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Conus geographus, the “cigarette snail”

Abstract:

Conus geographus, Linnaeus 1758, is a cone snail commonly called the geography cone. It is a Mollusca in the gastropod class, in the order of neogastropoda and the family of conidae. The neogastropoda are gastropods that live in marine environments. Gastropods typically include snails, and slugs. The family conidae refers to all cone shell subspecies. All cone snails are capable of delivering a sting or an injection, to hunt their prey. It uses a proboscis, which extends out of the shell and punctures the prey with a harpoon like tooth, known as a radula, which is capable of delivering a cocktail of neurotoxins and peptides that send the prey into an immobile state. These toxins are synthesized in the epithelial cells of venom gland that can be unfolded to reach four times the length of the cone snail (Hall). The radula has a barb that allows the snail to bring the prey to it and engulf the entire body. It is also able to release the toxins containing peptides (conotoxins or conopeptides) into the surrounding water, to cause paralysis in schools of small fish. It can then engulf the entire school, commonly referred to as net feeding. It is one of the few cone snails (~10%) that are piscivorous, or prey only on fish (Johnson and Stablum, 1971).

Conus geographus is unique among the conidae family because its sting is powerful enough to kill a human. *Conus geographus* has commonly been referenced to as the “cigarette snail” because it is rumored that once injected with its venom, a human has about same the time to live as it takes to smoke a cigarette before dying. However, according to Taylor, a human stung by *Conus* geographus has a 30% chance of living and will die in about 30 minutes to one hour after injection. *Conus geographus* is also unique among other cone snails because of its many different proteins present in its venom. It is capable of delivering a short chain form of insulin, during injection, which sends the prey into a physical state known as ‘hypoglycemic shock’. This is the result of the prey’s blood sugar levels dropping rapidly, making it nearly

immobile (Osborne). Within the conopeptides are certain proteins called conantokins, each with a unique action on the nervous system. A certain conantokin protein in *Conus geographus* acts directly on mammalian skeletal muscles, causing a form of paralysis. These skeletal muscles were unresponsive to stimulation up to 30V. It did not present signs of effect on slow fibers or cardiac muscle tissue (Endean et al, 1974). There is currently no known antivenin for *Conus geographus.* However, the conotoxins in the venom can be used for a wide variety of medical purposes. Conantokin proteins, such as conantokin-G and contulakin-G have been extracted from the toxins and have been tested for their use as pain medications in cancer and AIDS patients, as a receptor-selective and non-addictive substitute for morphine. Other conantokin proteins have been tested for their provided medical benefits for prevention of epilepsy and seizures (Hall).

Introduction:

Common habitats for *Conus geographus* include tropical and non-tropical, benthic and coastal zones ranging from 0m to 200m in depth (Hall). *Conus geographus* are commonly found along living or fragmented coral reef structures, similar to other cone snails. Most of the *Conus geographus* are found in shallow areas, 6-18m, compared to the deep, benthic zones. Once matured, it can reach sizes of 65-166mm in length. *Conus geographus* is indigenous to the indo-pacific oceans; mostly around the northern, eastern, and western shores of Australia. It is also been found around many tropical waters with coral reef systems, where other cone snails reside (Hall).

In reproduction, *Conus geographus* releases egg sacs after mating, containing approximately 40 eggs per sac. These eggs attach to a hard substrate and develop after 10 to 15 days. After development, they become meroplankton, only spending part of their life cycle as plankton. The cone snails have a transparent shell for the first 20 days, and most do not make it past the larval stages; many of those do not make it into adulthood. Sexual dimorphism is not present in *Conus geographus*. The weather, temperature and salinity, affect the larval stages and survivability of *Conus geographus* (Hall). In the meroplanktonic stages the cone snail is predated by nektonic fishes and filter feeders. Once in its mature stage, turtles and rays can feed on *Conus geographus* and very careful humans will hunt them for collection of the shells.

Conus geographus will commonly hide itself under the sand or rocks, at the bottom layer of the ocean, and will extend its proboscis (Figure 5). It is able to submerge itself under the sand by using its muscular foot, and swaying side to side until it is covered. Using chemoreceptors on its siphon, it can sense the vibrations and chemicals in the water once a fish swims by. It will then release its harpoon filled with toxins to capture the prey. *Conus geographus* only uses its harpoon once, ingesting it along with the prey. It was noted by Johnson and Stablum, 1971, that the toxins were also used as a defensive mechanism. It was reported that when the shell of the snail was touched or damaged, the proboscis would extend towards the damaged area without opening the rhynchodaeum, the mouth and digestive tract, implying a defensive technique when not needing to feed. Johnson and Stablum also noted that the prey is nocturnal and can feed once a night, if eaten small prey. It will feed every other night if the previous prey captured was of a larger size. The cone snails can feed on fish from sizes 30mm up to 140mm. Only the larger cone snails, 80mm or larger, feed on fish greater than 130mm (Hall).

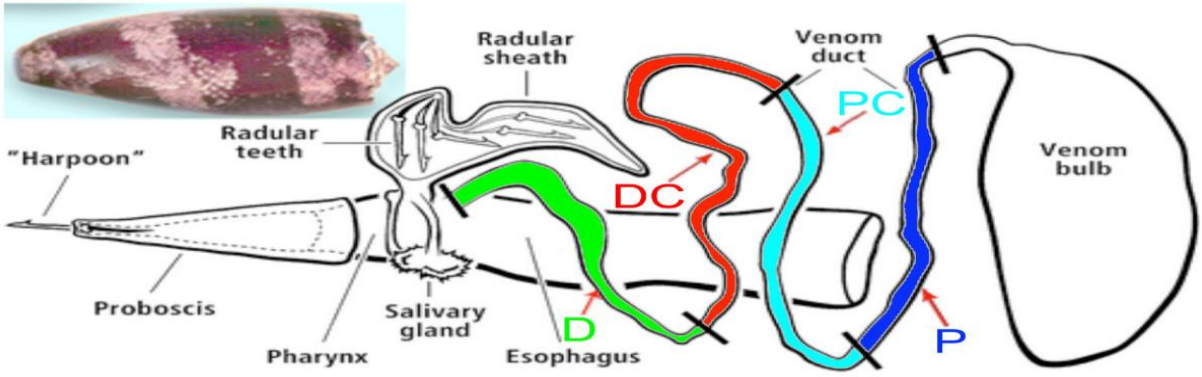
The geographic cone is currently not endangered and is listed as Least Concern under the International Union for Conservation of Nature (IUCN) Red List of National Species. The shell of the species is however sought out for its beauty. It is generally a cream or pink color, with brown or red spots and streaks. The snail trade is common, despite the protection these cone snails have. Some cone shells can reach prices of up to 5000$ while the shell of *Conus geographus* is worth only about 3 to 75 dollars according to the size, color, and seller (Taylor). Inside of the shell is a series of convoluted ducts and organs. *Conus geographus*, like other venomous cone snails, has a venom bulb and a venom duct that can grow to be 4 times longer than the shell of the snail. It also has its esophagus and radular sheath, where the radula are held, protected inside of the shell.

Figure 1: *Conus geographus* with organs

Figure 2: Apical view of *Conus geographus*

The shell of *Conus geographus* is pictured above (Figure 1 and 2). The color patterns are unique to the geography cone. The pink or cream base, with a series of brown streaks or spots (Figure 1 and 2) can be seen. The organs are seen just below the shell (Figure 1). The venom bulb can be seen to the left, connected to the esophagus by the long, convoluted, venom duct. The esophagus is connected to the radular sheath and the proboscis. Of the organs pictured, only the proboscis would be exposed in a live cone snail. The rhynchodaeum and the foot are not pictured.

Figure 3: Diagram of organs

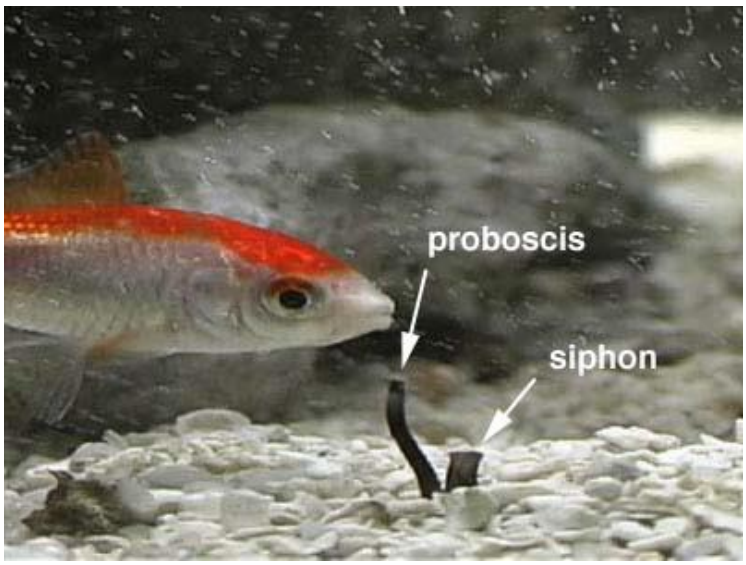


The organs pictured in figure 1 are labeled here. P represents the proximal section of the venom duct, connected directly to the venom bulb. PC shows the proximal central, DC is the distal central, and D is the distal region. The Distal region is the region closest to the pharynx (Hu et al., 2012).

Figure 4: *Conus geographus*

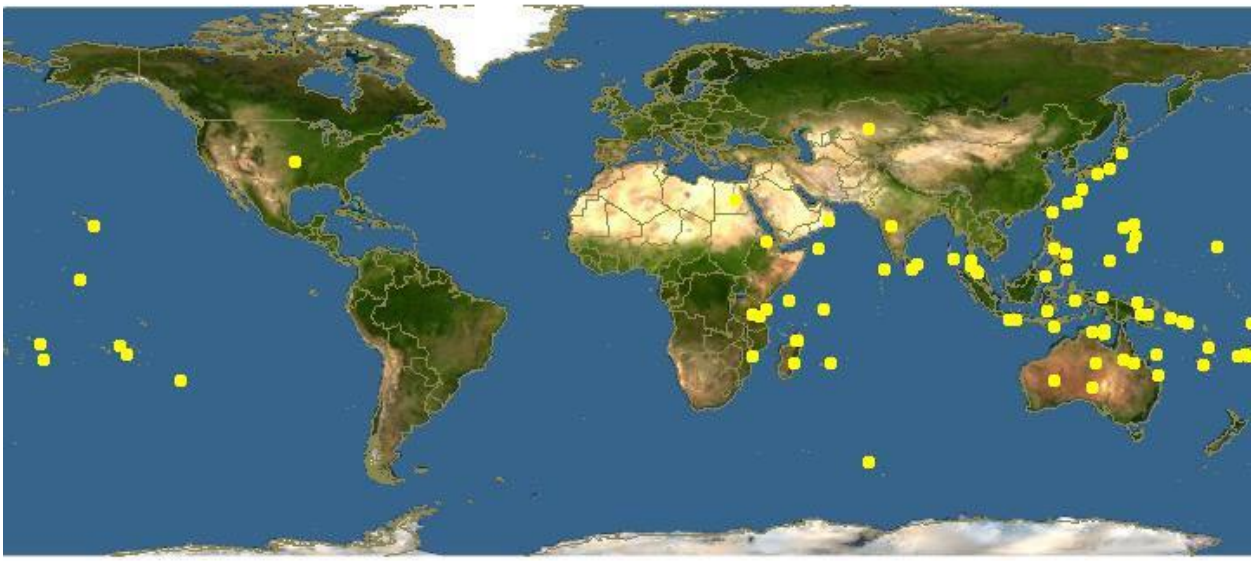
Conus geographus is pictured above. The proboscis is pictured in the center of the right side of the image. The rhynchodaeum is the opening (analogous to a mouth and stomach) pictured in the center of the image, which is used by the snails to engulf the fish. The eyestalk, which has the eye at the tip, can be seen just below the shell to the left of the rynchodaeum. The foot can be seen at the bottom left. The foot is a large muscle mass that is able to move and bury the snail. The proboscis is extendable and is capable of moving in any direction; making it an excellent defense mechanism.

Figure 5: Predation techniques



When not feeding, the snail will bury itself under the sand and will extend the siphon, which has chemoreceptors on it to detect any nearby fish. It will then release its harpoon-like tooth, radula, from the proboscis and inject the prey with hundreds of conotoxins that each work on certain nerve receptors and ion receptor channels, to subdue the prey for feeding.

Figure 6: National Geographic map of known cone snail locations.



Known locations of the cone snail, *Conus geographus,* are plotted as yellow dots on the map. The cone snail tends to stay in the indo-pacific oceans, near fragmented and live coral reef structures (National Geographic).

Discussion:

Since cone snails are able to defend themselves, it is very hard to extract the venom of many cone snails. There is no safe way to hold *Conus geographus* since the proboscis can bend to almost any angle. The radula is also capable of piercing through a diver's wetsuit, so there is not much protection available in collection and handling of cone snails. A common method of venom extraction is by holding a small fish with forceps, and allowing it to get stung and injected with venom. The venom is then extracted from tissue of the dead fish. "In some cone snails, the venom needed to kill a dozen people would fit into the head of a pen" (Sprackland, 2005). The LD_{50} (lethal dosage that caused fatality in 50% of trials) of the venom of *Conus* geographus, in milligrams of toxins per kilogram of human weight, is 0.004, compared to anthrax which is 0.0002 and the common Death Adder, with an LD_{50} of 0.500. In comparison, the venom of *Conus geographus* is only 20 times weaker than anthrax, but 125x stronger than the common Death Adder (Sprackland, 2005).

It has been discovered in highly successful predatory cone snails a form of insulin that is very similar to the structure of insulin found in fish. *Conus geographus* and *Conus tulipa* have been shown to use insulin as a major component of their conotoxin release. This form of insulin is very useful in predation. Since the structure is so similar to the insulin found in fish, upon injection or ingestion (net feeding) the exposed fish enters a state of hypoglycemic shock, a result of rapid decrease in blood glucose levels. The insulin found has great importance, not only the fact that it is shockingly similar to the insulin found in fish, but that it also has post-translational modifications to the conotoxins, specifically γ-carboxylglutamate (Hu et al., 2012).

The protein conantokin-G, present in the venom of *Conus geographus,* is a selective inhibitor of N-methyl-D-aspartate (NMDA) receptors and is an analgesic. It has shown the ability to induce sleep in young mice by intracranial injection (IC) (Jeminez, 2009). Conantokin-G and other conantokins have commonly been referenced to as "sleeper peptides" due to their ability to induce sleep and paralysis. The word "conantokin" comes from the Filipino word "antokin" which translates into "sleepy". Multiple post-translational modified amino acids residues of γ-carboxyglutamate are present in conantokin-G. It has been shown that these modified amino acids provide most of the biochemical actions in conantokins. When the multiple residues of

γ-carboxyglutamate of conantokin-G were decarboxylated the induced sleep activity in mice by IC was not present, showing that these modifications to the amino acid complexes are necessary, or important, to the specific biochemical reactions taking place in the nervous muscle tissues and ion receptor channels (Jeminez, 2009).

Since conantokin-G lacks a disulfide bond, the glutamate residues are very important to the structural framework of the protein and the biological processes that take place after injection. There are five glutamate residues present in conantokin-G: Gla3, Gla4, Gla7, Gla10, and Gla14. Using Nuclear Magnetic Resonance imagining (NMR) and Circular Dichroism (CD) conantokin-G showed random conformational structure, but has the ability to readily change into an α-helix formation in the presence of different divalent cations.

Upon presence of either Ca^{2+} or Mg^{2+} the curvilinear formation of conantokin-G was changed into a linear α-helix structure. In the presence of high Ca^{2+} cations, the distorted helix structure of conantokin-G was reformed at a high rate to a linear α-helix formation. Examination of conantokin-G structure in presence of Ca^{2+} using NMR and CD showed that upon reformation into α-helix glutamate residues were able to bind to one another on one face of the helix. Gla4 and Gla7 bound, while Gla10 and Gla14 bound together, causing a hydrophobic layer of amino acids to form on the opposite face of conantokin-G (Jeminez, 2009). In the presence of Mg^{2+}, using NMR and CD to examine the structural framework of conantokin-G, it was observed that one ion of Mg^{2+}bound to each of the oxygen atoms on the γ-carboxylates of Gla3, Gla4, and Gla7. While one ion of Mg^{2+} bound to three oxygen atoms in the γ-carboxylates of Gla10 and Gla14. NMR showed that each glutamate residue plays a major role in the structural transformation from a curvilinear helix to a linear α-helix structure in the presence of Mg^{2+} cations (Jeminez, 2009).

N-methyl-D-aspartate (NMDA) is a subtype of the ionotropic glutamate receptor. NMDA has two main subunits; NR1 and NR2. There are four subunits of NR2: NR2A, NR2B, NR2C, and NR2D. The protein conantokin-G acts selectively on NR2B subunit of NMDA (Jeminez, 2009). NMDA is blocked by Mg^{2+} ions, which must be removed to elicit glutamate and glycine currents. Upon injection of the conotoxins, conantokin-G binds to these Mg^{2+} ions and opens the Mg^{2+} ion blocked glutamate channels, allowing current to flow.

Conclusion:

Conantokin-G has been used in human clinical trials under the brand name Cognetix, CGX-1007, as an anticonvulsant and as an analgesic. The selectivity of conantokin-G has allowed pain relief, at very low dosages, without addiction or side effects commonly seen from the long-term use of morphine. It has also been shown to provide neuroprotection for post-ischemia in rat models. Conantokin-G has made it to phase II of clinical trials, where fewer than 100 patients are treated and the specific effects of the drug are being tested. After passing phase III the drug goes under FDA approval and would be released to the market.

The NMDA receptors are very permeable to Ca^{2+} ions and in high presence of Ca^{2+} excitoxicity and death of neuronal cells will occur (Jeminez, 2009). Due to this action, there has been much interest in using conantokins for targeting NMDA receptors and subunits to prevent this neuronal damage. The selectivity of conantokin-G has been examined for safe and effective use of targeting NMDA receptors to prevent chronic pain, convulsions, and post-ischemia (inadequate blood supply to an organ or part of the body, specifically the heart and brain).

In cerebral neurons conantokin-G was able to alleviate the excitotoxic responses of NMDA in the presence of Ca^{2+} cations. It was also shown in studies that it could be used for neuroprotection against injury induced by NMDA, glutamate, veratridine (that targets sodium receptors) and hypoglycemia/hypoxia. Studies on the use of conantokin-G for rat brain-ischemia have shown an 89% reduction of infarction (death of tissue due to lack of oxygen) with an 8 hour window of treatment from the onset of the injury (Jeminez, 2009).

The selectivity of conantokin-G on NDMA receptors is very critical to its use as an analgesic. Common analgesics that do not target specific NMDA receptors have shown the ability to reduce pain from injury, but undesired side effects such as lowered mental capacity, sedation, and lowered motor function ability were seen. Conantokin-G exhibited pain relief without these undesired side effects at doses 17 to 27 times lower than commonly used analgesics (Jeminez, 2009). In trials using rats, with severe spinal compression, conantokin-G expressed the ability to alleviate nociceptive responses, using intrathecal injection. It was also shown to reduce edema (buildup of fluids) of the paw in mice (Jeminez, 2009).

Due to the ion receptor selectivity of specific conopeptides and conotoxins, the medical field has taken a great interest in *Conus geographus*. Conantokin-G was found to be highly selective to the NMDA subtype NR2B. It is idealized to target the specific actions in conantokin-G, and other conopeptides, that allow for the highly selective activities on certain ion receptors. Once these specific actions or mechanisms can be isolated, medicine can be synthesized to target these receptors for various medical benefits. The selective abilities seen in conopeptides, specifically conantokin-G, can be correlated to the post-translational modifications to the amino acids. The modified glutamates, γ-carboxyglutamate, were studied and seen using NMR and CD to be critical components in the transformation of the curvilinear structure into a linear α-helix structure. The stability of the α-helix is important in the subunit selectivity and current flow of NMDA subunit receptors. Without the subunit selectivity of conantokin-G, and other conopeptides, the efficacy and importance of conopeptides would not be seen as a solution, or for drug substitutions, in the medical field.

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