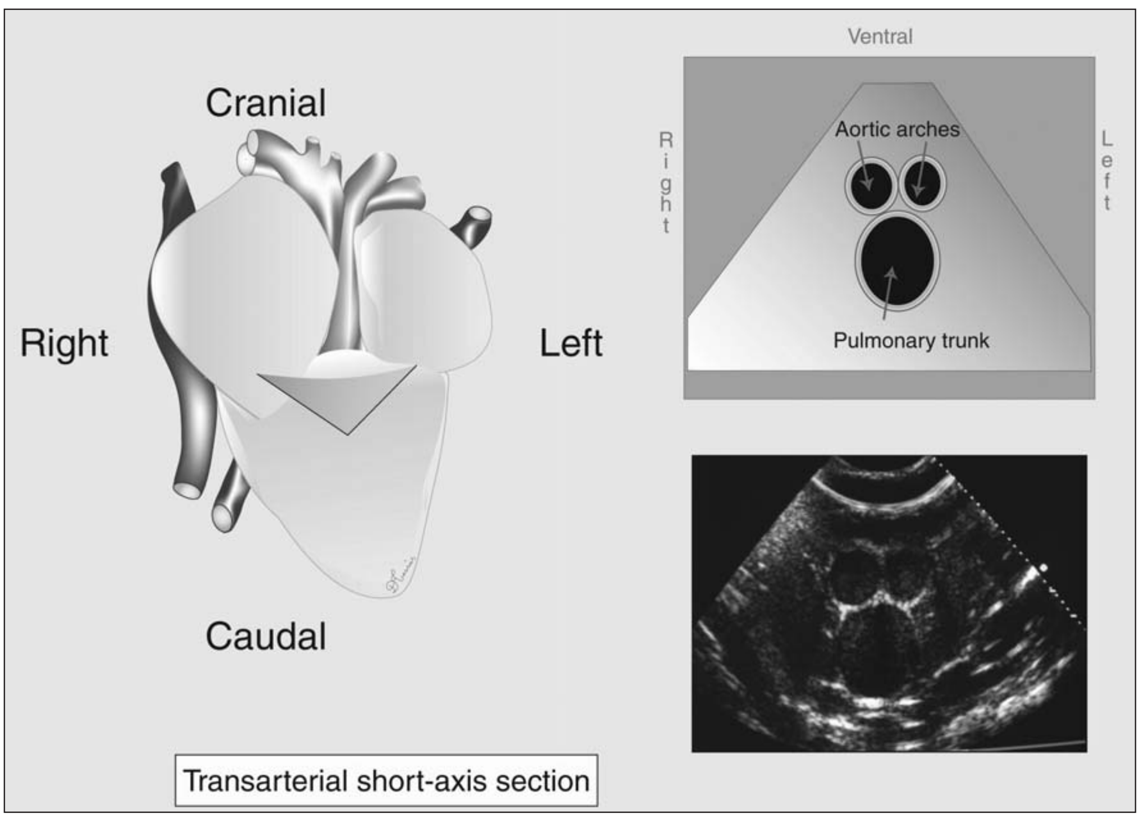

Figure 7. Transarterial short-axis section (N°2). This short-axis view shows the two aortic arches and the pulmonary trunk.

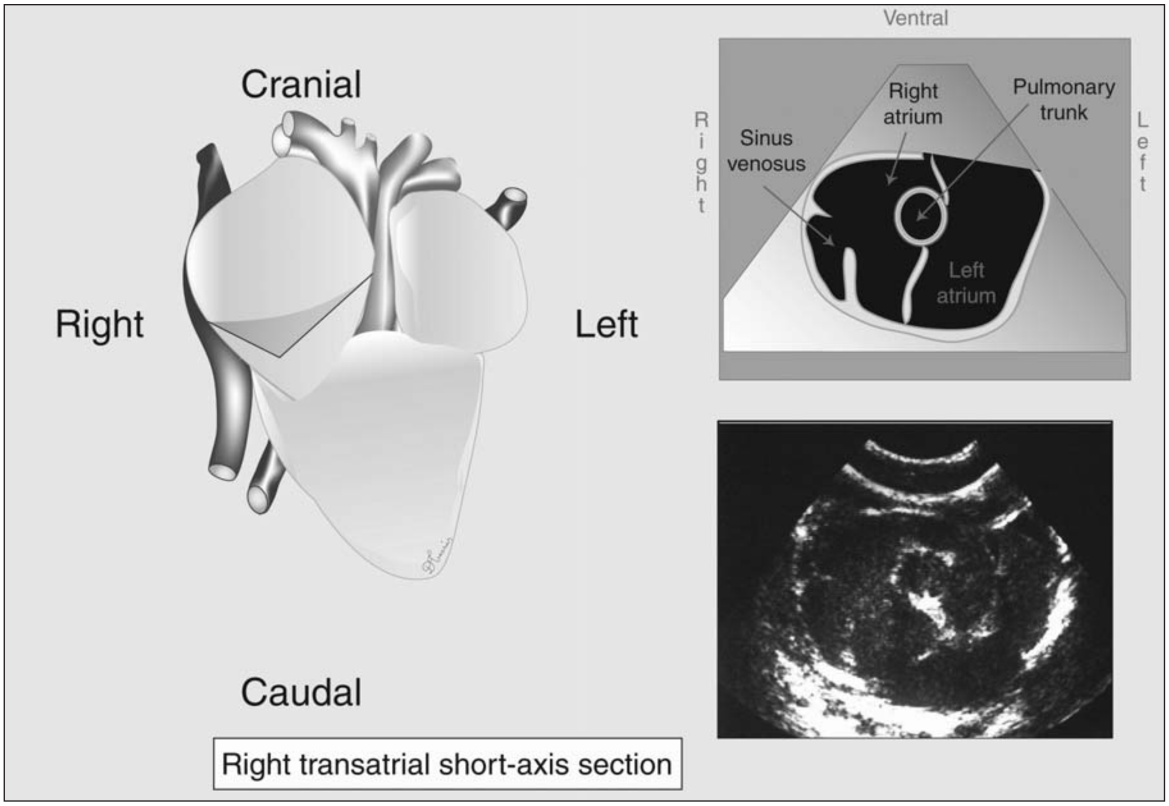

Figure 8. Transatrial short-axis section (N°3). This short-axis view shows the right atrium, the sinoatrial valves and the sinus venous on the right and the left atrium on the left.

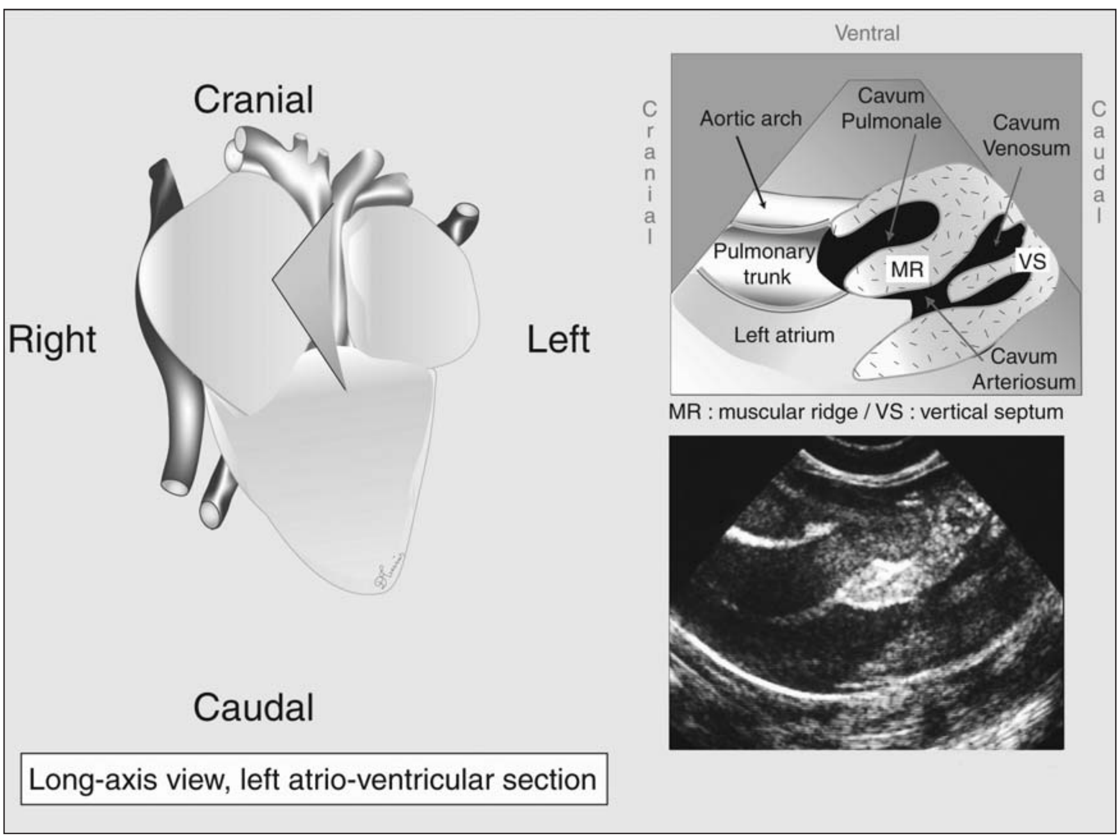

Figure 9. Long-axis section (N°4a) of the left atrioventricular junction. One of the two aortic arches and the pulmonary trunk are observed.

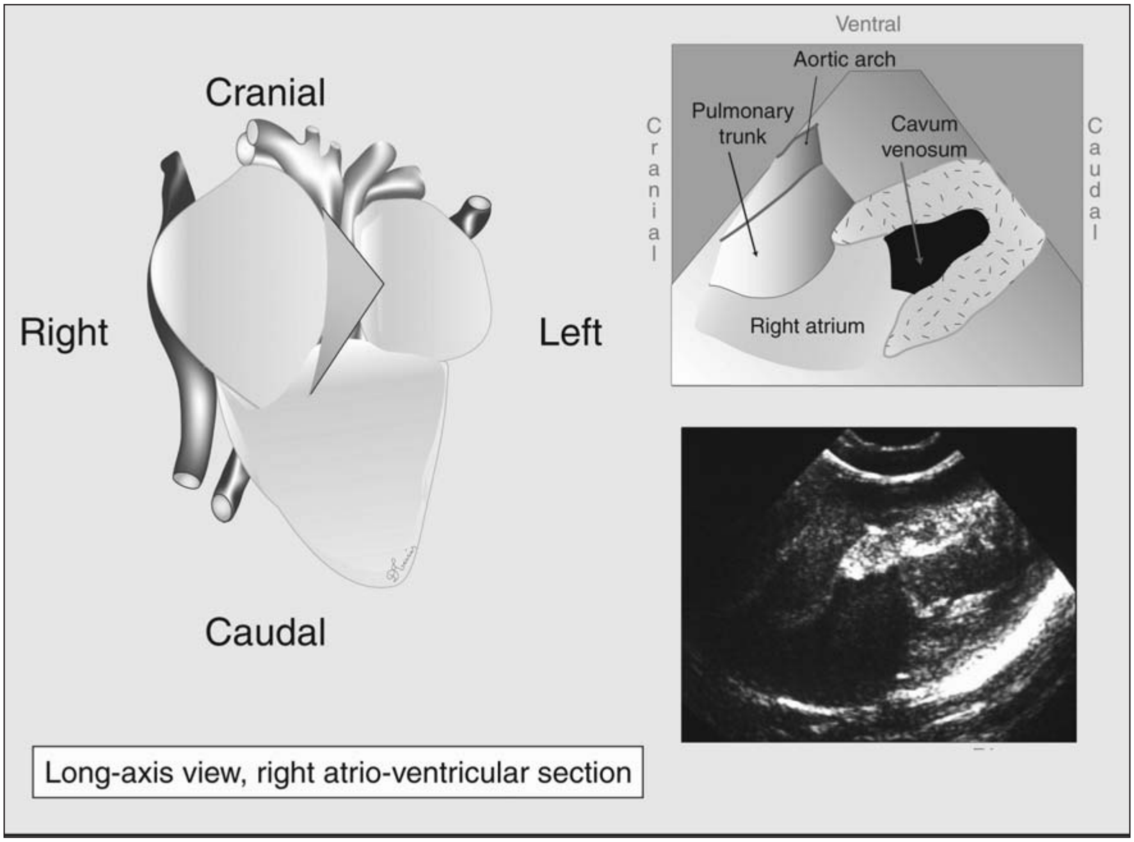

Figure 10. Long-axis section (N°4b) of the right atrioventricular junction.

Similarly, starting from the short-axis transarterial section (section 2-Figure 4), the probe is rotated 90° to obtain a **long-axis transarterial section** (section 5-Figure 4) which reveals the right aortic arch and the pulmonary trunk with parallel paths (Figure 11). The path of the pulmonary artery can be located by moving the probe caudally. The path of this artery comes closer to the probe ventrally and then opens out on the right with the cavum pulmonale (Figure 12).

Lastly, starting from the right transatrial short-axis section (section 3-Figure 4), the probe is rotated 90°, and moved caudally, to display the path of the caudal vena cava parallel to the pulmonary vein and a **long-axis transcaval section** (section 5-Figure 4), starting from the sinus venosus can be obtained (Figure 13).

II.3.2. Two-dimensional sections obtained by intercostal approach.

When imaging small snakes, the examination may be completed by two intercostal approaches. Thus the transarterial long-axis section, obtained by right intercostal approach, provides clear visualization of the left atrium. The probe is placed laterally on the right so the cross-section is parallel to the animal's body, and the left atrium is removed from the proximal field occupied by the large arterial trunks. Conversely, the left symmetrical intercostal section provides a good approach for observing the right atrium.

Discussion

In our opinion, the ventral approach in ophidians is the main ultrasound display window that provides an almost complete exploration of the heart. The single ventricle and the three cava, the pericardium, the sinus venosus, both atria, arterial efference and venous afference can be observed and analyzed along long-axis (sagittal) or short-axis (transverse) projections. The two intercostal approaches complete the data supplied by the above projections, especially in the examination of atria.

Previous studies mainly attempted to identify the anatomical cardiac structures visualized by echocardiography in snakes (Frye, 1991b, 1994a, Isaza, *et al*, 1993, Schildger, *et al*, 1994, Rishniw and Carmel, 1999, Snyder, *et al*, 1999, Sklandsky, *et al*, 2001). In this paper, our goal is to pursue these studies by standardizing logically and methodically this technique of examination. The adopting of ultrasound standards such as these should help to improve the veterinarian's knowledge of ophidian cardiology, fine-tune the understanding of the common heart diseases encountered in these animals and lastly facilitate the communication of clinical discoveries in snake cardiac studies amongst professionals.

Acknowledgements

Dominique Tessier for the technical quality of the graphics he performed for us.

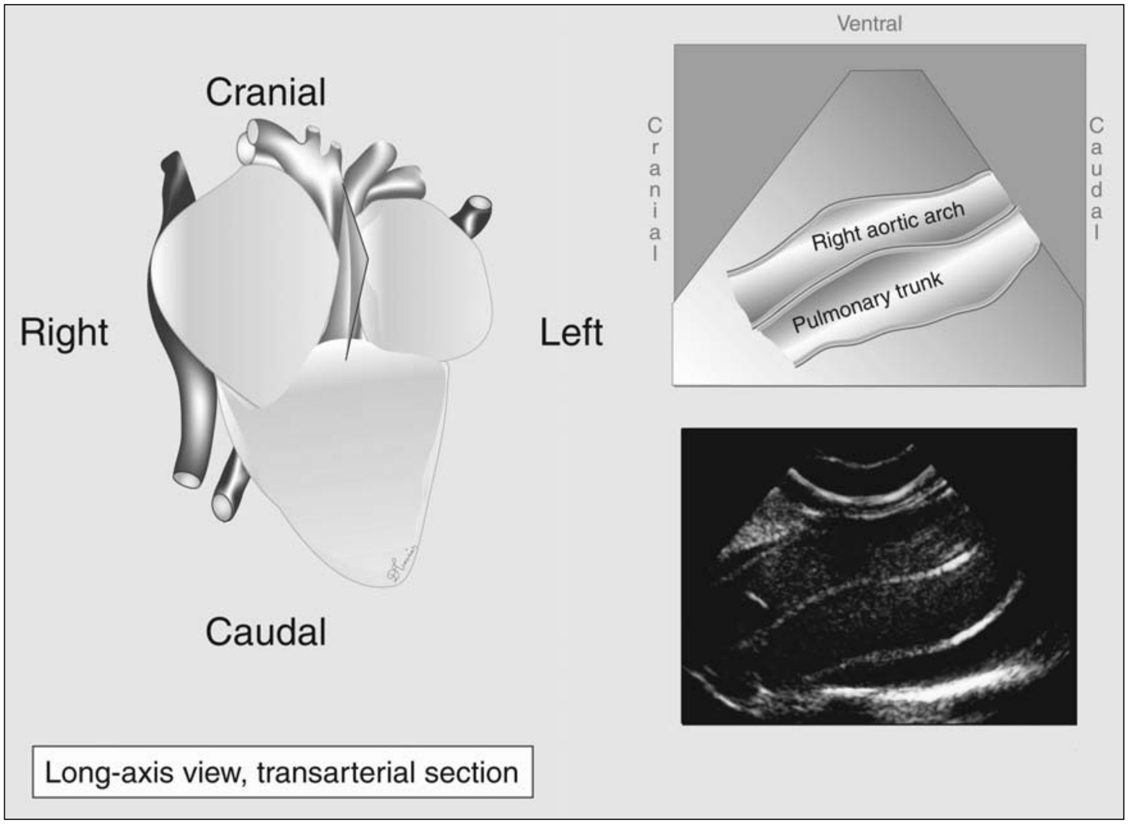

Figure 11. Transarterial long-axis section (N°5). The distal view shows the path of the dorsal pulmonary trunk parallel to the ventral right aortic arch.

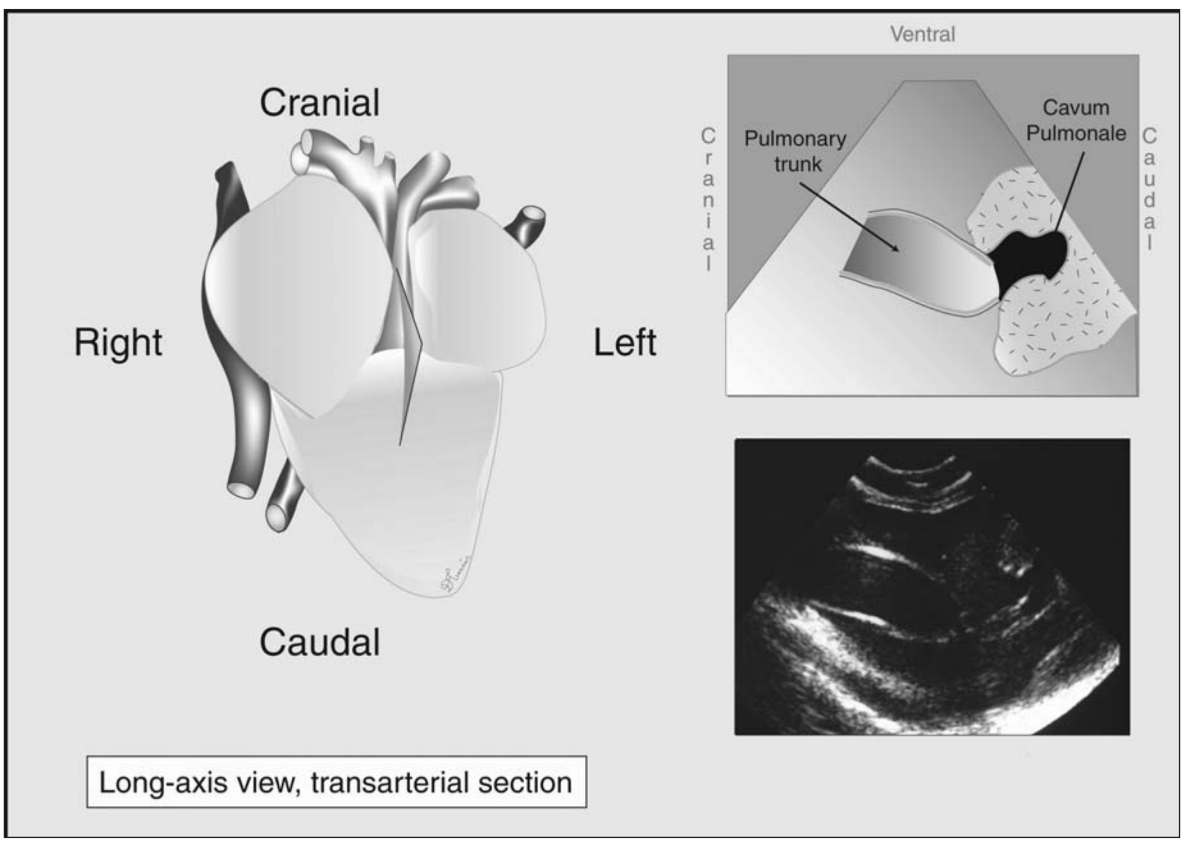

Figure 12. Transarterial long-axis section (N°5). On the proximal view, the junction between the cranial pulmonary trunk and the caudal *Cavum Pulmonale* is observed.

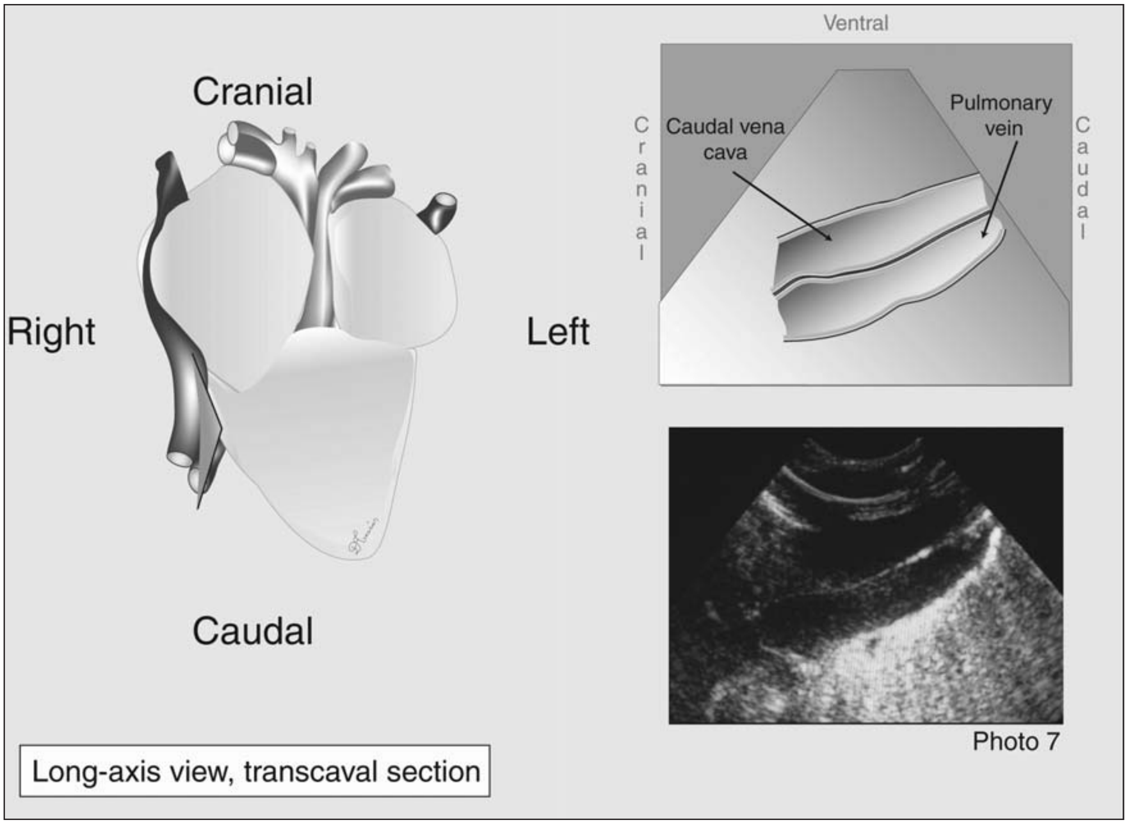

Figure 13: Transcaval long-axis section (N°6). This long-axis lateral view shows the ventral caudal vena cava parallel to the more dorsal pulmonary vein

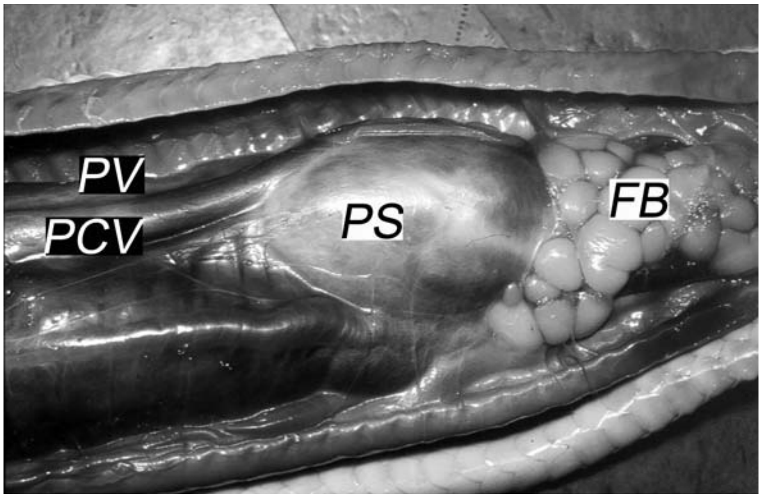

Photo 1. Ventral aspect of the heart in an albino burmese python, *Python molurus bivittatus*, after coeliotomy: note the whitish-looking and of fibrous consistency pericardial sac (PS), the fat bodies (FB) usually located at the base of the heart, and the post caval vein (PCV) lying just in front and close to the pulmonary vein (PV).

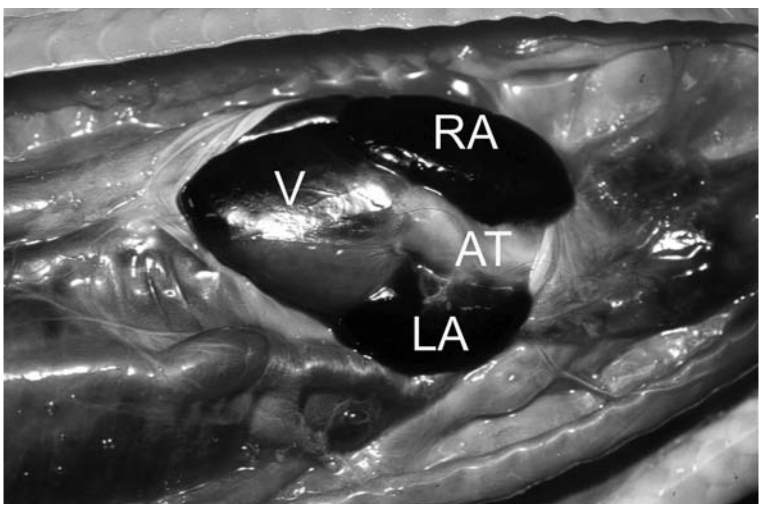

Photo 2. Ventral aspect of the heart in a burmese python, *Python molurus bivittatus*, after resection of the pericardial sac: note the two atria (the right one [RA] being more developed than the left one [LA], separated one from each other by the three arterial trunks [AT], rotating towards the right) and the single ventricle (V).

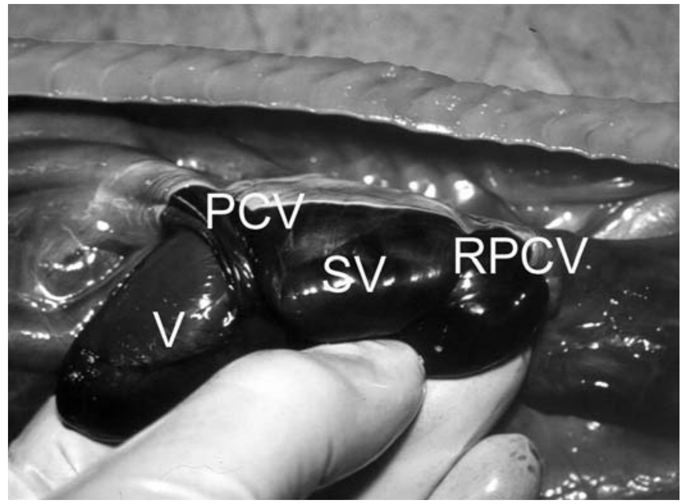

Photo 3. Photograph showing the aspect of the tubular shaped sinus venosus (SV) in a burmese python, *Python molurus bivittatus*, located on the dorsal face of the right atrium, at the confluence of the three venae cavae (RPCV = right precaval vein, PCV = post-caval vein, V = ventricle)

Photo 4. Cross section of the three efferent arterial trunks at the base of the heart in a burmese python, *Python molurus bivittatus*: the left aortic arch (LAoA), the right aortic arch (RAoA) and the pulmonary trunk (PT) (which shows the largest diameter).

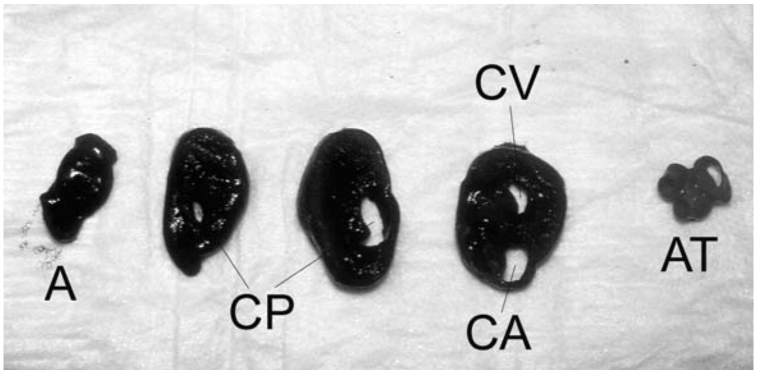

Photo 5. Cross sections of the ventricle of the heart in a burmese python, *Python molurus bivittatus*, showing its three main cavities, from the apex [A] (on the left) to the arterial trunks [AT]of the heart's base (on the right) [CP = *Cavum Pulmonale*, CV = *Cavum Venosum*, CA = *Cavum Arteriosum*].

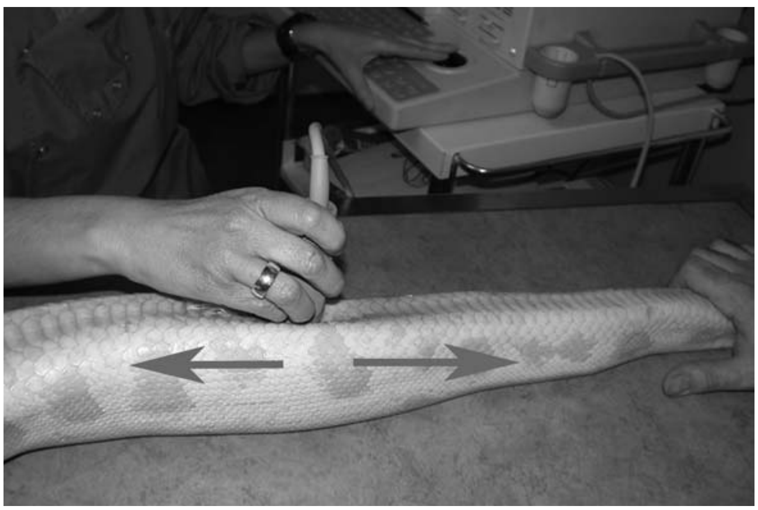

Photo 6. Position of a snake for an echocardiographic examination.

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