HORSESHOE CRAB NECROPSY GUIDE

A Guide to the Examination and Necropsy of the Horseshoe Crab Using *Limulus polyphemus* as Limulidae Prototype

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ACKNOWLEDGMENTS

This free-access guide was made possible by the Wildlife Disease Association Small Grants Committee award to the National Aquarium in 2018.

Thank you to Roxanna Smolowitz, DVM, Assistant Professor of Biology, and Director of the Aquatic Diagnostic Laboratory at Roger Williams University, for providing useful materials on horseshoe crab anatomy and necropsy guidelines.

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INTRODUCTION

The Atlantic, or American, horseshoe crab (*Limulus polyphemus*) has existed largely unchanged for over 100 million years. Millions of individuals are commonly observed ashore in spring and summer months during spawning events along the entire North American coastline expanding from the East to the Gulf coasts of the United States and Mexico. Other species can be found in the Indian and Pacific Ocean. The massive deposit of eggs in near-shore sand provides a critical source of food for endangered migrating birds, especially the Red Knot (*Calidruis canutus rufa*) in the Delaware Bay. Horseshoe crabs are also an important component of the sea turtle diet. In addition to the ecological importance, horseshoe crabs are used commercially for bait in eel and conch fisheries and for biomedical purposes in the production of Limulus Amebocyte Lysate (LAL) to detect bacterial toxins in injectable drugs and implantable devices. Commercial demands have led to population declines in some regions. Fisheries are regulated by state and the current International Union for Conservation of Nature (IUCN) listing for *L. polyphemus* is vulnerable.

A small number of individuals are housed in public aquaria for educational purposes. With growing interest in animal welfare, the health and stability of populations, and potential stressors that can contribute to decline , it is important to have clear and detailed descriptions of horseshoe crab anatomy and necropsy techniques. The purpose of this guide is to illustrate the normal anatomy and the step-by-step technique for dissection of horseshoe crabs. The contents are largely excerpts of the master's thesis of artist, Katie (Bergdale) Roorda, which was based on photographs from C. Meteyer documenting the sequence and procedure used for necropsy dissection.

GENERAL ANATOMY

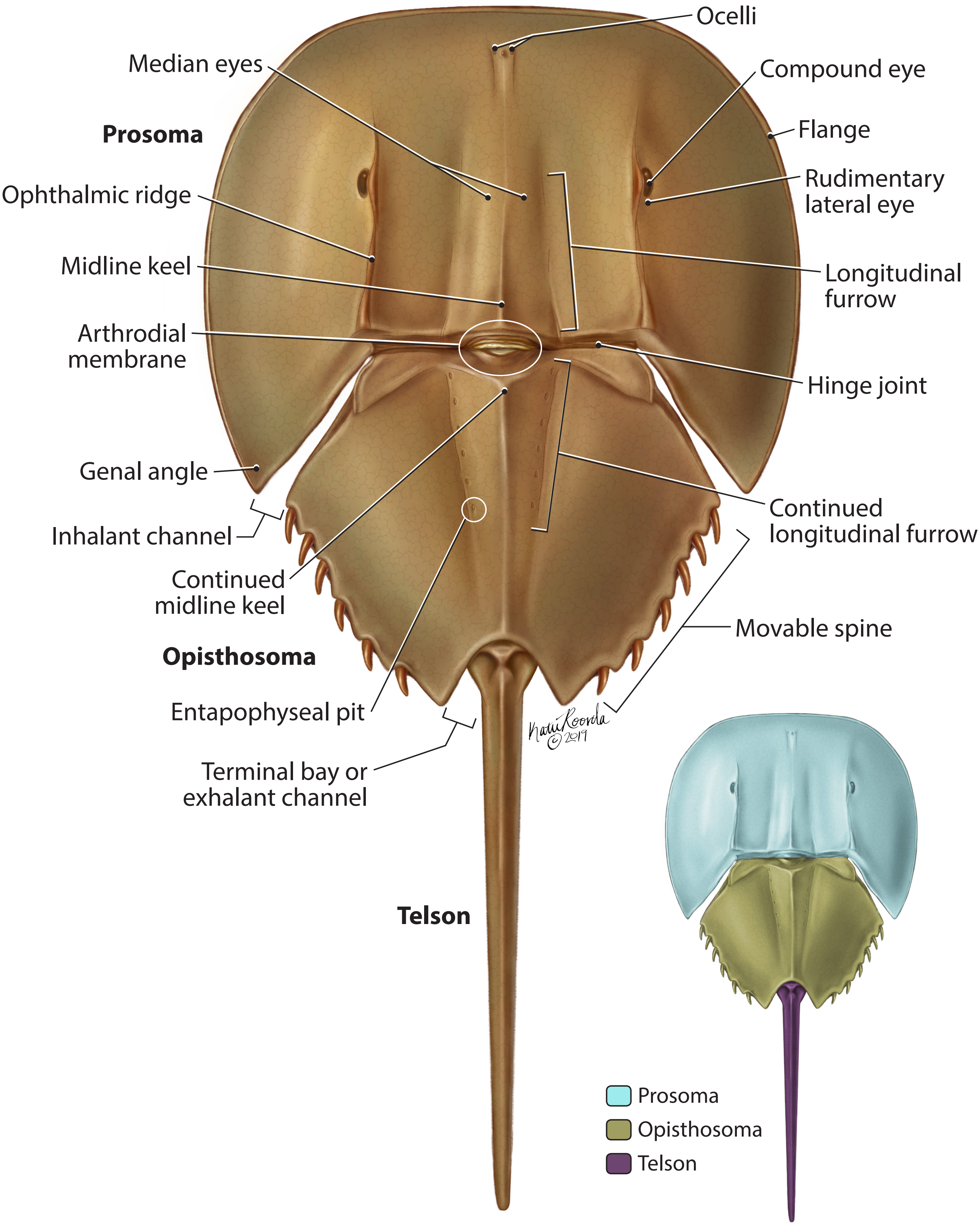
The four extant species of horseshoe crabs are in the Phylum Arthropoda, subphylum Chelicerata, class Merostomata and subclass Xiphosura. This guide uses the Atlantic, or American, horseshoe crab *Limulus polyphemus*, but the concepts may be applied to the Asian species.

EXTERNAL ANATOMY - DORSAL VIEW

Fig. 1A: External Anatomy: Dorsal view
Fig. 1B: Segments of the Body: Dorsal view

The horseshoe crab has a protective continuous cuticular body cover, or carapace, separated into three main sections: prosoma (cephalothorax), opisthosoma (abdomen), and telson (tail). The carapace is bilaterally symmetrical, divided by a midline keel extending from the prosoma, interrupted by the central hinge joint, then continues to the opisthosoma and telson. Two longitudinal furrows are located parallel and lateral to the keel. Within each furrow on the prosoma sections, there are six entapophyseal pits that function as attachment points for the ventral genital operculum and gill opercula. Two compound eyes are just medial to the ophthalmic ridges on the prosoma, and two median eyes are on either side of the keel. The outermost edge of the carapace, the flange, continues around the entire prosoma. The triangular edges of the prosoma are called the genal angles.

The two main body sections are separated by the hinge joint, which has a central arthrodial membrane and lies superficial to the pericardial sac and heart. This is a commonly used phlebotomy site for hemolymph extraction. The lateral edges of the opisthosoma form a movable spine. Each has six individual small extensions that are not controlled voluntarily but are flexible at the point of attachment and aid in protection. There are two channels that aid in respiration. The space between the posterior triangular tip of the opisthosoma and the telson is called the terminal bay or the exhalant channel, while the space between the genal angle of the prosoma and the anterior edge of the opisthosoma is called the inhalant channel.


Ocelli
Median eyes
Compound eye
Flange
Prosoma
Rudimentary lateral eye
Ophthalmic ridge
Midline keel
Longitudinal furrow
Arthrodial membrane
Hinge joint
Genal angle
Continued longitudinal furrow
Inhalant channel
Continued midline keel
Movable spine
Opisthosoma
Entapophyseal pit
Terminal bay or exhalant channel
Kani Roorda © 2019
Telson
Prosoma
Opisthosoma
Telson

EXTERNAL ANATOMY - VENTRAL VIEW

Fig. 2A: External Anatomy: Ventral view
Fig. 2B: Segments of the Body: Ventral view

The anterior most section of the prosoma is a flat, triangular region called the subfrontal area and the outer ridge lying anterior is known as the doublure. Extending laterally, the doublure forms an indented area known as the exuviation suture. Posterior to the subfrontal area, just anterior to the first set of legs, is the ventral sense organ.

The horseshoe crab has 14 pairs of specialized appendages. The anterior six are segmented legs residing in the vault of the prosoma, while the posterior eight comprise the opisthosomal appendages: genital operculum, gill opercula, and the telson.

PROSOMA APPENDAGES

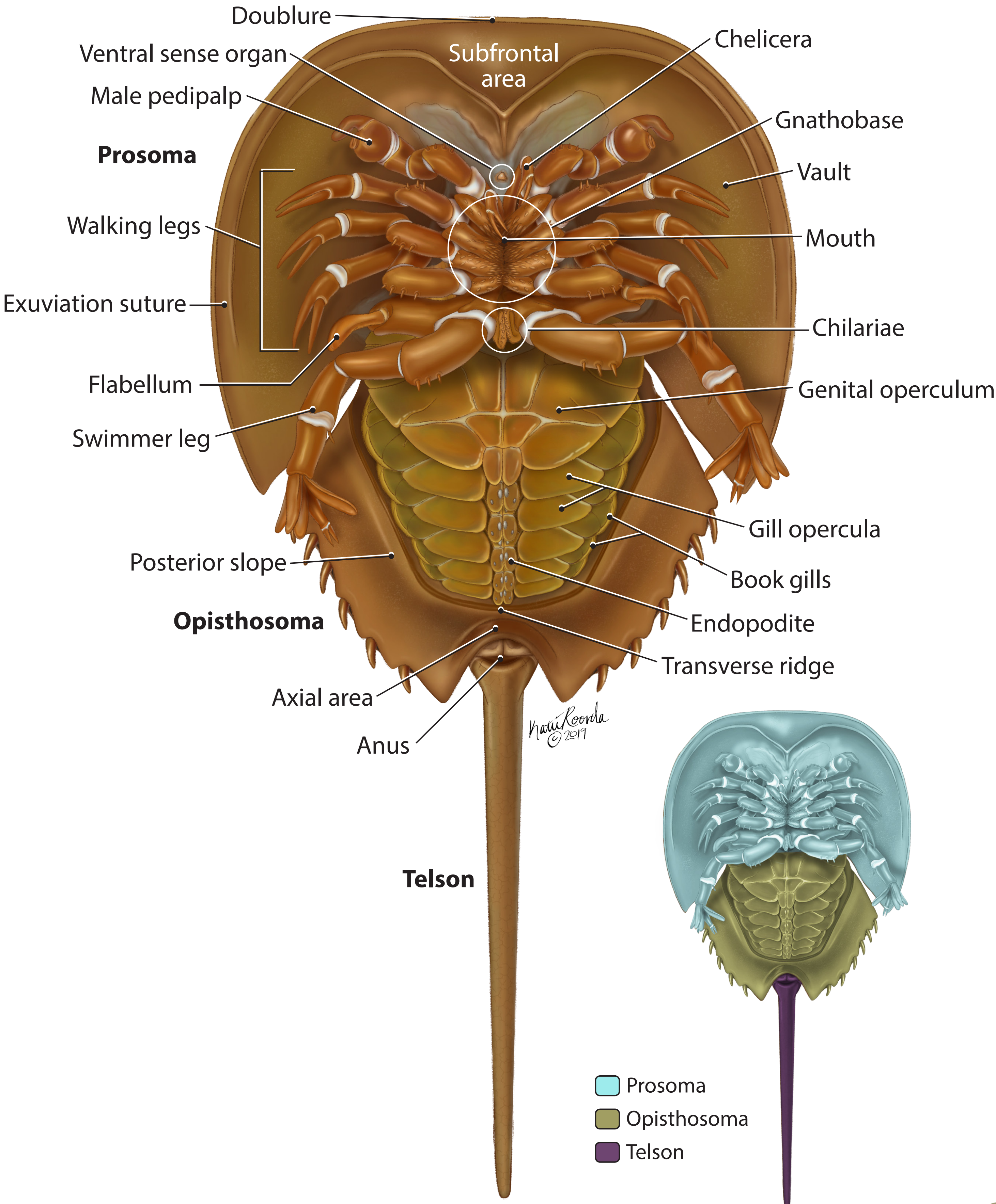
- **First Pair** (Fig. 2A): Chelicerae, or small pinchers specialized for guiding food to the mouth.
- **Second Pair** (Fig. 3, A): Pedipalps, anatomically different for males (with large bulbous claw) and females (same as the walking or ambulatory legs).
- **Third – Fifth Pairs** (Fig. 3, B): Walking or ambulatory legs.; The coxa leg segments of the second through fifth have regions that together form the gnathobase used for grinding and passing food to the centrally located mouth.
- **Sixth Pair (Fig. 3, C):** Swimmer legs are used to propel water to swim forward and between the pair are the chilariae.

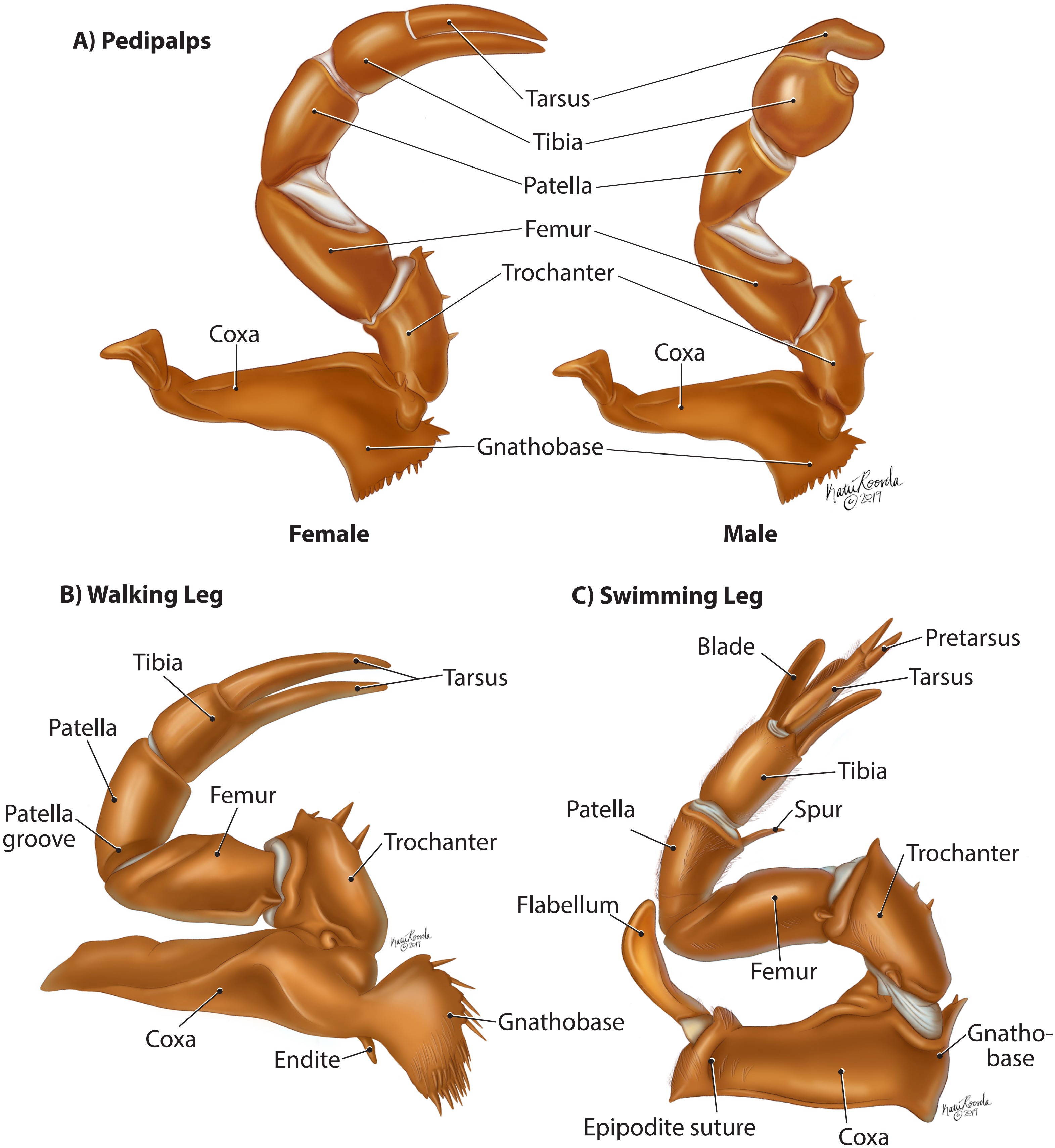
The flabellum, or epipodite, articulates with the coxa leg segment. The function of the flabellum is still unclear. It is suggested that it may aid in determining low oxygen conditions by aiding passing water into the book gills through the inhalant water channel to test the water composition (Ecological Research & Development Group, 2002; Fox, 2007; Shuster, et. al., 2003)

OPISTHOSOMA APPENDAGES

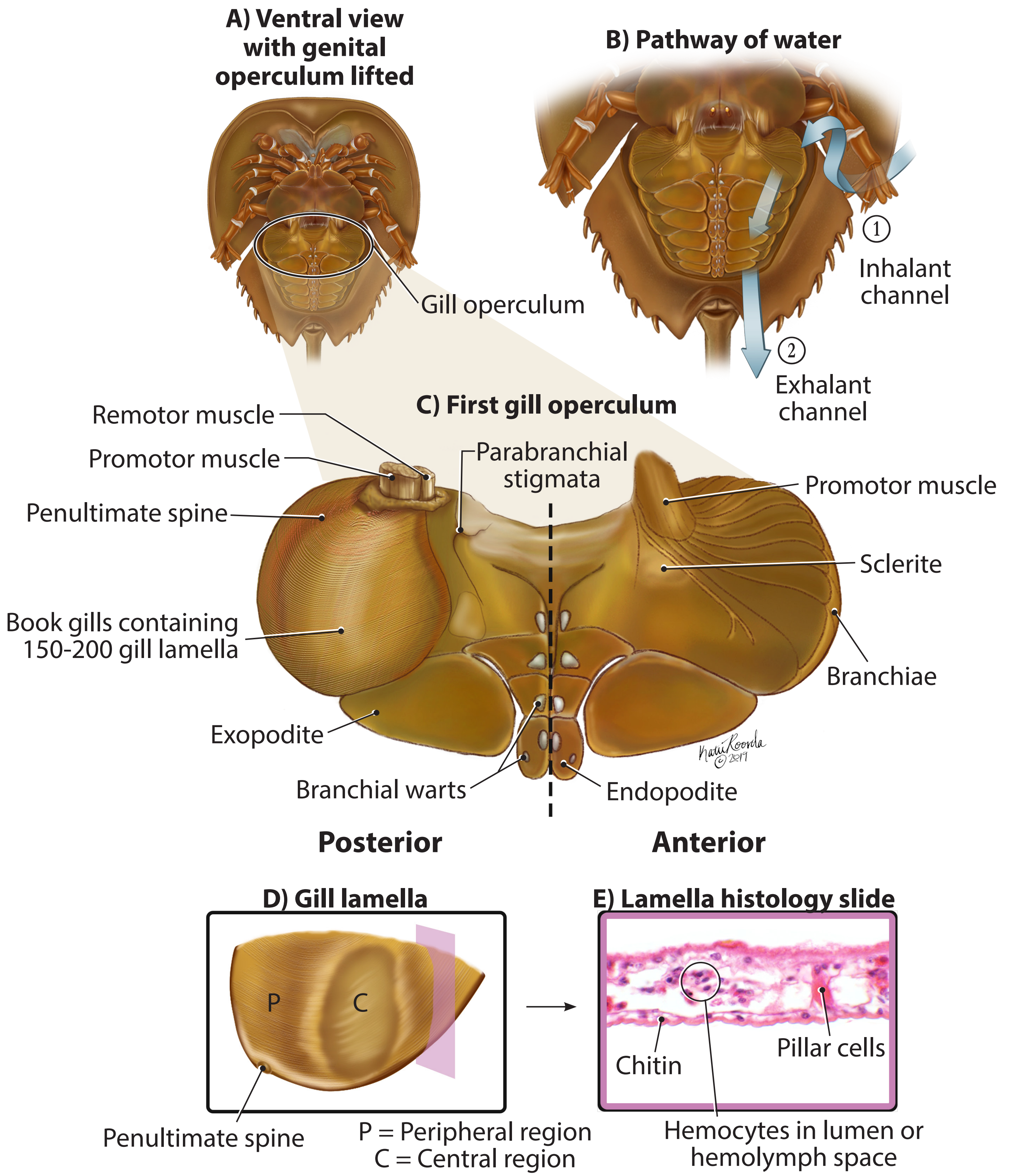
- **Seventh Pair** (Fig. 2A): Genital operculum, which covers and protects the book gills
- **Eighth – Thirteenth Pairs** (Fig. 4): Gill opercula, which protect and contain ventrolateral paired book gills, each contain hundreds of lamellae, or leaflets, and are used for respiration, osmoregulation, and propulsion. Each set of book gills has two centrally located endopodites, containing branchial warts.
- **Fourteenth Appendage** (Fig. 2A): The telson, used to aid the overturned horseshoe crab to right itself and to ward off predators.

The smooth, slightly raised area on the opisthosoma around the gill opercula is known as the posterior slope, which contains the transverse ridge. Anterior to the base of the telson is the anus, which is protected by a small ridge known as the axial area.


Doublure
Ventral sense organ
Subfrontal area
Chelicera
Male pedipalp
Gnathobase
Prosoma
Vault
Walking legs
Mouth
Exuviation suture
Chilariae
Flabellum
Genital operculum
Swimmer leg
Gill opercula
Posterior slope
Book gills
Opisthosoma
Endopodite
Transverse ridge
Axial area
Anus
Katie Roorda © 2019
Telson
Prosoma
Opisthosoma
Telson



Leg Anatomy: A) Pedipalps: Female and Male: The second prosomal appendage; B) Walking (or ambulatory) leg: The third through fifth prosomal appendages; C) Swimmer leg: The sixth prosomal appendage



Gill Anatomy: A) Ventral view of a horseshoe crab with the genital operculum lifted; B) Pathway of water between prosoma and opisthosoma at inhalant and exhalant channels; C) Posterior and anterior view of the first gill operculum; D) Single gill lamella; E) Histology slide of a single gill lamella

INTERNAL ANATOMY AND SYSTEMS

Fig. 5: **Cross Section at Midline**

This illustration shows the four anatomical systems of the horseshoe crab in relation to each other.

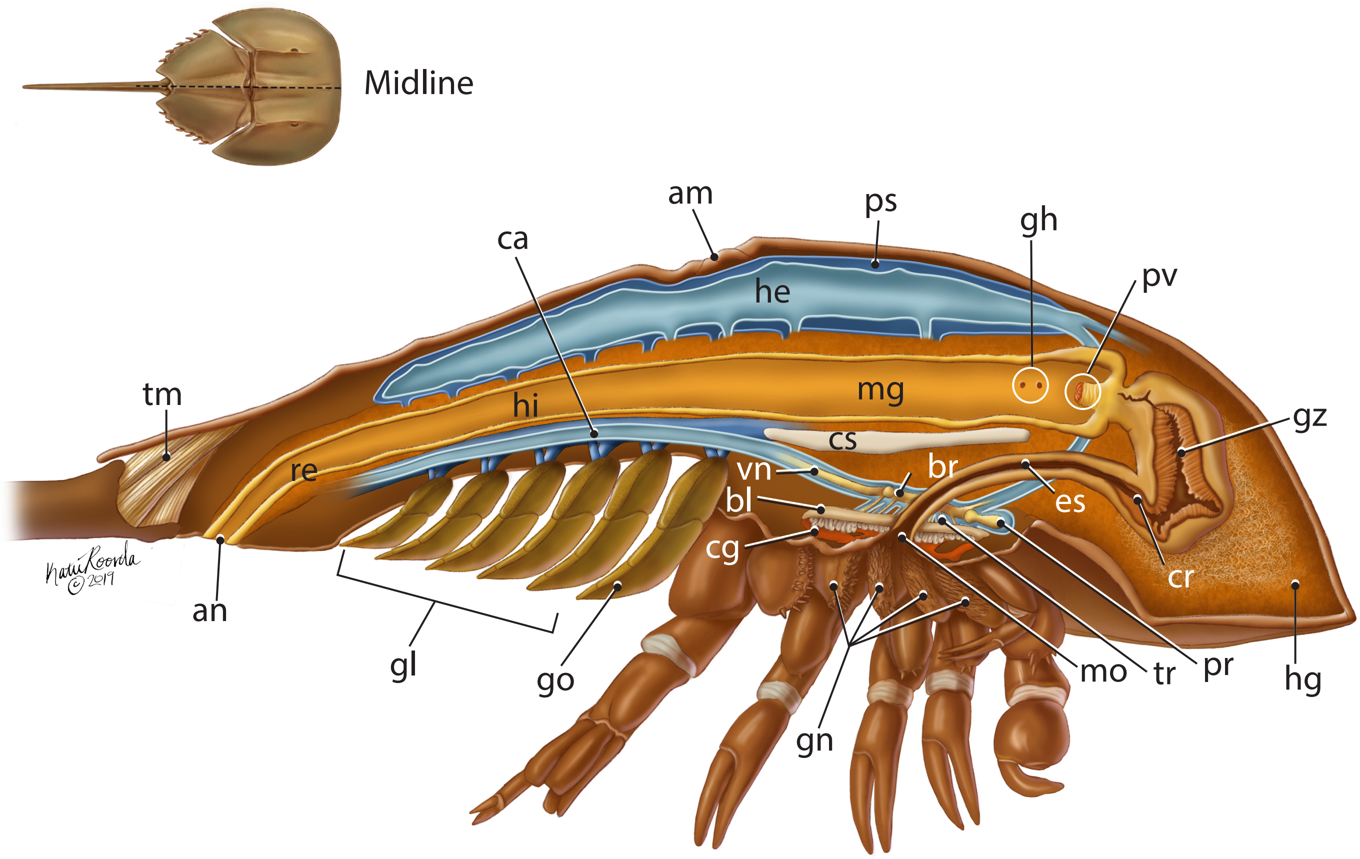
Digestive (Fig 5, 17): Food is ground by the gnathobase and pushed through the mouth opening, aided by the chelicerae. Food then travels through the sclerotized esophagus, which contains longitudinal folds that extend to the dilated crop. The ventriculus (commonly known as gizzard), or grinding chamber, immediately follows and contains denticles on the longitudinal circular folds that further grind the food. Any large, undigestible particles are regurgitated through the esophagus. The digestible food continues through the pyloric valve into the intestine, specifically the non-sclerotized midgut. Two pairs of ducts called glandular hepatic ceca ramify throughout the hepatopancreas. Food passes through the hindgut and out through the sclerotized rectum and anus.

Excretory (Fig 5, 11): Waste first collects in the coxal glands, four pairs of bright red-orange glands located within the musculature of the coxa leg segments (Shuster et al., 2003). From the coxal glands, the waste is transferred into a common chamber and then into the convoluted tubules to the paired elongated bladders. Waste is finally excreted through a small duct termed the excretory pore, or nephropore, on the posterior surface of the fifth appendage (Smolowitz, 1999).

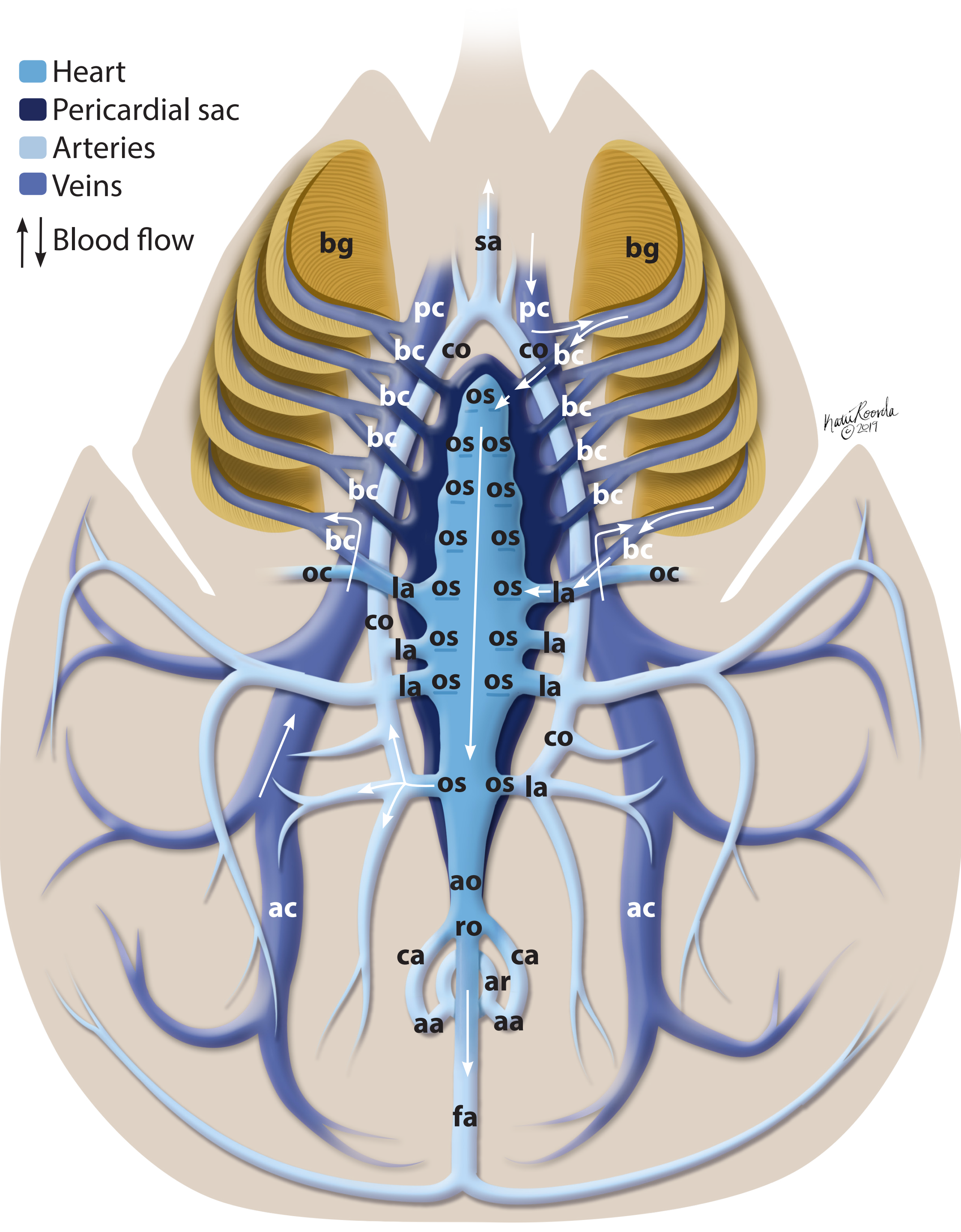
Nervous (Fig 5, 12): The nervous system is unique in the horseshoe crab because it is entirely enclosed within the arterial system. The brain is positioned within the arterial ring, forming a collar around the esophagus. Anteriorly, the brain forms a bulge called the protocerebrum and extends a thick posterior branch, called the ventral nerve cord, that continues posteriorly within the abdominal artery to the telson. The ventral nerve cord has five ganglia and lateral nerves that supply the opisthosoma and its appendages (Smolowitz, 1999).

Circulatory (Fig 6): The dorsal tubular heart is encased within the pericardium but is shown diagrammatically in the illustration to emphasize the borders of each structure. This illustration also uses realistic instructional colors for the blue hemolymph (blood) to distinguish between the arterial and venous supply while the colors of the heart and pericardial sac remain consistent with the other illustrations in this guide. Hemolymph collects anteriorly in the anterior cardinal sinuses and flows to the book gills. Arrows indicate the pathway of hemolymph flow.

Fig. 5: Cross Section at Midline



Cross Section at Midline: am: arthrodial membrane; an: anus; bl: bladder; br: brain within arterial ring; ca: coelomic artery (abdominal artery); cg: coxal gland; cr: crop; cs: "cartilage" shelf or endosternite; es: esophagus; gh: glandular hepatic ceca; gl: gill opercula; gn: gnathobase; go: genital operculum; gz: ventriculus or gizzard; he: heart; hg: hepatopancreas and gonadal tissue; hi: hindgut; mg: midgut; mo: mouth; pr: protocerebrum; ps: pericardial sac; pv: pyloric valve; re: rectum; tm: telson muscles; tr: tritocerebrum; vn: ventral nerve cord



Circulatory System: **aa** aortic arch; **ac** anterior cardinal artery; **ao** aortic valve; **ar** arterial ring; **bc** branchio-cardiac canals; **ca** cephalic artery; **co** collateral artery; **fa** frontal artery; **la** lateral artery; **os** ostia; **bg** book gill; **sa** superior abdominal artery; **pc** posterior cardinal vein; **oc** opercular canal; **ro** rudimentary ostium

EXTERNAL EXAMINATION

Commonly observed lesions, shell pathology, and inhabiting organisms include:

- **Eyes** (Fig. 7): The surface of the eye can erode and form ulcers.
- **Shell lesions** (Fig. 8): Large, discolored, and irregular patches of erosion on the surface of the carapace are commonly observed both in free-ranging horseshoe crabs and those in managed care. Other lesions may occur from physical trauma of crushing or fractures of the carapace. Trauma of the carapace and appendages may also be caused by predators, improper handling, or environmental factors. Smaller erosive patches may be found on the legs, telson and arthrodial membrane due to possible infectious agents such as algae, fungi, bacteria and parasites (Smith, 2012). A condition known as "gill rot" causes decay of the book gills and appears as yellowing of the gill opercula and blackening of the individual lamellae. The fungus, *Fusarium*, can be seen as fast growing, large, white, raised, irregular, proliferative tissue around the leg segments.
- **Inhabiting organisms** (Fig. 9): Many sessile organisms inhabit the carapace and/or book gills of horseshoe crabs in an interaction known as epibiosis, a nonsymbiotic relationship between the host (horseshoe crab) and the epibiont (sessile organism) (Shuster et al, 2003). Commonly observed epibionts include:
  - Common sponge
  - Mussels (*Mytilus*)
  - Barnacles (*Semibalanus*)
  - Scuds (*Gammarus*)
  - Oyster Drill (*Urosalpinx*)
  - Limulus leech (*Bdelloura*)
  - Slipper limpets (*Crepidula*)
  - Algae (*Ulva*)
  - Tube worms

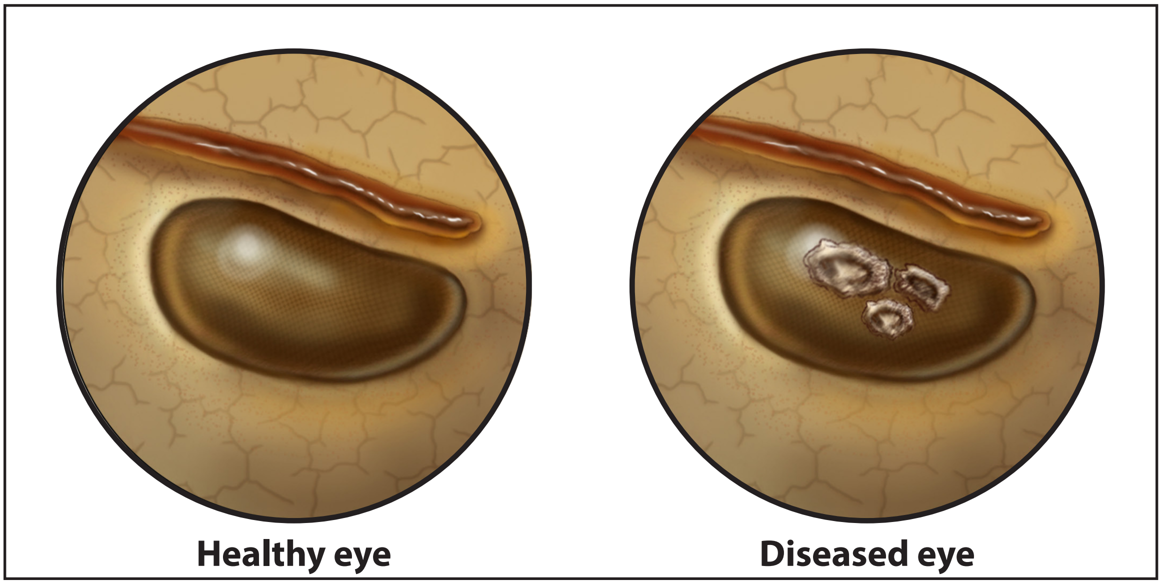
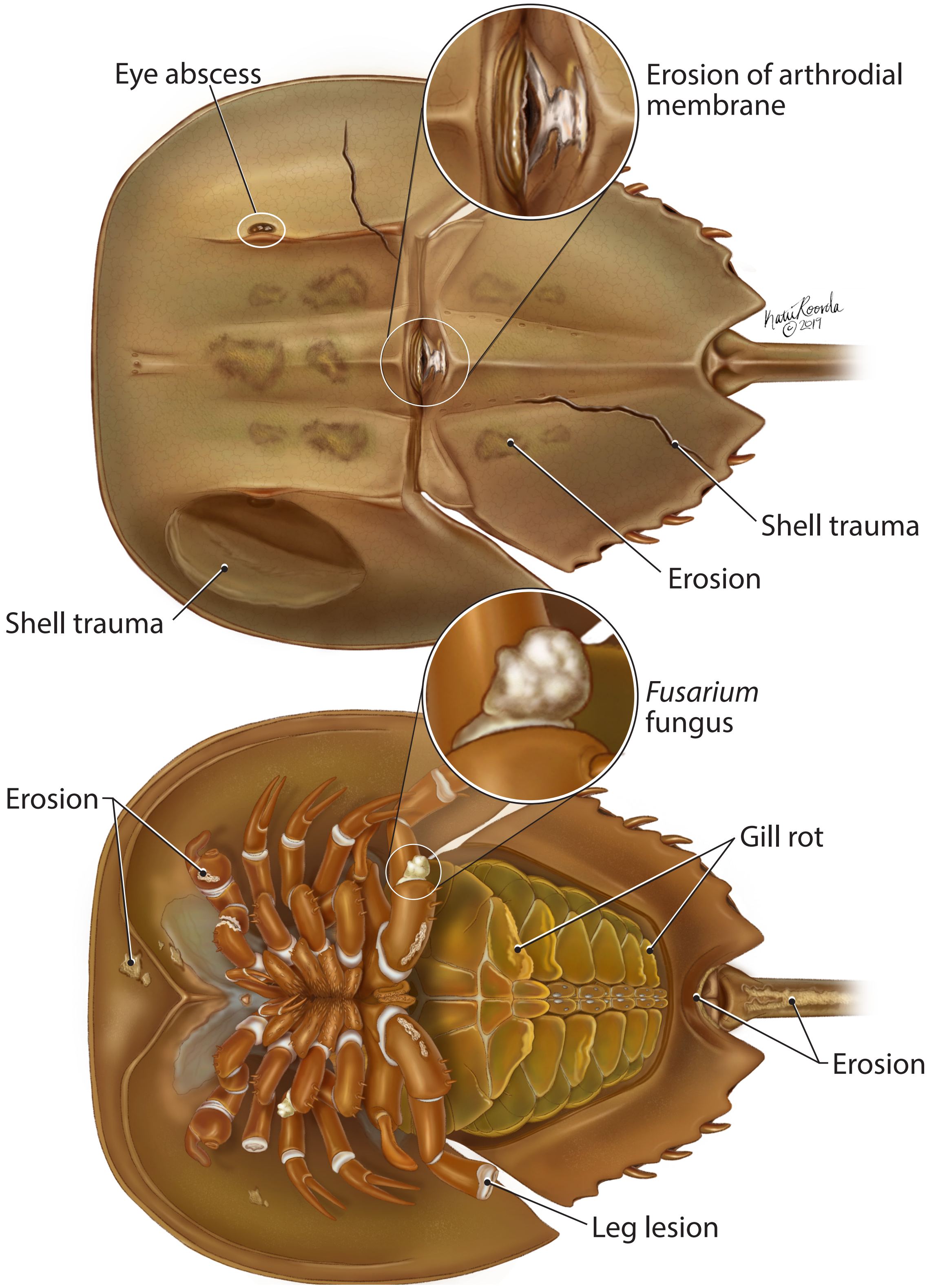
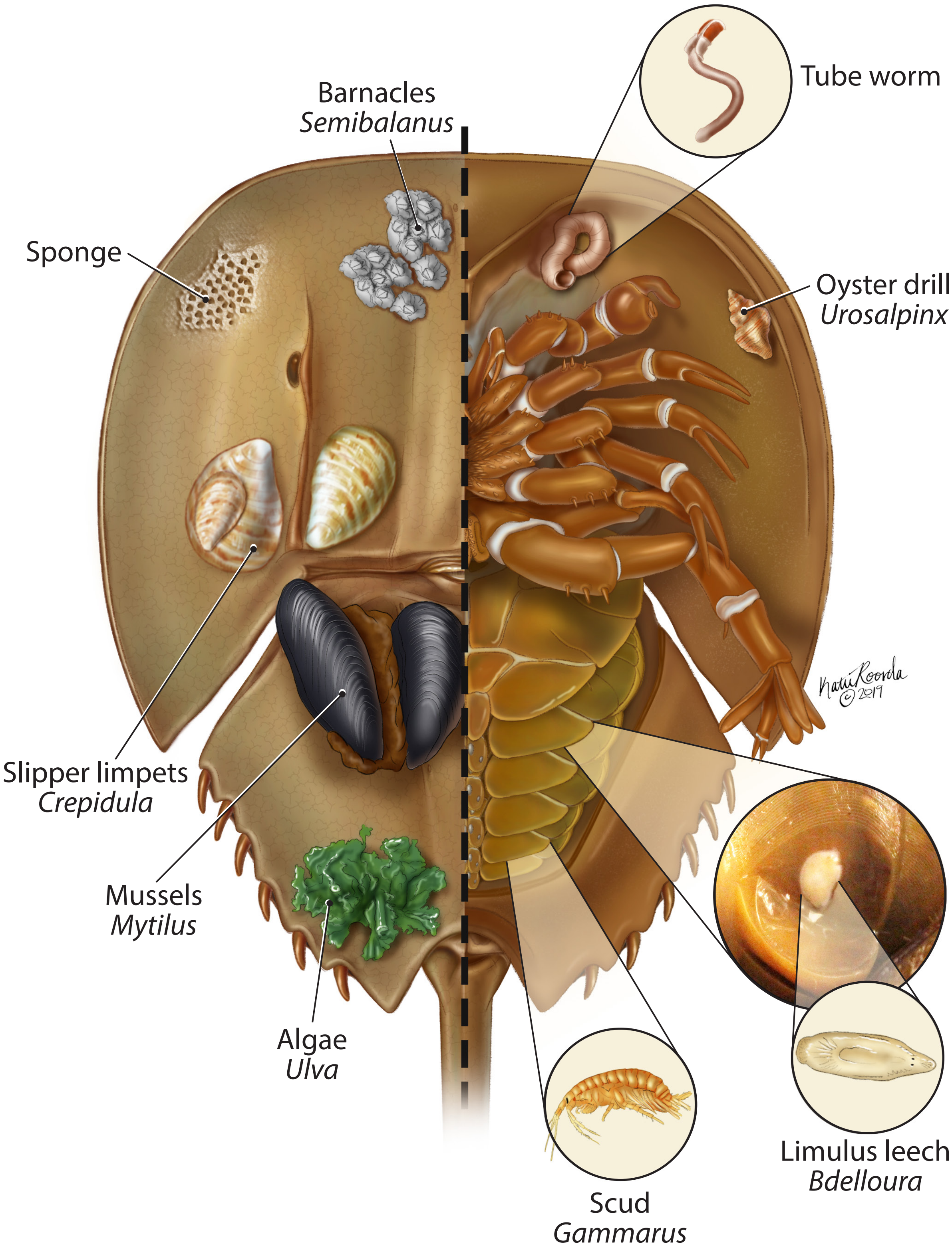


Fig. 7: Eye Comparison: A diseased eye has ulcers and erosion present compared to a healthy eye.



Common Lesions in Horseshoe Crabs: These occur on the carapace, arthrodial membrane, appendages, eyes and telson.



Inhabiting Organisms: Commonly observed sessile organisms include: common sponge; mussels: *Mytilus*; barnacles: *Semibalanus*; scud: *Gammarus*; oyster drill: *Urosalpinx*; limulus leech: *Bdelloura*; Slipper limpets: *Crepidula*; algae: *Ulva;* tube worms.

INTERNAL EXAMINATION

Figs. 10-18: **Necropsy Guide**

Step 1: Remove legs and gnathobase (Fig. 10)

- Make two linear incisions along each side of the gnathobase and the promesosternite
- The brain is beneath (dorsal) and encircles the mouth so care must be taken during dissection
- Avoid the chelicera and chilariae
- With gentle lateral traction on the legs, remove the gnathobase, legs, and promesosternite

Step 2: Carefully remove promesosternite to expose coxal glands (Fig. 11)

- Grasp both chelicera with hemostats and pull up gently on the chelicera to apply traction while cutting
- Carefully remove the promesosternite, including the chelicera and chilaria, staying as close to the promesosternite as possible
- The coxal glands will be visible and can be sampled
- Remove the coxal glands and associated excretory tissue (difficult to visualize). This exposes the brain

Step 3: Remove the brain with the mouth for sampling (Fig. 12)

- Examine the arterial ring, which encloses the brain
- Do not touch to avoid contamination and destruction of the tissue
- To ensure integrity of neural tissue, grasp the fibrous mouth with a forceps applying traction to underlying tissue, and cut under the brain tissue to remove with mouth

Step 4: Expose upper and lower intestine (Fig. 13)

- Cut the "cartilage" that lies underneath the arterial ring and brain
- Reflect the "cartilage" to expose the upper intestine
- Remove genital opercula, gill opercula, and gill lamellae, examining and sampling as needed
- Remove the "cartilage" underlying the book gills

Step 5: Expose entire intestinal tract (reflect but do not remove) (Fig. 14)

- Cut through the caudal opisthosoma on either side of the axial area through the transverse ridge and remove cut shell
- Clamp the end of the intestine with a hemostat
- Cut caudal to the hemostat but proximal to the cloaca and anus to reflect the intestine

Step 6: Expose and remove heart (Fig. 15)

- The heart lies underneath (dorsal) to the intestine. Note the heart tissue is more transparent than the illustration displays.
- Cut the vessels on either side of the heart
- Grasp the most caudal point of the heart (abdominal aorta) with forceps, gently lifting and freeing it from the surrounding tissue
- Examine and remove heart as proximal as feasible without disrupting the intestine

Step 7: Expose the ventriculus and remove the intestine (Fig. 16)

- Remove subfrontal area of the carapace
- Examine the regional hepatopancreas
- The ventriculus will be embedded within the hepatopancreas
- Bluntly dissect the ventriculus with a finger to remove
- Examine the exposed ventriculus

Step 8: Examine the gastrointestinal tract (Fig. 17)

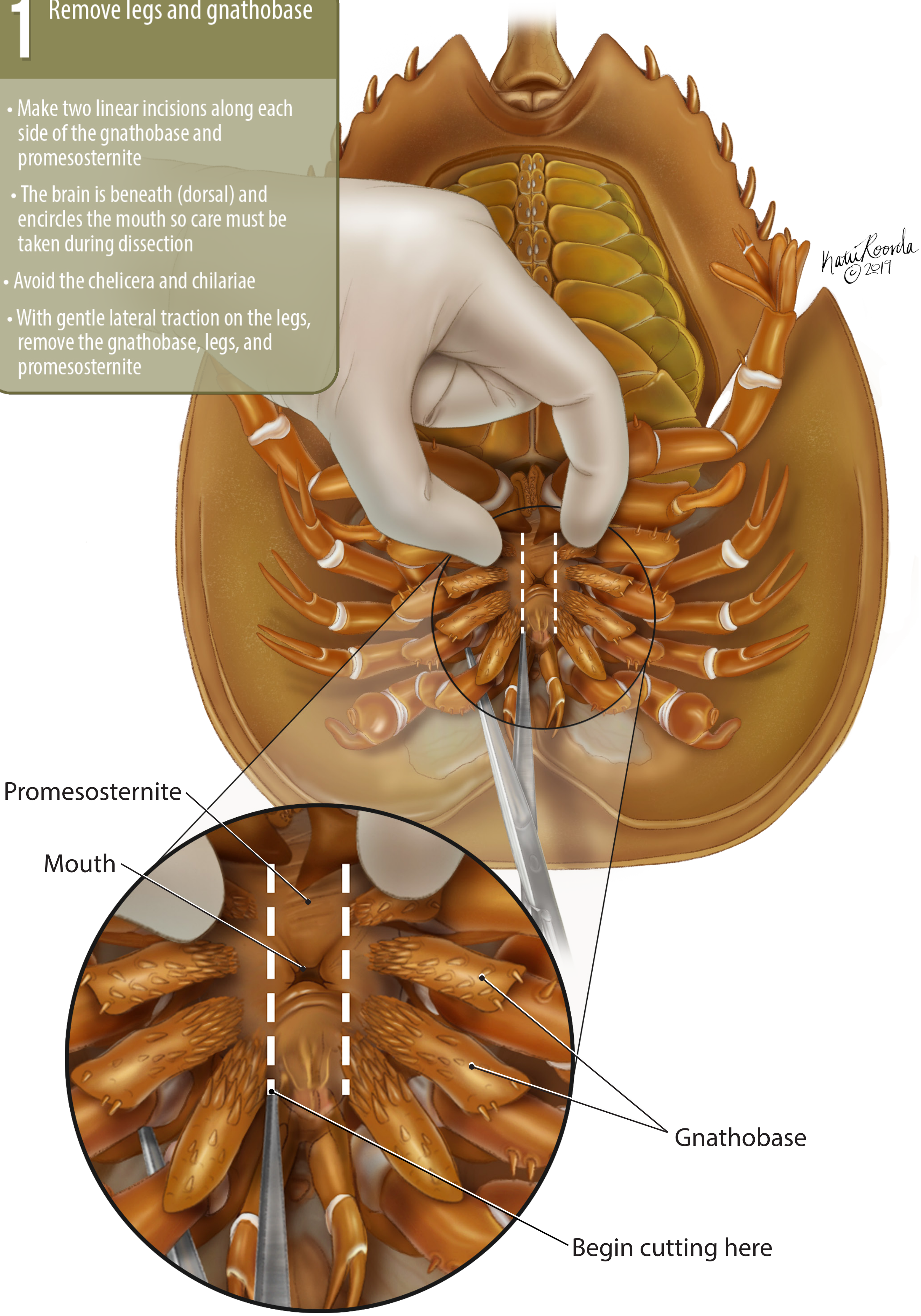
- Cut through the glandular hepatic ceca connections to remove the intestine
- Examine for any abnormalities before opening
- Take some sections of the unopened intestine for histology
- Open and examine the esophagus, ventriculus, and pylorus.

Step 9: Examine hepatopancreas (Fig. 18)

- Enter the vault using sharp scissors, or similar, to cut the ventral carapace as close to the dense exuviation suture without cutting through it
- Lift the ventral carapace (or use hemostats) as you gently dissect the hepatopancreas away from the inner surface
- Evaluate the hepatopancreas 'in situ' taking samples if necessary
- Separate the hepatopancreas from the vault digitally to remove (scoop out) and complete the examination
- Eggs will be present in spawning females

1 Remove legs and gnathobase

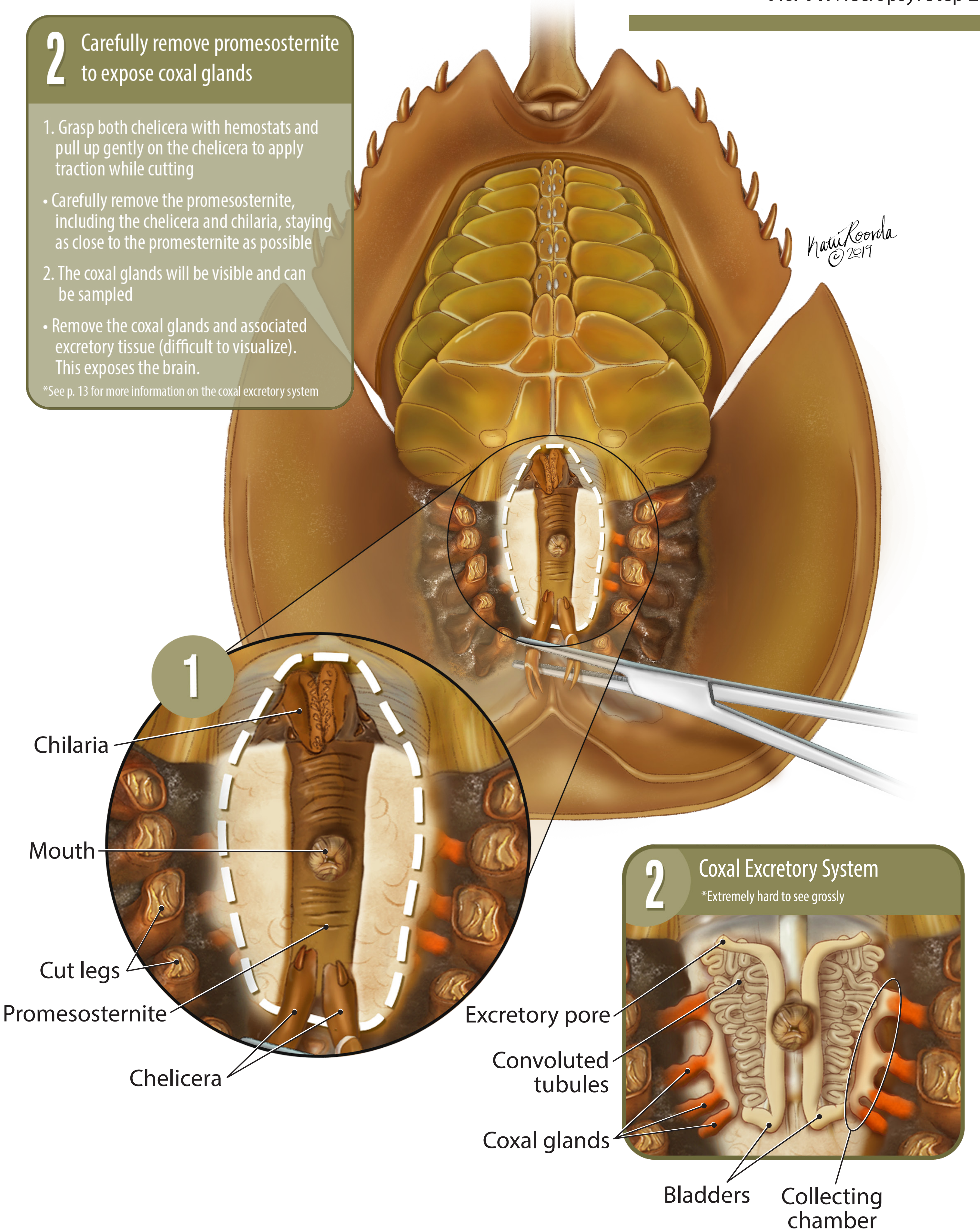
- Make two linear incisions along each side of the gnathobase and promesosternite
- The brain is beneath (dorsal) and encircles the mouth so care must be taken during dissection
- Avoid the chelicera and chilariae
- With gentle lateral traction on the legs, remove the gnathobase, legs, and promesosternite



2 Carefully remove promesosternite to expose coxal glands

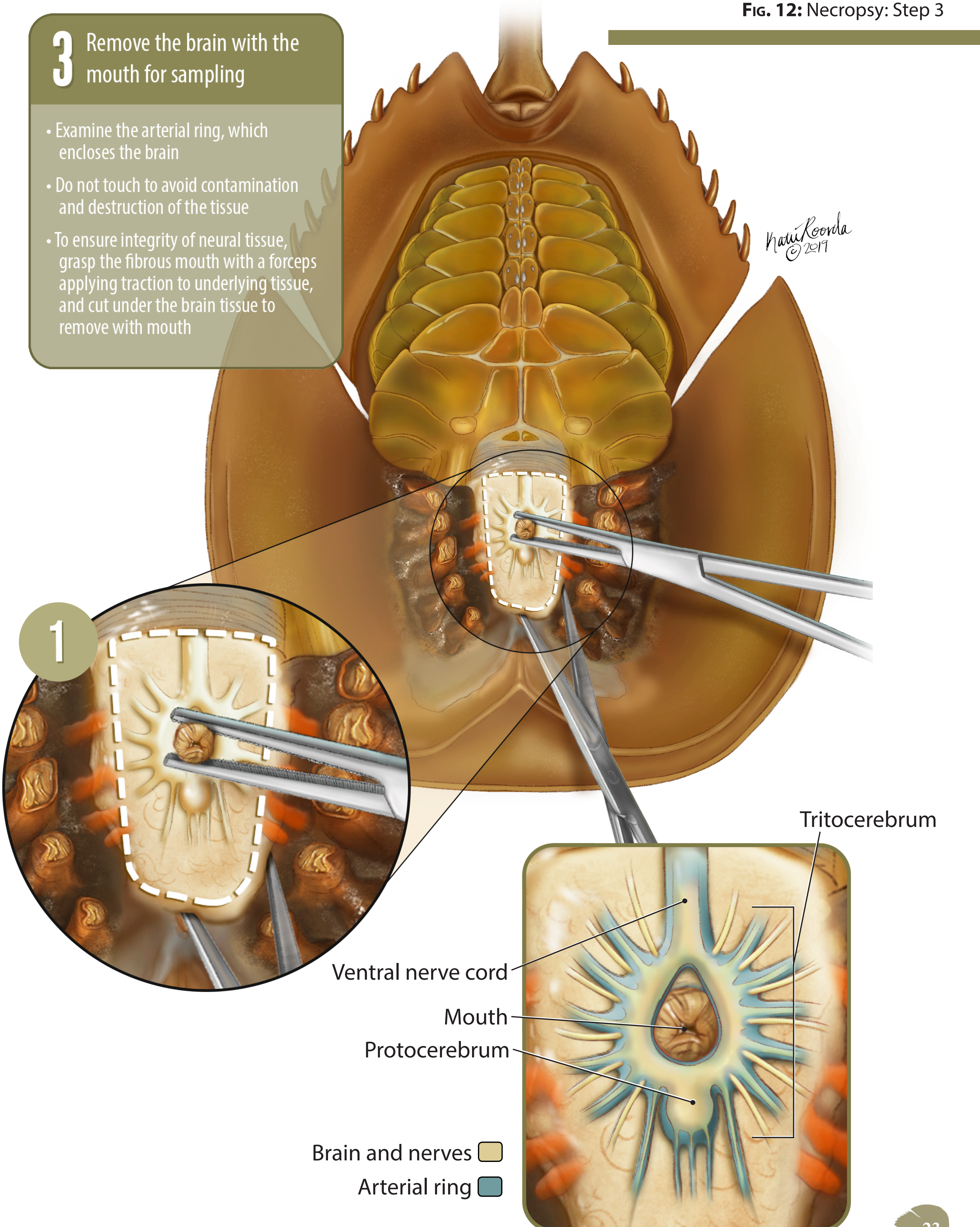
1. Grasp both chelicera with hemostats and pull up gently on the chelicera to apply traction while cutting
   - Carefully remove the promesosternite, including the chelicera and chilaria, staying as close to the promesternite as possible
2. The coxal glands will be visible and can be sampled
   - Remove the coxal glands and associated excretory tissue (difficult to visualize). This exposes the brain.

*See p. 13 for more information on the coxal excretory system



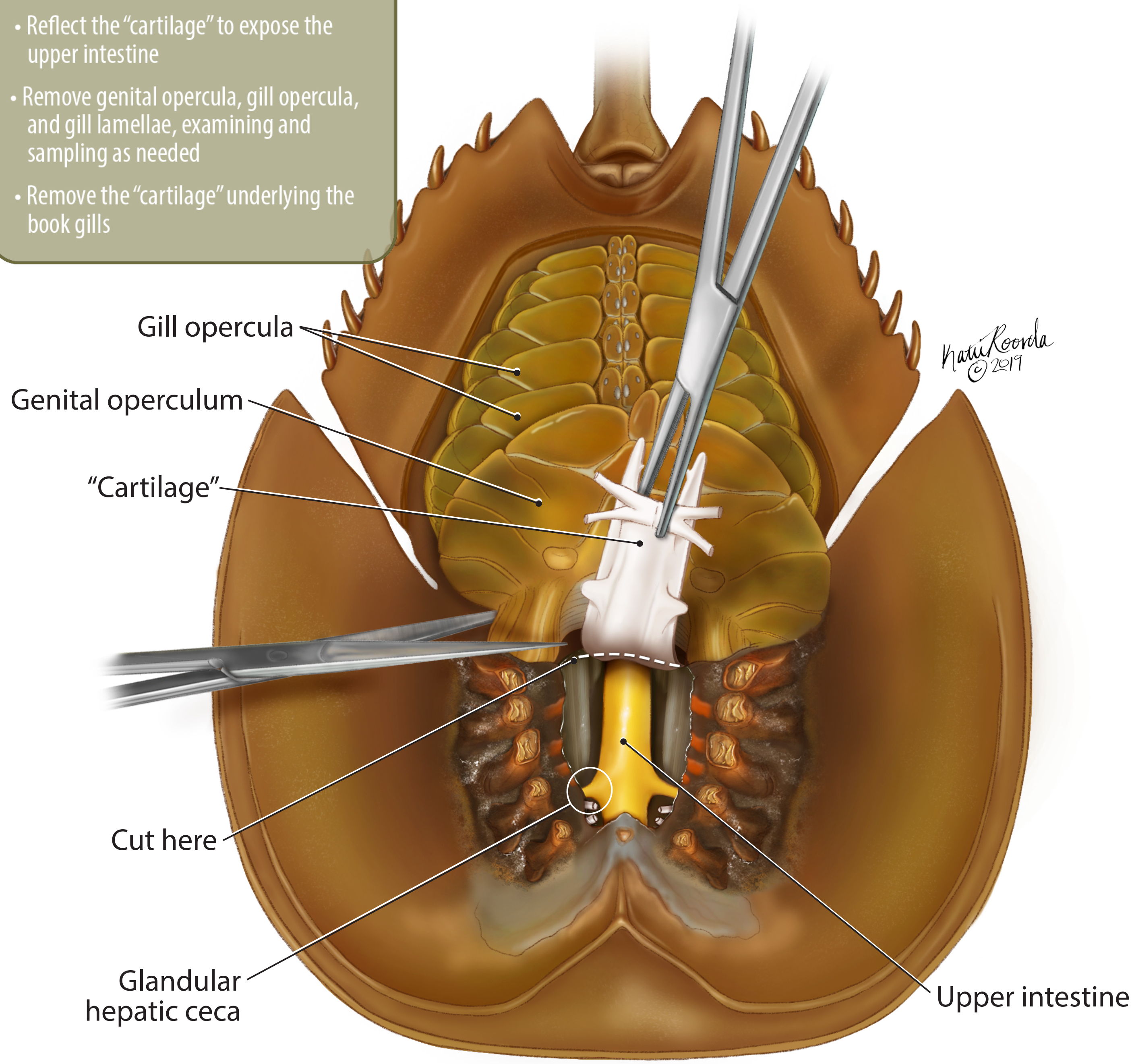
3 Remove the brain with the mouth for sampling

- Examine the arterial ring, which encloses the brain
- Do not touch to avoid contamination and destruction of the tissue
- To ensure integrity of neural tissue, grasp the fibrous mouth with a forceps applying traction to underlying tissue, and cut under the brain tissue to remove with mouth



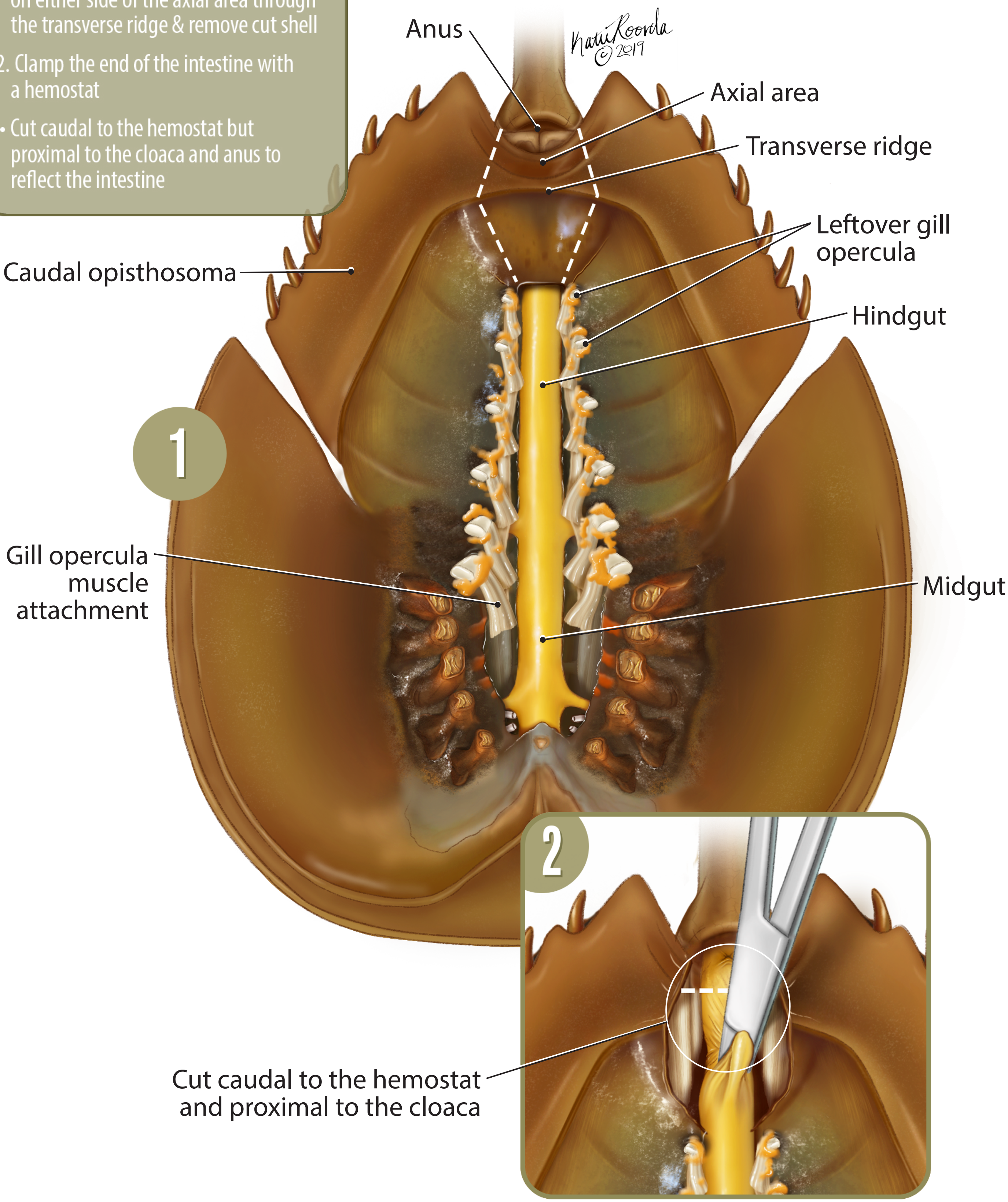
4 Expose upper and lower intestine

- Cut the "cartilage" that lies underneath the arterial ring and brain
- Reflect the "cartilage" to expose the upper intestine
- Remove genital opercula, gill opercula, and gill lamellae, examining and sampling as needed
- Remove the "cartilage" underlying the book gills



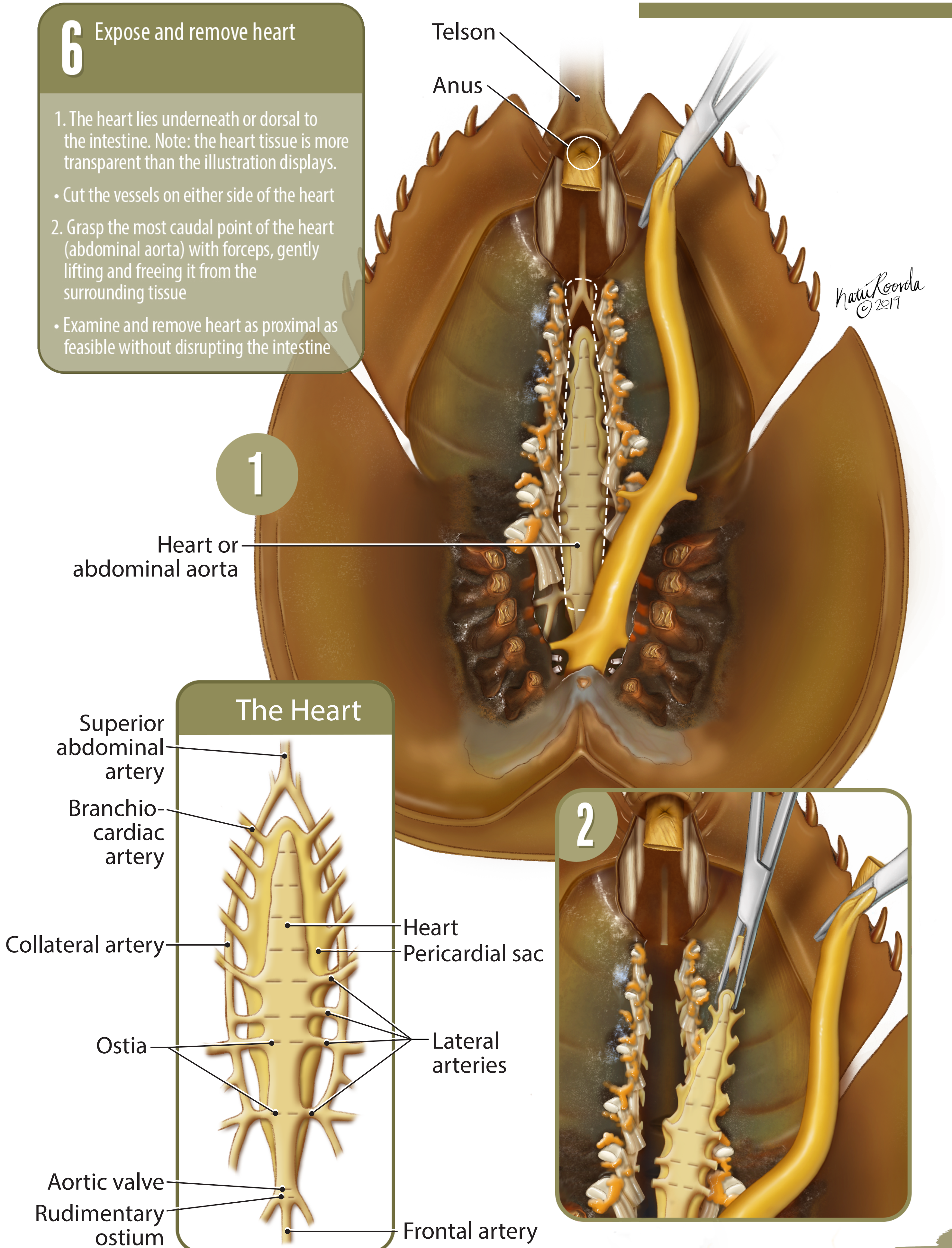
5 Expose entire intestinal tract (reflect but do not remove)

1. Cut through the caudal opisthosoma on either side of the axial area through the transverse ridge & remove cut shell
2. Clamp the end of the intestine with a hemostat
   - Cut caudal to the hemostat but proximal to the cloaca and anus to reflect the intestine



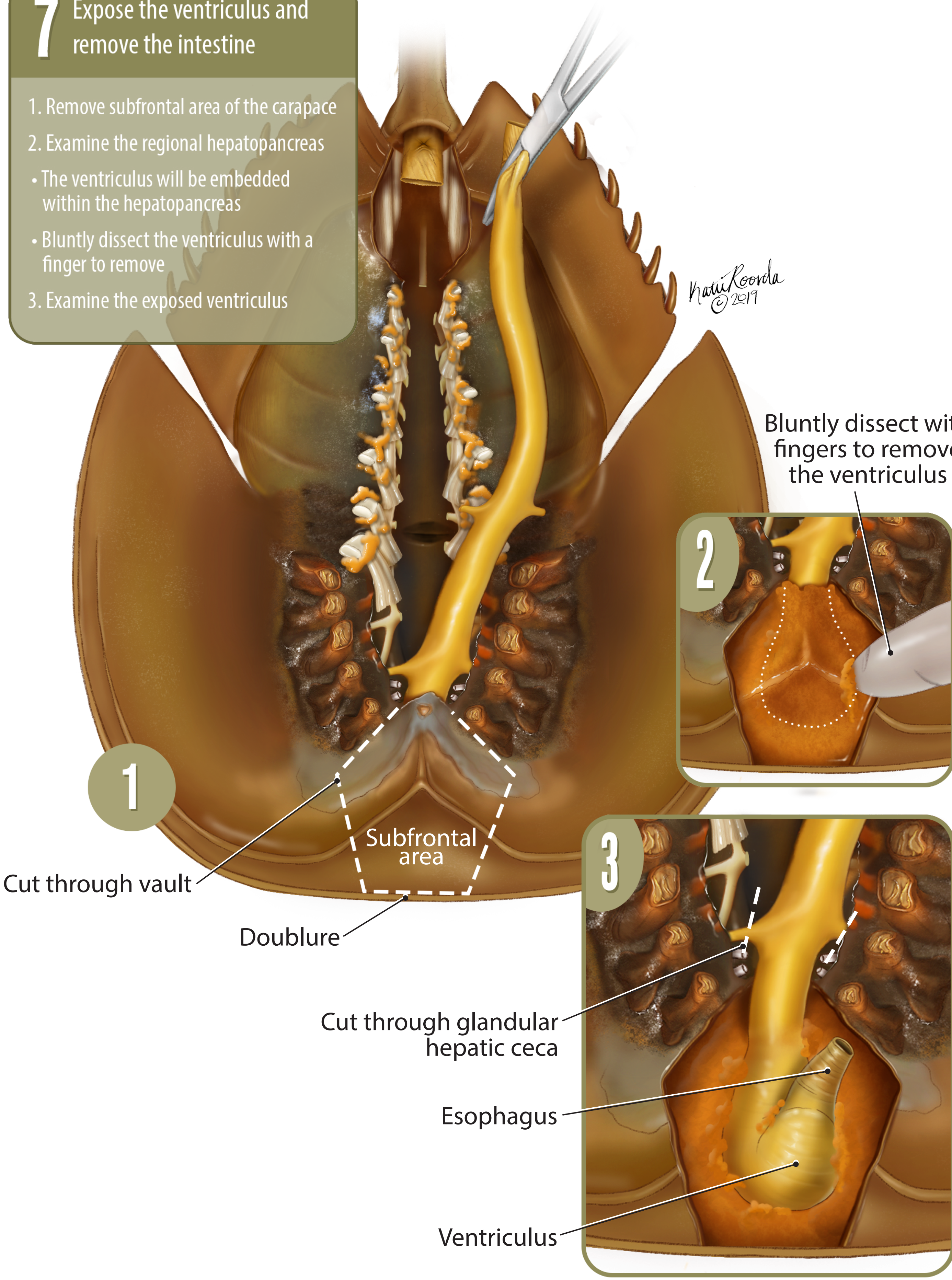
6 Expose and remove heart

1. The heart lies underneath or dorsal to the intestine. Note: the heart tissue is more transparent than the illustration displays.
   - Cut the vessels on either side of the heart
2. Grasp the most caudal point of the heart (abdominal aorta) with forceps, gently lifting and freeing it from the surrounding tissue
   - Examine and remove heart as proximal as feasible without disrupting the intestine



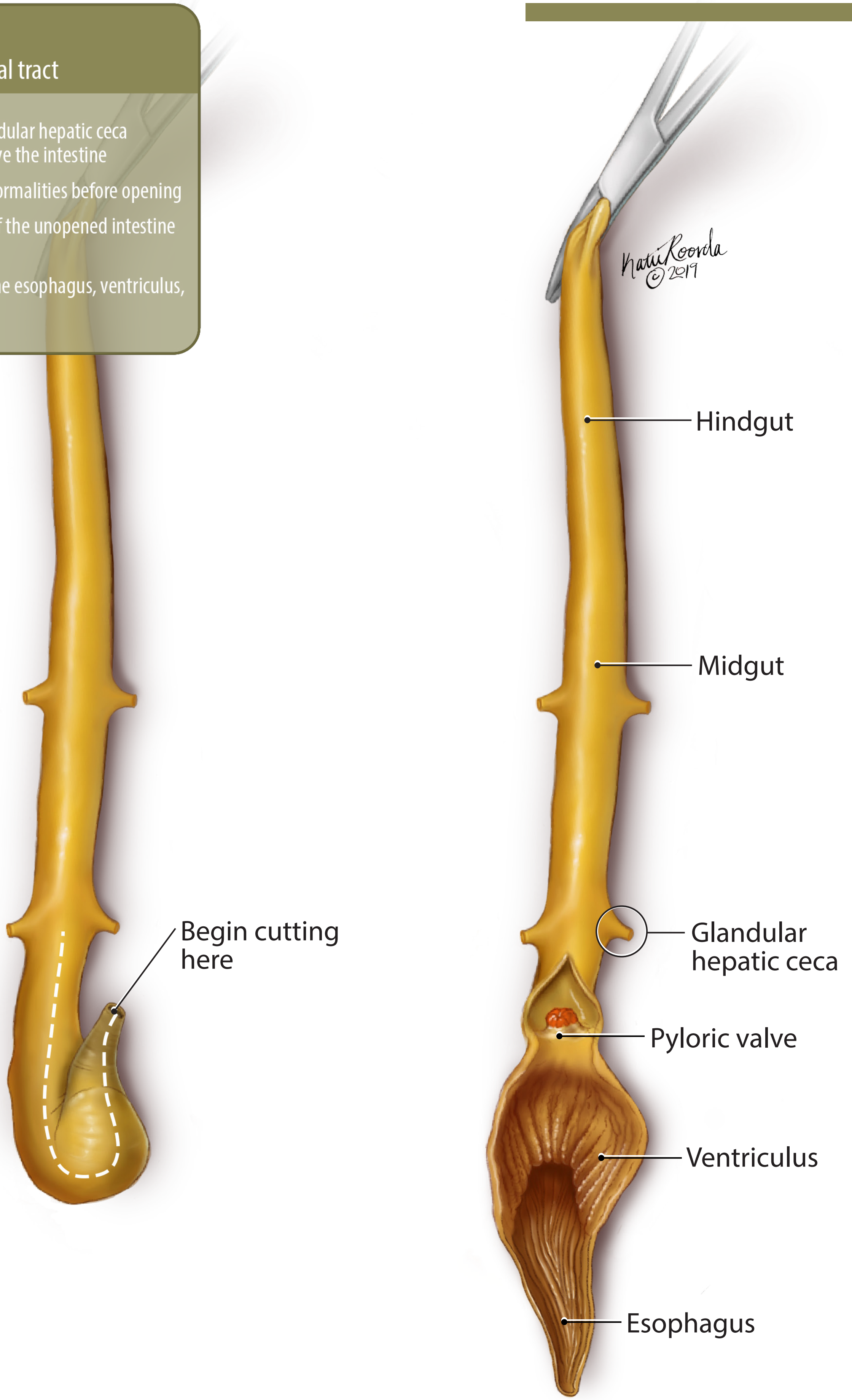
7 Expose the ventriculus and remove the intestine

1. Remove subfrontal area of the carapace
2. Examine the regional hepatopancreas
   - The ventriculus will be embedded within the hepatopancreas
   - Bluntly dissect the ventriculus with a finger to remove
3. Examine the exposed ventriculus



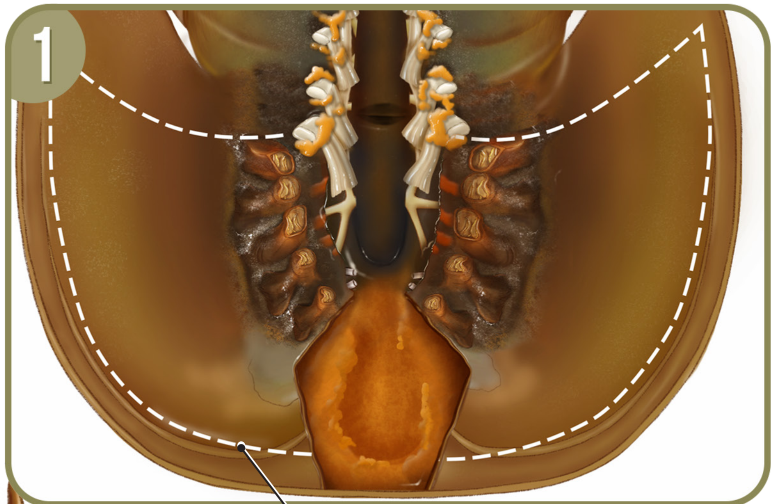
8 Examine the gastrointestinal tract

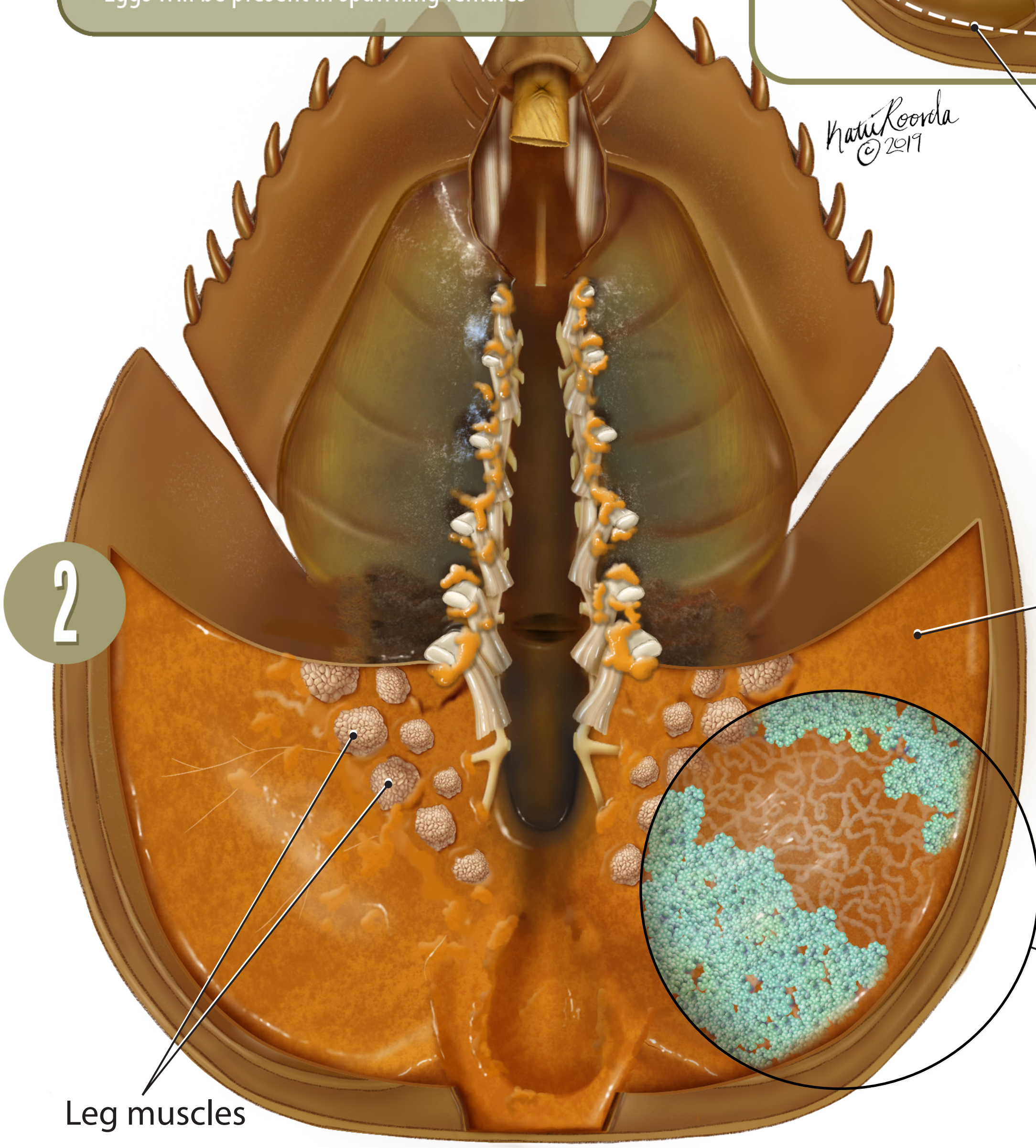
- Cut through the glandular hepatic ceca connections to remove the intestine
- Examine for any abnormalities before opening
- Take some sections of the unopened intestine for histology
- Open and examine the esophagus, ventriculus, and pylorus.



9 Examine hepatopancreas

1. Enter the vault using sharp scissors, or similar, to cut the ventral carapace as close to the dense exuviation suture without cutting through it
   - Lift the ventral carapace (or use hemostats) as you gently dissect the hepatopancreas away from the inner surface
2. Evaluate the hepatopancreas 'in situ' taking samples if necessary
   - Separate the hepatopancreas from the vault digitally to remove (scoop out) and complete the examination
   - Eggs will be present in spawning females





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