

**BIODIVERSITY  
OF THE GENUS *CONUS* (FLEMING, 1822):  
A RICH SOURCE OF BIOACTIVE PEPTIDES**

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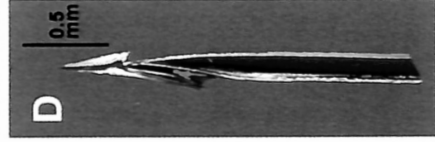
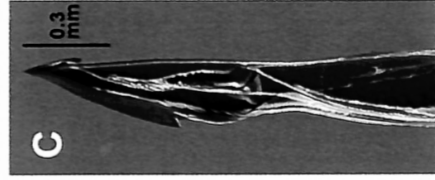
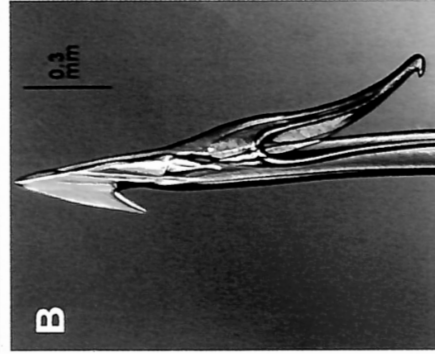
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**Abstract.** In this paper, we present an overview of the biodiversity of both marine snails of the large genus *Conus* and their venoms. After a brief survey of Conidae malacology, we focus on the high degree of biodiversity of this genus, its specific biogeography as well as its habitat, and the relatively strict diet of its members. The venom of Conidae species contains a large number of peptides that can interact selectively with key elements of the peripheral and central nervous systems of vertebrates and invertebrates. Emphasis is on summarizing our current knowledge of the specific actions of venom components on ionic channels, receptors and other key elements of cellular communication. The peptides isolated from venoms, called conotoxins, form different families according to both their primary structure and their specific pharmacological targets. Three families encompassing the  $\mu$ -,  $\mu$ O- and  $\delta$ -conotoxins target voltage-sensitive sodium channels but with different modes of action or tissue selectivity. Another important class of conotoxins is the  $\omega$ -conotoxin family which acts on voltage-sensitive calcium channels. The  $\alpha$ -conotoxin family is represented by several peptides blocking muscular or neuronal nicotinic acetylcholine receptors. Finally, a blocker of potassium channels is presented as well as two conotoxins acting on the N-Methyl-D-Aspartate receptor. Primary structures and cysteine frameworks of all these conotoxins are shown and compared. At the end of the review, we report the contribution of molecular biology to identification of new conotoxins having original pharmacological properties. In conclusion, conotoxins have received increasing attention from physiologists, pharmacologists, biochemists and physicians because of their selectivity as well as their pharmacological and therapeutic potential.

**Key words :** Conidae ; *Conus* malacology ; *Conus* venoms ;  $\mu$ -conotoxins ;  $\delta$ -conotoxins ;  $\omega$ -conotoxins ;  $\alpha$ -conotoxins ;  $\kappa$ -conotoxins ; conantokins.



## THE BIODIVERSITY OF CONIDAE

The Conidae (FLEMING, 1822) is a family of prosobranch gastropod molluscs which, together with the Turridae and the Terebridae, make up the Conoidea superfamily (genus and species names are according to COOMANS *et al.* (1979-1986), RÖCKEL *et al.* (1995) and RICHARD, (1990)). These animals paralyse their prey with specialized mouth parts that inject venom via highly modified radular teeth.

The more abundant species of Conidae can easily be found on the infra-littoral level of the inter-tropical zone. Their elegance and the large variety of colours of their shells make them very popular among amateurs, while for the researcher they represent experimental material that is now being used ever more frequently. A vast number of samples have been accumulated and there is a wealth of literature devoted to the Conidae, making its members ideal and original models both for the study of evolutionary biogeography and for the development of pharmacological applications based on the knowledge of how cone venom functions.

A BRIEF SURVEY OF CONIDAE MALACOLOGY  
AND CONCHOLOGY

In its natural environment, the only visible part of the cone is very often its inhalent siphon, although sometimes the sheath of the proboscis bearing the ocular peduncle, lying immediately below the siphon and sticking out from the anterior end of the animal, can also be seen (Fig. 1A). Cones crawl over the substrate using a muscular foot that is largely hidden by the shell. The posterior end of the animal has a small, nail-shaped infolding operculum. The colours of the siphon, the proboscis and the foot vary widely between different members of the family, but are extremely uniform within many species, an example being the red tones of *Conus imperialis*. In some cases, however, colours are very poor indicators of specific rank.

Like many neogastropods, cones are dioecious, their reproduction involving internal fertilisation after mating between the two sexes. The fact that the female has a seminal receptacle and that sexual partners gather at certain seasons (KOHN & PERRON, 1994 – personal observation) makes it possible, or even likely, that in numerous species the female is inseminated by several different males. The female can lay from a few hundred to several million eggs that are contained in egg capsules (KORN, 1994). Capsules are in the shape of a flat purse, and are placed by the female under blocks of coral or rock to protect them. The first stage of larval development occurs inside these capsules. For species with a

*Legend to the figure (see page 18)*

Fig. 1. — (A). *Conus consors*, a piscivorous species collected in Chesterfields Islands and acclimated in aquarium. Radula tooth morphology from *Conus consors* (piscivorous species). — (B). *Conus imperialis* (vermivorous species). — (C) and *Conus textile* (molluscivorous species). — (D). Note the presence in (B) of a long harpoon, posteriorly-directed with a curved tip. — (E). *Conus consors* stinging a fish by using the «harpooners» strategy.

« direct development », the young larvae look almost like miniature adults when they leave the capsules. They can be distinguished by the small protoconch shell with a small number of spirals and with roughly the shape of a conical cap with the top bent forward, as can be seen, for example, on *Conus magellanicus* (POINTIER *et al.*, 1987). Sometimes the larvae go through a planktonic stage of development of varying length. In Conidae, this stage may last from a few days (species probably undergoing lecithotrophic development) to weeks or months (species with larvae that feed in the plankton): in the latter case, at the top of the adult shell (or teleoconch), a multi-spiral protoconch is present which is larger (has more spirals) than that of the species with direct development. *Conus catus* is an example of a cone which has a planktonic developmental stage, lasting in its case for approximately three weeks. Very little is presently known about the growth of cones, in contrast to the many families of tropical molluscs (RICHARD, 1982, 1986). Data that until now have not been published put the life span of a few species of cones living on reef flats in French Polynesia at between ten and twenty years.

It is essentially only the last whorl of the teleoconch that can be used to observe the shell characteristics; the upper whorls, hidden by the last whorl, form the spire. In its general aspect, the shell of a cone can be turbinate (*e.g.* *C. monile*, *C. thomae*, *C. bayani*), conical (*C. pertusus*, *C. dalli*, *C. pretiosus*), biconical (*C. arcuatus*, *C. excelsus*, *C. cancellatus*), obconical (*C. lenavati*, *C. hirasei*, *C. sugimotoi*) or even fusiform (*C. glans*, *C. coccineus*, *C. nucleus*). The spire, which can have various numbers of whorls (such as *C. kintoki*, with a flattened spire, or *C. milneedwardsi*, with a very high spire) may have a convex (*C. rolani*, *C. bulbus*), straight (*C. sulcocastaneus*, *C. dayriti*) or concave profile (*C. armadillo*, *C. schepmani*). The last whorl may be totally smooth (*C. dusaveli*, *C. eburneus*) or it may have many ribs (*C. proximus*, *C. raoulensis*); its texture may also vary substantially between members of the same species, as is the case with *C. muriculatus* and *C. mucronatus*. The shoulder can be either smooth and angular (*C. striatus*, *C. thalassiarachus*), rounded (*C. omaria*, *C. zebra*) or scalloped (*C. imperialis*, *C. marielae*). The aperture is either narrow and covers the whole length of the peristome (*C. coelinae*, *C. shikamai*) or is vase-shaped toward the anterior (*C. geographus*, *C. tinianus*); on the inside it is lined with a columella that is usually smooth but it can also have a fold at its base that varies in size but is constantly present in a small number of species (*C. angasi*, *C. lozeti*, *C. luciae*). In some species the periostracum is rather thin and transparent, and the underlying polychromatic patterns can be seen through it (*C. textile*, *C. plinthis*), but in most species it is thick and relatively opaque, more or less totally hiding the coloration of the shell (*C. leopardus*, *C. virgo*). During the last few years the siphonal canal (anterior) and the anal canal (posterior) have been studied with a view to providing additional distinctive elements for the specific rank.

### A HIGH DEGREE OF BIODIVERSITY

The first Conidae (genus *Conorbis*) appeared right at the beginning of the Eocene period, almost 60 million years ago. They evolved from an ancestral group belonging to the same family as earlier Strombidae; the direct ancestor of the cones was more than likely a member of the Turridae family. During that period, the world's ocean was uni-

formly warm, with surface water temperatures of at least 22°C at low latitudes. However it was during the Lutetian period that the family began to expand, with the appearance of *Conospira*, *Cryptoconus*, *Hemiconus*, *Mamiconus*; even the *Lithoconus* group appeared during this period. It is possible to collect a large number of species dating from this period in France, particularly in the Paris basin (*C. calvimontensis*, *C. diversiformis*, *C. granatinus*, *C. glabratus*) and also in Normandy (*C. douvillei*). A little later, during the Miocene period (Helvetian), during which time many of the modern groups of cones appeared, the first fish-eating cones made their appearance with the diversification of the *Chelyconus* group. *C. aldrovandi*, *C. pelagicus* and *C. ponderosus* were among the cones that existed at that time. In Europe, the diversity of the family seemed to decline somewhat during the Pliocene (*C. antiquus*, *C. brocchii* and *C. striatus* are from this period), before expanding rapidly again during the Pleistocene. Nevertheless, compared with other families of molluscs, very few Conidae fossils have been found, primarily because their favoured habitats are seldom fossilized.

Today, the Conidae family is prospering in its natural environment, particularly in the Indo-Pacific province and in the southern Atlantic. The ease with which scientists can now travel and obtain material, the improved access to the scientific literature and to standard specimens, together with the ability to go prospecting in new areas (an example being the trawling of the bathyal zone) are some of the reasons why the last few decades have seen such large increase in the descriptions of new taxa (genus, sub-genus and species). Many scientists and several extremely competent and enlightened amateurs have attempted, with varying degrees of success, to propose an up-to-date cone taxonomy (COOMANS *et al.*, 1979 to 1986; KOHN, 1963 to 1992; WALLS, 1979; RICHARD, 1990). The work of Alan KOHN, professor at the University of Seattle (USA), is undoubtedly among the most interesting, and the recent book by RÖCKEL, KORN & KOHN (1995) is an almost exhaustive survey of what is known at the present time about the taxonomy of cones from the Indo-Pacific province, down to the specific rank, based on morphological and biometric criteria of the shell. Although this book is now an obligatory starting point for all the taxonomists of this family, it is not wholly satisfying, and work is in progress that will attempt to improve cone taxonomy and make it congruent with the evolution of the family.

It has been estimated that there are at least 600 extant species, with at least 700 species and sub-species divided into about thirty «groups», which are currently under study to see if they correspond to monophyletic taxa. It is already well established that a large number of these taxa correspond at most to sub-genus rank. The table below indicates the «working groups» that RICHARD (1990) defined, and shows a few examples of present-day species that belong in each group; most of the groups are either species groups or sub-genera.

*AFRICONUS* GROUP: *C. borgesii*, *C. cuneolus*, *C. grahami*, *C. irregularis*.

*ASPRELLA* GROUP: *C. aculeiformis*, *C. comatosa*, *C. elegans*, *C. insculptus*.

*CHELYCONUS* GROUP: *C. achatinus*, *C. aemulus*, *C. catus*, *C. ermineus*.

*COCHLICONUS* GROUP: *C. centurio*, *C. emarginatus*, *C. gradatus*, *C. poormani*.

*CONASPRELLA* GROUP: *C. acutangulus*, *C. baileyi*, *C. memiae*, *C. nereis*.

*CONUS* GROUP: *C. araneosus*, *C. bandanus*, *C. marmoreus*, *C. nocturnus*.

*CYLINDER* GROUP: *C. abbas*, *C. gloriamaris*, *C. telatus*, *C. textile*.

- CYLINDRELLA* GROUP: *C. marielae*, *C. moluccensis*, *C. sibogae*, *C. sulcocastaneus*.  
*DARIOCONUS* GROUP: *C. crocatus*, *C. magnificus*, *C. omaria*, *C. pennaceus*.  
*DAUCICONUS* GROUP: *C. ferrugineus*, *C. planorbis*, *C. striatellus*, *C. swainsonii*.  
*DENDROCONUS* GROUP: *C. betulinus*, *C. figulinus*, *C. genuanus*, *C. glaucus*.  
*EMBRIKENA* GROUP: *C. pergrandis*, *C. potusmarumai*, *C. stupa*, *C. stupella*.  
*ENDEMOCONUS* GROUP: *C. boucheti*, *C. dayriti*, *C. otohimeae*, *C. spirofilis*.  
*FLORACONUS* GROUP: *C. anemone*, *C. peronianus*, *C. spectrum*, *C. tinianus*.  
*GASTRIDIDIUM* GROUP: *C. cuvieri*, *C. eldredi*, *C. geographus*, *C. tulipa*.  
*HERMES* GROUP: *C. artoptus*, *C. nussatella*, *C. viola*, *C. violaceus*.  
*LAUTOCONUS* GROUP: *C. californicus*, *C. taslei*, *C. unifasciatus*, *C. ventricosus*.  
*LEPORICONUS* GROUP: *C. cylindraceus*, *C. mitratus*, *C. nucleus*, *C. tenuistriatus*.  
*LEPTOCONUS* GROUP: *C. amadis*, *C. ammiralis*, *C. locumtenens*, *C. splendidulus*.  
*LITHOCONUS* GROUP: *C. eburneus*, *C. leopardus*, *C. litteratus*, *C. tessulatus*.  
*PHASMOCONUS* GROUP: *C. janus*, *C. neptunus*, *C. ochroleucus*, *C. pretiosus*.  
*PIOCONUS* GROUP: *C. consors*, *C. fischoederi*, *C. magus*, *C. richeri*.  
*PROFUNDICONUS* GROUP: *C. lani*, *C. profundorum*, *C. scopulisqla*, *C. smirna*.  
*PUNCTICULIS* GROUP: *C. arenatus*, *C. pulicarius*, *C. vautieri*, *C. zeylanicus*.  
*REGICONUS* GROUP: *C. aulicus*, *C. auratinus*, *C. aureus*, *C. auricomus*.  
*RHIZOCONUS* GROUP: *C. capitaneus*, *C. miles*, *C. mustelinus*, *C. namocanus*.  
*RHOMBUS* GROUP: *C. imperialis*, *C. zonatus*.  
*STEPHANOCONUS* GROUP: *C. archon*, *C. cedonulli*, *C. mappa*, *C. regius*.  
*STRIOCONUS* GROUP: *C. barthelemyi*, *C. gauguini*, *C. gubernator*, *C. striatus*.  
*TEXTILIA* GROUP: *C. bullatus*, *C. cervus*, *C. dusaveli*, *C. vicveei*.  
*TURRICONUS* GROUP: *C. exelsus*, *C. gemmulatus*, *C. sowerbii*, *C. subaequalis*.  
*VARIOCONUS* GROUP: *C. cepasi*, *C. fuscolineatus*, *C. naranjus*, *C. nobrei*.  
*VIRGICONUS* GROUP: *C. berdulinus*, *C. coelinae*, *C. kintoki*, *C. martensi*.  
*VIRROCONUS* GROUP: *C. chaldaeus*, *C. coronatus*, *C. ebraeus*, *C. miliaris*.  
*XIMENICONUS* GROUP: *C. paraguana*, *C. perplexus*, *C. tornatus*, *C. ximenes*.

#### A RELATIVELY STRICT DIET

The Conidae are relatively strict carnivores. Based on the study of KOHN (1959), they can be put into three categories: vermivorous (*C. coronatus*, *C. imperialis*, *C. lividus*, *C. striatellus*, ...), which feed principally on annelids and polychaetes: Eunicidae, Terebellidae, Sabellidae; piscivorous (*C. catus*, *C. ermineus*, *C. geographus*, *C. consors*, ...) which feed on fish; and molluscivorous (*C. textile*, *C. dalli*, *C. retifer*, *C. auratinus*), which feed on molluscs such as Strombidae, Cymatiidae and other Conidae (RICHARD personal observations). LIM (1969) estimated that 65% of cones were worm-eaters, 18% were fish-eaters and 16% mollusc-eaters (figures rounded off). More recent

studies have tended to confirm these findings. At least two types of identification keys have been proposed, one of which is based on diet. The first relies on the morphology of the radular teeth, which have now been described in numerous species (ROLAN & RAYBAUDI MASSILIA, 1994) and which seem to be highly adapted to predator preference (ENDEAN & RUDKIN, 1965) and to the strategy used by *Conus* to envenomate their prey (LE GALL *et al.*, 1999). The second is based on the presence or absence of a few specific shell characteristics (LIM, 1969). Nevertheless, there are a few exceptions that do not fulfil these conditions and certain observations show that the existence of a fourth category, including omnivorous cones with a more varied diet (*C. californicus*, *C. pictus*, ...), should be considered. Finally, several species are known to frequently stray from their diet, *C. regius* (considered to be vermivorous) being a well-known example.

Cones first paralyse their prey by firing a harpoon-like radular tooth (Fig. 1B-D), a veritable poisoned dart through which the venom is injected. The tooth is fired by the protrusible proboscis, which is then used to ingest the prey. These darts have their origin in the transformation of the radular ribbon which, during the course of evolution, may have lost its lateral and median teeth while the outside teeth grew longer, became hooked and jagged. They are well separated and stored, ready for use, in a radular sac at the back of the pharynx.

In fact, the venomous device of the Conidae consists of four organs (Fig. 2): the venom gland, the venom duct, the radular sac and the pharynx-proboscis complex.

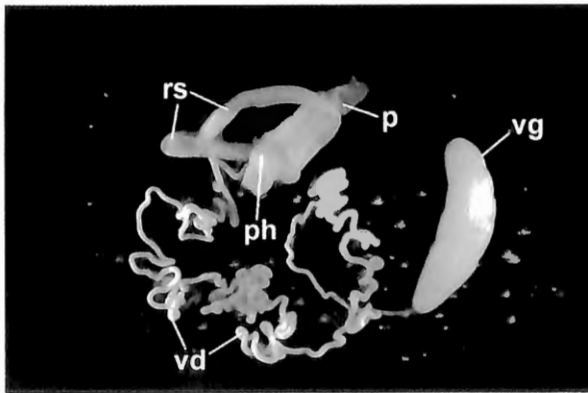


Fig. 2. – Venom apparatus of Conidae. It comprises the proboscis (p), the pharynx (ph), the radula sac (rs), the venom gland (vg) and the venom duct (vd). The role of these various components is detailed in the text.

*The venom gland*, otherwise known as the gland of Leiblin, is the largest organ of the venomous apparatus. Histological observations have confirmed the mechanical function of this gland, which was once considered to be responsible for secreting the poison. A transverse section shows that the internal structure has three layers: two layers of polygonal cells between which lies a fibrous ring about one hundred microns thick that acts as

a kind of skeleton, reinforcing the cohesion of the outer layer. The crescent shape gland is whitish, lies at right angles to the axis of the cone's body, slightly to the left side, and with the concave side facing forward.

*The venom duct* is the main organ of the venomous apparatus. It is a long yellowish tube, 4 to 6 cm long and a few hundred micrometers in diameter, wound into a ball. It emerges from the back of the pharynx, on the right side, just behind the muscular ring that forms the base of the rostrum (this description corresponds most closely to *C. lividus*). A section through this duct shows a large luminous area filled with an abundance of venom in the form of strings of coloured granules, surrounded by a thin epithelium of cuboidal secretory cells lying on a fibrous base. The wall of the tube comprises an intermediary layer of smooth ring muscles and an outer layer of longitudinal muscles. It is, as HERMITTE pointed out as far back as 1946, not just a simple duct for the transport of venom to the envenomation apparatus but the organ where the venom is made.

*The radular sac* consists of a short arm whose front end emerges on the right side of the pharynx, and a long, curved arm that starts at the middle of the short arm and continues toward the right front edge of the interior cavity.

The odontoblasts at the bottom of the radular sac are responsible for synthesizing the teeth. The teeth are initially chitinous and flexible but then become hard during their migration from the long to the short arm (MARSCH, 1977). En route to the short arm, the radular teeth are organised into two parallel and longitudinal rows, with their sharp ends pointing toward the bottom of the sac. Once inside the short arm, the mature and rigid teeth now face towards the opening into the pharynx. They are held there by a flexible and transparent ligament comprising a cylindrical stack of acellular strips attached to the base of the tooth. The teeth, ready for use, are in fact thin calcified sheets, from a few tens of micrometers to one or two centimeters long, rolled up to form cylinders and flared at the edges. This configuration allows them to accumulate venom.

The morphology of the radular teeth can vary enormously between species and is strictly related to diet (ENDEAN & RUDKIN, 1965), indicating a high degree of functional adaptation. The teeth of the vermivorous species are generally smaller and simpler (straighter, with fewer, simpler barbels) (Fig. 1C). Some piscivorous *Conus* tether their prey before engulfing them, and are named «harpooners» (Fig. 1E). In contrast to the vermivorous and molluscivorous species, these piscivorous species have the most complex radular tooth morphology, which in all likelihood they require to perforate the fish tegument (see Fig. 1B).

*The proboscis-pharynx complex* is at the front end of the Conidae venomous apparatus. The end of the short arm of the radular sac emerges on the right side of the pharynx. It then continues toward the front as a pre-pharynx, surrounded by a long protrusible proboscis. The latter is itself contained within a rostrum, which is a kind of sheath bearing among other things the ocular peduncles.

It would appear that, during an attack, the animal invaginates the end of its proboscis down as far as the front opening of the short arm of the radular sac to load a tooth. The tooth is then ejected in the direction of the prey by a rapid devagination of the proboscis. Histological sections of the proboscis show that the wall is covered with a thick layer of



muscle-type cells, which are all sheathed in a thick tegument of transversally striated fibres. This structure gives the organ its phenomenal capacity for extension and contraction.

The toxicity of cones has been known for a very long time. As early as 1705, RUMPHIUS reported the death of a native woman on the island of Banda (the Molucca islands) after she was stung by a *Conus textile*. Sir Edward Belcher was himself the victim of a *Conus aulicus* while collecting specimens of marine molluscs during the famous scientific expedition on the H.M.S. Samarang; luckily, the sting was not fatal. The first accidents in eastern Polynesia reported in the literature were due to *Conus tulipa*, a species that is abundant on certain atolls of the Tuamotu Archipelago.

Although all cones are capable of stinging the imprudent collector working on sunken reefs, the sting of most species only results in slight pain (*C. eburneus*, *C. virgo*, and the vermivorous species in general). Cones that provoke the most serious consequences are *C. geographus*, *C. omaria*, *C. striatus*, *C. tulipa*, *C. textile* and *C. magus*, i.e. mainly fish-eating, followed by mollusc-eating cones.

#### BIOGEOGRAPHY

The geographic range of a species depends on its capacity to adapt to imposed external factors, and especially on its means of dissemination. A majority of Conidae species have a high rate of reproduction but are rather stenotopic in relation to ecological factors and have a relatively short larval life, although some species do have planktonic periods lasting about a month (*C. coronatus*, for example). As a result, none of the Conidae species is cosmopolitan or even circumtropical. Pan-provincial species do, however, exist and poorly distributed species can be found in all of the great oceanic subdivisions of the globe, although the situation varies according to the biogeographical province. In the Indo-Pacific, the number of planktonic species is 4 to 5 times higher than that of non-planktonic species, whereas in the southern Atlantic the ratio is inverted between the two categories of larval development (it is doubled in the case of the Caribbean and the ratio is even greater in the case of West Africa).

For the Indo-Pacific, at least 50 of the 330 species (number rounded off) occur throughout this biogeographical region. These species (including *C. catus*, *C. chaldaeus*, *C. ebraeus*, *C. lividus*) represent a percentage of the specific wealth in Conidae that increases as one leaves the Philippines (25%) in the direction of Madagascar (50%) or French Polynesia (75%). This corresponds to an axis of impoverishment in species, more pronounced to the east than to the west, going away from the Philippines. As a result, almost all of the species of Conidae in western Polynesia are pan-Indo-Pacific or endemic.

Generally speaking, less than fifty species of Conidae have a distribution zone which covers all of the biogeographical province to which they belong. More than half of the others are species that are distributed over several biogeographical regions, not necessarily adjoining, of the same province. In addition, each biogeographical region has species of Conidae that are present solely in that region.

Furthermore, each region has a collection of various numbers of macroendemic species. The distribution of these species can cover the whole region (*C. taeniatus*, in the north-west of the Indian Ocean and the Red Sea), or just an archipelago (*C. julii*, in the Mascarene Islands). More often than not, however, endemism depends on geographical insularity. Some archipelagos have numerous endemic species and/or sub-species, such as the Marquesas Islands, which are the islands furthest away from a continent. Some of these micro-endemic species are restricted to a group of islands (*C. gauquini* on the Marquesas Islands (RICHARD & SALVAT, 1973)), to one island (*C. magellanicus*, in Guadeloupe) or even to a single bay (*C. nobrei*, in Angola). The table below provides a few examples of endemic species in several regions of the inter-tropical zone :

PANAMANIAN REGION : *C. archon*, *C. bartschi*, *C. purpurascens*, *C. vittatus*  
 CARIBBEAN REGION : *C. cardinalis*, *C. cedonulli*, *C. granulatus*, *C. jucundus*  
 BRAZILIAN REGION : *C. clerii*, *C. carioca*, *C. scopulorum*, *C. selenae*  
 SENEGALESE REGION : *C. adansonii*, *C. cloveri*, *C. mercator*, *C. taslei*  
 PERSIAN REGION : *C. ardisiaceus*, *C. melvilli*, *C. milesi*, *C. stocki*  
 CALEDONIAN REGION : *C. boucheti*, *C. lamberti*, *C. lienardi*, *C. luciae*  
 POLYNESIAN REGION : *C. encaustus*, *C. marchionatus*, *C. marielae*, *C. vautieri*  
 SOUTH AFRICAN REGION : *C. infrenatus*, *C. natalis*, *C. pictus*, *C. tinianus*

#### EACH CONE HAS ITS OWN HABITAT

We are now beginning to have a good insight into the lifestyle of the Conidae that live in shallow water, particularly those that live in the coral reefs of Melanesia and western Polynesia, where one of the authors of this article has been on numerous missions. In these areas, cones are to be found on dead coral substrates, such as slabs (*C. balteatus*, *C. ebraeus*, *C. miliaris*), on madrepora clumps (*C. circumcissus*, *C. magnificus*, *C. omaria*), on rough detrital accumulations (*C. coffeae*, *C. flavidus*, *C. imperialis*) or in sandy basins (*C. arenatus*, *C. bullatus*, *C. tessulatus*). Certain species have very specific habitats, such as *C. legatus*, which lies buried in the sand with *Halimeda*, on the outer slopes of the Society Islands, or *C. miles*, which lives nestled in the grass situated behind the algal crest, on the outer reefs of the Tuamotu atolls.

In other regions of the reef environment, vast stretches of the sea bed sediment have been colonised by different species : *C. ammiralis*, *C. aulicus*, *C. generalis* ; in the thick mass of grass, *C. ammiralis* seems to have been replaced by *C. pseudocedonulli* (observations made in the Amirante Islands, which form part of the Seychelles islands).

The rocky coasts also have their own species of Conidae, which take up residence under blocks of rock (*C. ardisiaceus*, *C. monachus*), in mud (*C. artopus*, *C. viola*) or among algae (*C. klemae*, *C. orion*). Some members of the family have even settled in mangroves (*C. trigonus*).

The above examples correspond to situations very frequently observed during field missions, but they are not exhaustive. Every cone has at some time been observed outside of its zone of predilection. Generally speaking, the majority of species (at least 60%) are considered to be part of the epifauna. In French Polynesia, for example, about twenty

species of cone from a total of seventy, *i.e.* less than half, are part of the endofauna. However, for paleogeographical reasons, this region is particularly poor in endofauna in the lagoons of its atolls.

Each zone of the reef also has its dominant, characteristic and exclusive species. In French Polynesia, *C. coronatus* and *C. quercinus* can be more easily found on the fringing reef, whereas *C. pulicarius* and *C. ratus* are more abundant on the barrier reef. To find *C. catus* and *C. retifer*, it is best to look on the outer reefs of atolls where the conglomerates lie on slabs. *C. moreleti* can be found in the depressions under rocky outcrops on the outer slopes. On the outer slopes, below low tide mark, numerous discoveries are still to be made. It is from this poorly accessible zone that the few rare samples of *C. adamsonii*, *C. aurisiacus* and *C. luteus* come.

The ocean islands of the central Pacific are surrounded by steep slopes. However Australia, for example, has a continental shelf with numerous species of endemic Conidae, including *C. rufimaculosus*, *C. sculetti* and *C. wallangra*.

Even further down, light is now being shed on the Conidae fauna of the bathyal level, particularly around New Caledonia (*C. boucheti*, *C. estivali*, *C. kanakinus*, *C. luciae*), in the south-west of the Indian Ocean (*C. patens*, *C. gradatulus*, *C. caillaudi*) and along the coast of Brazil (*C. capricorni*, *C. candidus*, *C. selenae*). The samples trawled in the New Caledonia region are so abundant that they have enabled a bionomic diagram to be proposed for a bathymetric gradient that goes from a depth of 100 to 700 m. (RÖCKEL *et al.*, 1995). Of the 17 species of cones trawled below 450 m., eight were captured alive: *C. alisi* (460 m., max. depth), *C. boucheti* (500 m.), *C. ichinoseana* (490 m.), *C. loyaltiensis* (480 m.), *C. luciae* (485 m.), *C. pergrandis* (509 m.), *C. profundorum* (500 m.) and *C. teramachii* (675 m.). At these depths, none of the species found so far are cosmopolitan. However *C. orbignyi* has been trawled in the Mozambique channel and near Madagascar (*C. orbignyi elokismenos*), off Indonesia and the Philippines (*C. orbignyi orbignyi*), and off New Caledonia (*C. orbignyi coriolisi*) and it forms, together with *C. emersoni* (American Pacific), *C. macgintyi* and *C. mazei* (southern Atlantic), a group of circum-tropical species.

## CONIDAE VENOMS

The venoms of cone snails contain short peptides called conotoxins. Each cone produces a mixture of conotoxins that have multiple biological activity (OLIVERA, 1997; LE GALL, 1999). In general these peptides have a highly conserved structure that will be reviewed according to the molecular targets involved in their specific actions.

### Conotoxins acting on sodium channels: the $\mu$ -, $\mu\text{O}$ - and $\delta$ -conotoxins

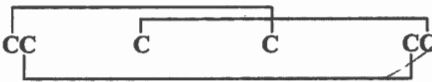
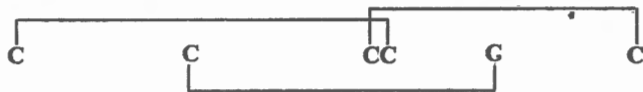
Sodium channels are transmembrane proteins that play a fundamental role in membrane excitability. At least six different toxin receptor binding sites have been identified on the sodium channel-protein (reviewed by CATERALL, 1986; GORDON, 1997). From a general standpoint two families of conotoxins have been reported to interact with voltage-dependent sodium channels: the  $\mu$ -conotoxins, which block the channels by binding to

their receptor-site 1, and the  $\delta$ -conotoxins, which mainly modify channel inactivation by binding to their receptor-site 6.

**The  $\mu$ -conotoxins from the venom of piscivorous Conus**

The  $\mu$ -conotoxins, first purified from the venom of *C. geographus*,  $\mu$ -GIIIA,  $\mu$ -GIIIB and  $\mu$ -GIIIC (CRUZ *et al.*, 1985) are basic peptides comprising 22 amino-acid residues folded by three disulfide bridges and include an amidated C-terminal (Table 1). Electrophysiological experiments, performed on sodium channels purified from rat muscle or brain and incorporated into lipid bilayers, revealed that  $\mu$ -GIIIA reversibly inhibits muscle sodium channels, in a voltage-dependent manner, without affecting neuronal sodium channels (CRUZ *et al.*, 1985). The specificity of action of  $\mu$ -conotoxins on muscle sodium channels was further confirmed by MOCZYDLOVSKY *et al.* (1986), who reported that the toxins have no affinity for neuronal sodium channels, but instead inhibit the specific binding of  $^3\text{H}$ -saxitoxin and  $^3\text{H}$ -tetrodotoxin on receptor-site 1 of muscle sodium channels.

TABLE 1  
*Sequences of  $\mu$ -conotoxins that block voltage-gated sodium channels*

$\mu$ -conotoxins	Sequences*
<b>Piscivorous Conus</b>	
<i>C. geographus</i>	
$\mu$ -GIIIA	RDCCTOOKKCKDRQCKOQRCCA
$\mu$ -GIIIB	RDCCTOORKCKDRRCCKOMKCCA
$\mu$ -GIIIC	RDCCTOOKKCKDRRCCKOLKCCA
<i>C. purpurascens</i>	
$\mu$ -PIIIA	ZRLCCGFOKSCRSRQCKOHRCC
Framework	
<b>Molluscivorous Conus</b>	
<i>C. marmoreus</i>	
$\mu$ O-MrVIA	ACRKKWEYCI VPI I GF I YCCPGLI CGPFVCV^A
$\mu$ O-MrVIB	ACS K KWEYCI VPI L GF VYCCPGLI CGPFVCV^A
Framework	

\* See references in text.

Following the chemical syntheses of  $\mu$ -conotoxins (CRUZ *et al.*, 1989), these conopeptides proved to be tools of particular interest both for studying synaptic transmission

mechanisms at the vertebrate neuromuscular junction (SOSA & ZENGEL, 1993), and to functionally discriminate between the different types of voltage-dependent sodium channels, *i.e.* muscle or neuronal (CRUZ *et al.*, 1989).

Recently, a novel polypeptide ( $\mu$ -PIIIA) was isolated from the venom of *C. purpurascens* (SHON *et al.*, 1998a). As reported for  $\mu$ -conotoxins,  $\mu$ -PIIIA comprises 22 amino-acid residues folded by three disulfide bridges (see Table 1). Although  $\mu$ -PIIIA was shown to block muscle sodium channels by binding to their receptor-site 1, its action was not reversible. Moreover, in contrast to previously reported  $\mu$ -conotoxins,  $\mu$ -PIIIA also reversibly inhibited rat brain type II sodium channels expressed in *Xenopus* oocytes. This result raises questions about the specificity of  $\mu$ -conotoxins, purified from piscivorous cone snails, on muscle sodium channels.

Here again, the conotoxin  $\mu$ -PIIIA provides a good tool to pharmacologically differentiate between different sub-types of voltage-dependent sodium channels: (1) the sodium channels from skeletal muscle sensitive to  $\mu$ -PIIIA and  $\mu$ -GIIIA, (2) the neuronal sodium channels (type II) that are sensitive to  $\mu$ -PIIIA and resistant to  $\mu$ -GIIIA, and (3) the neuronal  $\mu$ -PIIIA and  $\mu$ -GIIIA-insensitive sodium channels (see SHON *et al.*, 1998a).

### ***The $\mu$ - and $\mu$ O-conotoxins from the venom of molluscivorous Conus***

The recent characterization of two  $\mu$ -conotoxins,  $\mu$ -PnIVA and  $\mu$ -PnIVB, purified from the venom of the molluscivorous cone snail *C. pennaceus* (see Table 1), introduces another specificity in the blocking action of  $\mu$ -conotoxins (FAINZILBER *et al.*, 1995a). Indeed, these toxins specifically act on mollusc neuronal sodium channels without altering mammalian neuronal sodium channels, *i.e.* those of either bovine chromaffin cells or rat brain synaptosomes.

Finally, two conotoxins, the  $\mu$ O-conotoxins MrVIA and MrVIB, have been purified from the venom of the molluscivorous cone *C. marmoreus*. These toxins differ from those described above firstly, because they are composed of 31 amino-acid residues (instead of 22, see Table 1), and secondly, because they do not bind to the receptor-site 1 of sodium channels. In particular,  $\mu$ O-MrVIA blocks rat brain type II sodium channels expressed in *Xenopus* oocytes, but neither affects nor modifies the specific binding of  $^3$ H-saxitoxin to rat brain membranes or to *Torpedo* electric organ (FAINZILBER *et al.*, 1995b; MCINTOSH *et al.*, 1995, TERLAU *et al.*, 1996a). Therefore,  $\mu$ O-MrVIA inhibitory action on the neuronal sodium channel occurs through the binding to a receptor-site that is different from site 1.

### ***The $\delta$ -conotoxins from the venom of molluscivorous Conus***

The identification of the receptor-site 6 of voltage-dependent sodium channels was made possible by using a novel conotoxin purified from the venom of *C. textile* named  $\delta$ -TxVIA, (FAINZILBER *et al.*, 1994). This hydrophobic peptide of 27 amino-acid residues is folded by three disulfide bridges (Table 2) (HILLYARD *et al.*, 1989; FAINZILBER *et al.*, 1991). Specific binding studies, involving an iodinated derivative of  $\delta$ -TxVIA, revealed that  $\delta$ -TxVIA interacts with high affinity, and in a voltage-independent manner, with the

receptor-site 6 of rat brain sodium channels. It should be noted that  $\delta$ -TxVIA was reported to have no toxic activity when injected into mammals (ZLOTKIN *et al.*, 1996; SHICHOR *et al.*, 1996). Until now, there has been no clear explanation to account for this discrepancy.

TABLE 2  
Sequences of  $\delta$ -conotoxins that inhibit sodium channel inactivation

$\delta$ -conotoxins	Sequences*
<b>Piscivorous Conus</b>	
<i>C. nigropunctatus</i> $\delta$ -NgVIA	S K C F S O G T F C G I K O G L C C S V R C F S L F C I S F E
<i>C. purpurascens</i> $\delta$ -PVIA	E A C Y A O G T F C G I K O G L C C S E F C L P G V C F G
<b>Molluscivorous Conus</b>	
<i>C. textile</i> $\delta$ -TxVIA	W C K Q S G E M C N L L D Q N C C D G Y C I V L V C T
$\delta$ -TxVIB	W C K Q S G E M C N V L D Q N C C D G Y C I V F V C T
<i>C. gloriamaris</i> $\delta$ -GmVIA	V K P C R K E G Q L C D P I F Q N C C R G W N C V L F C V ^
Framework	

\* See references in text.

### The $\delta$ -conotoxins from the venom of piscivorous Conus

In contrast to  $\delta$ -TxVIA, the two  $\delta$ -conotoxins,  $\delta$ -NgVIA and  $\delta$ -PVIA, purified from the venom of *C. nigropunctatus* and *C. purpurascens* respectively (see Table 2), were reported to have a toxic activity when injected into mammals (FAINZILBER *et al.*, 1995c; SHON *et al.*, 1995). In addition,  $\delta$ -PVIA and  $\delta$ -NgVIA were shown to suppress and/or slow sodium channel inactivation in rat hippocampal cells and in mollusc neurons respectively (TERLAU *et al.*, 1996b; FAINZILBER *et al.*, 1995c). Although both  $\delta$ -PVIA and  $\delta$ -NgVIA were reported to inhibit the specific binding of  $\delta$ -TxVIA on rat and mollusc neuronal membranes,  $\delta$ -NgVIA is supposed to bind to sodium channels on a receptor-site different from site 6.

### Conotoxins acting on calcium channels: the $\omega$ -conotoxins

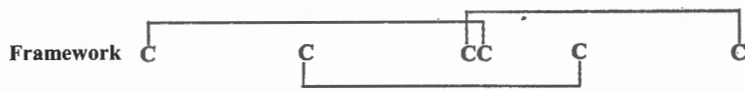
Because calcium ions play a crucial role in the regulation of various cell functions including neurotransmitter release, enzyme activation, axonal growth, muscle contraction, membrane excitability and gene expression, voltage-dependent calcium channels have

been studied in great detail. The understanding of the physiological function of calcium channel sub-types, notably those named N, P/Q, has been made possible by using toxins isolated from the venom of various cones. Indeed, the  $\omega$ -conotoxins purified from the venom of piscivorous cones, due to their high specificity, have been essential tools to characterize the different sub-types of calcium channels in nerve cells and chemical synapses (see for reviews, CRUZ & OLIVERA, 1986; MC CLESKEY *et al.*, 1987; RIVIER *et al.*, 1987; MYERS *et al.*, 1990; OLIVERA *et al.*, 1994; MILJANICH & RAMACHANDRAN, 1995).

The first  $\omega$ -conotoxin that was isolated and purified from the venom of *C. geographus* was named  $\omega$ -GVIA (OLIVERA *et al.*, 1984). Then, two other  $\omega$ -conotoxins were directly purified from the venom of the cone *C. magus*:  $\omega$ -MVIIA and  $\omega$ -MVIIB (OLIVERA *et al.*, 1987) and two others were characterized ( $\omega$ -MVIIC and  $\omega$ -MVIID) from cDNA gene sequences, extracted from the venom duct (KOCH *et al.*, 1990; MONJE *et al.*, 1993). Other  $\omega$ -conotoxins have been isolated:  $\omega$ -SVIA and  $\omega$ -SVIB from the venom of *C. striatus*,  $\omega$ -TVIA from the venom of *C. tulipa* and  $\omega$ -RVIA from the venom of *C. radiatus* (MILJANICH *et al.*, 1991; RAMILO *et al.*, 1992). All these  $\omega$ -conotoxins are basic peptides composed of 24 to 29 amino-acid residues folded by three disulfide bridges (Table 3) (NISHIUCHI *et al.*, 1986).

TABLE 3  
 *$\omega$ -conotoxins that block voltage-gated sodium channels*

$\omega$ -conotoxins	Sequences *	<i>Conus</i> species
$\omega$ -GVIA	C K S O G S S C S O T S Y N C C R S C N O Y T K R C Y	<i>C. geographus</i>
$\omega$ -MVIIA	C K G K G A K C S R L M Y D C C T G S C R S G K C	<i>C. magus</i>
$\omega$ -MVIIB	C K G K G A S C H R T S Y D C C T G S C N R G K C	
$\omega$ -MVIIC	C K G K G A P C R K T M Y D C C S G S C G R R G K C	
$\omega$ -MVIID	C Q G K G A S C R K T M Y N C C S G S C N R G R C	
$\omega$ -SVIA	C R S S G S O C G V T S I C C G R C Y R G K C	<i>C. striatus</i>
$\omega$ -SVIB	C K L K G Q S C R K T S Y D C C S G S C G R S G K C	
$\omega$ -TVIA	C L S O G S S C S O T S Y N C C R S C N O Y S R K C	<i>C. tulipa</i>
$\omega$ -RVIA	C K P O G S O C R V S S Y N C C S S C K S Y N K K C G	<i>C. radiatus</i>



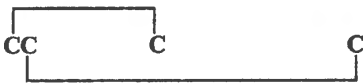
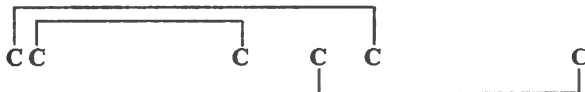
\* See references in text.

The conotoxin  $\omega$ -GVIA exerts a specific action on N-type calcium channels. Indeed, it irreversibly blocks calcium channels in various mammalian neuronal preparations (FELDMAN *et al.*, 1987; REGAN *et al.*, 1991). As a result of its specific action,  $\omega$ -GVIA inhibits neurotransmitter release at the frog neuromuscular junction, as revealed by the blockade of nerve-evoked end-plate potentials, the spontaneous miniature end-plate potentials being unaffected by the toxin (KERR & YOSHIKAMI, 1984; KOYANO *et al.*, 1987). Surprisingly,  $\omega$ -GVIA was reported to have no effect on the mammalian neuromuscular junction (ANDERSON & HARVEY, 1987). The  $\omega$ -MVIIA also blocks N-type calcium channels but, in contrast to  $\omega$ -GVIA, its effects are reversible (OLIVERA *et al.*, 1987).

More recently, the conotoxins  $\omega$ -MVIIC and  $\omega$ -MVIID were reported to block, with a high affinity, P/Q-type calcium channels in Purkinje cells of the mammalian cerebellum, as well as (although with a lower affinity) the N-type calcium channels (HILLYARD *et al.*, 1992; MONJE *et al.*, 1993; KRISTIPATI *et al.*, 1994). As a consequence,  $\omega$ -MVIIC inhibits neurotransmitter release at the mammalian neuromuscular junction, as revealed by the reduction and blockade of nerve-evoked end-plate potentials (SUGIURA *et al.*, 1995).

Therefore, it appears that the variability of intercysteine residues of  $\omega$ -conotoxins (see Table 3) is responsible for the specificity of action of these neurotoxins on the different presynaptic calcium channel sub-types. Thus,  $\omega$ -conotoxins are considered as essential tools, firstly to pharmacologically separate the distinct calcium channel sub-types, and secondly, to identify new ones.

TABLE 4  
 *$\alpha$ -conotoxins that inhibit muscular nicotinic acetylcholine receptors*

$\alpha$ -conotoxins	Sequences*
<i>C. geographus</i>	
$\alpha$ -GI	E C C N P A C G R H Y S C
$\alpha$ -GIA	E C C N P A C G R H Y S C G K
$\alpha$ -GII	E C C H P A C G K H F S C
<i>C. magus</i>	
$\alpha$ -MI	E C C N P A C G R H Y S C G K
<i>C. ermineus</i>	
$\alpha$ -SI	I C C N P A C G P K Y S C
$\alpha$ -SIA	Y C C H P A C G P K Y S C
<i>C. ermineus</i>	
$\alpha$ -EI	R D O C C Y H P T C N M S N P Q I C
Framework	
<i>C. purpurascens</i>	
$\alpha$ A-PIVA	G C C G S Y O N A A C H O C S C K D R O S Y C G Q
<i>C. ermineus</i>	
$\alpha$ A-EIVA	G C C G P Y O N A A C H O C G C K V G R O O Y C D R O S G G
$\alpha$ A-EIVB	G C C G K Y O N A A C H O C G C T V G R O O Y C D R O S G G
Framework	

\* See references in text.



### Conotoxins acting on nicotinic acetylcholine receptors: the $\alpha$ -conotoxins

Among the prominent conotoxins found in cone venom, the  $\alpha$ -conotoxins define a set of peptides sharing a similar cysteine pattern and pharmacological target: the nicotinic acetylcholine receptors (nAChRs). Unlike the  $\alpha$ -neurotoxins from snake venoms (comprising 60 to 80 amino-acids), the  $\alpha$ -conotoxins are small peptides of 12 to 30 amino-acid residues, usually folded by two disulfide bridges and showing a characteristic cysteine pattern (—CC—C—C—) (Tables 4 and 5). Due to their small peptide length, these conopeptides can be easily obtained by chemical synthesis. Moreover, the preparation of specific derivatives is relatively straightforward since  $\alpha$ -conotoxins have many chemical groups that can potentially be modified. As a consequence of the high variability in the sequence of  $\alpha$ -conotoxins from one species to another (OLIVERA, 1996), a great number of these toxins have been characterized. They present a high specificity for the different types of nAChRs, *i.e.* peripheral nAChRs of skeletal muscles and central nAChRs of neurons, and even for the different receptors sub-types.

TABLE 5

*$\alpha$ -conotoxins that inhibit neuronal nicotinic acetylcholine receptors*

$\alpha$ -conotoxines	Séquences*
<b>Pisvivorous <i>Conus</i></b>	
<i>C. magus</i>	
$\alpha$ -MI	G C C S N P V C H L E H S N L C
<b>Vermivorous <i>Conus</i></b>	
<i>C. imperialis</i>	
$\alpha$ -ImI	G C C S D P R C A W R C
<b>Framework</b>	

\* See references in text.

### The $\alpha$ -conotoxins acting on peripheral nAChRs

The  $\alpha$ -conotoxin GI, isolated from *C. geographus* (GRAY *et al.*, 1981), MI from *C. magus* (MCINTOSH *et al.*, 1982), SI from *C. striatus* (ZAFFARALLA *et al.*, 1988) and EI from *C. ermineus* (MARTINEZ *et al.*, 1995) target peripheral nAChRs that are composed of  $\alpha_2\beta\gamma\delta$  subunits. Some of these conotoxins recently aroused interest due to their ability to specifically inhibit one of the two acetylcholine binding sites of nAChRs. Indeed, in mammals, the  $\alpha$ -conotoxins MI and GI have been shown to specifically target the  $\alpha/\delta$  binding site compared to the  $\alpha/\gamma$  binding site of muscle nAChRs (KREINKAMP *et al.*, 1994; GROEBE *et al.*, 1995). However, in the fish *Torpedo marmorata*, these toxins preferentially bind to the  $\alpha/\gamma$  site (HANN *et al.*, 1994; GROEBE *et al.*, 1995). Site-directed mutagenesis of nAChRs

of mouse skeletal muscles led to the identification of three amino-acids that differ between  $\delta$  and  $\gamma$  subunits and that are involved in the binding of  $\alpha$ -conotoxins (SINE *et al.*, 1995).

The conotoxins  $\alpha$ A-EIVA and  $\alpha$ A-EIVB, purified from the venom of *C. ermineus*, as well as the conotoxin  $\alpha$ A-PIVA purified from the venom of *C. purpurascens*, are polypeptides comprising 25 to 30 amino-acid residues folded by three disulfide bridges (Table 4) (HOPKINS *et al.*, 1995; JACOBSEN *et al.*, 1997). As reported for the  $\alpha$ -conotoxin SI, the two conotoxins  $\alpha$ A-EIVA and  $\alpha$ A-EIVB block nAChRs of mammalian skeletal muscle by binding indifferently to their two sites, *i.e.*  $\alpha/\delta$  and  $\alpha/\gamma$ .

### *The $\alpha$ -conotoxins acting on neuronal nAChRs*

Various  $\alpha$ -conotoxins are high-affinity ligands for neuronal nAChRs (Table 5) which are composed of  $\alpha$  ( $\alpha_2$  to  $\alpha_6$ ) and  $\beta$  ( $\beta_2$  to  $\beta_4$ ) subunits. It is worth noting that the number of nAChR subunits, identified to date, allows the targeting of a great diversity of nicotinic receptors (by multiple combination of the subunits) in various tissues. The  $\alpha$ -conotoxin ImI, purified from *C. imperialis* (MCINTOSH *et al.*, 1994), selectively targets the  $\alpha_7$  subunit, which forms an homomeric nAChR. In contrast, nAChRs composed of  $\alpha_3\beta_2$  subunits are potently blocked by the  $\alpha$ -conotoxin MII isolated from the venom of *C. magus* (CARTIER *et al.*, 1996). The  $\alpha$ -conotoxins PnIA and PnIB, both purified from *C. penanceus*, block *Aplysia* neuronal nAChRs (FAINZILBER *et al.*, 1994). More recently, the  $\alpha$ -conotoxin EpI, isolated from *C. episcopatus*, was characterized as a specific inhibitor of  $\alpha_3\beta_2$  and  $\alpha_3\beta_4$  nAChRs (LOUGHAN *et al.*, 1998).

Finally,  $\alpha$ -conotoxins have been reported as useful tools for phylogenetic discrimination between vertebrate nAChRs (ZAFFARALLA *et al.*, 1988). In addition, these conotoxins have proved to be very effective in probing nAChRs by photoaffinity labeling with considerable accuracy (MYERS *et al.*, 1991, 1993). Therefore, it appears that  $\alpha$ -conotoxins represent useful tools for probing the surface of the acetylcholine receptor. Both the work so far carried out on  $\alpha$ -conotoxins and the wide variety of cone species give great promise for potential applications of these conotoxins. The discovery of new, highly specific ligands will improve the understanding of the pharmacology, physiology and structure-activity relationships of nAChRs.

### **Conotoxins acting on potassium channels: the $\kappa$ -conotoxin**

The conotoxin  $\kappa$ -PVIIA, purified from the venom of *C. purpurascens*, is a polypeptide comprising 27 amino-acid residues folded by three disulfide bridges and including an amidated C terminal (Table 6) (TERLAU *et al.*, 1996b). Electrophysiological studies revealed that  $\kappa$ -PVIIA reversibly blocks the Shaker-type of potassium channels expressed into *Xenopus* oocytes, without affecting either the  $Kv_{1.1}$  or the  $Kv_{1.4}$  type of potassium channels in rat brain (SHON *et al.*, 1998b). The conotoxin  $\kappa$ -PVIIA appears thus to be a specific tool to study the Shaker type of potassium channels. The conotoxins  $\kappa$ -PVIIA and  $\delta$ -PVIA act in synergy for rapid prey immobilization, as required by the « harpooners » strategy used by *C. purpurascens*.

TABLE 6

*Sequence of the κ-conotoxin that blocks the voltage-gated potassium channel*

κ-conotoxin	Sequence*
κ-PVIIA	C R I O N Q K C F Q H L D D C C S R K C N R F N K C V
Framework	

\* See references in text.

**Conotoxins acting on N-methyl-D-aspartate receptors : the conantokins**

The conantokins are a family of conotoxins including conantokin-G, purified from the venom of *C. geographus* (McINTOSH *et al.*, 1984 ; OLIVERA *et al.*, 1985), and conantokin-T, isolated from the venom of *C. tulipa* (HAACK *et al.*, 1990). These toxins have been reported to block N-methyl-D-aspartate receptors and, as a consequence, to inhibit calcium influx into central nervous system neurons.

Conantokins comprise 4 to 5 γ-carboxyglutamate residues (Table 7). These residues have been suspected to play an essential role in the formation, in the presence of calcium ions, of stable α-helices, which are necessary for the physiological action of conantokins. It is worth noting that conantokins do not contain cysteine residues.

TABLE 7

*Conantokins present in the venom of C. geographus and C. tulipa*

<i>C. geographus</i> Conantokine-G	G E γ γ L Q γ N Q γ L I R γ K S N
<i>C. tulipa</i> Conantokine-T	G E γ γ Y Q K M L γ N L R γ A E V K K N A

\* See references in text.

SEARCHING FOR NEW CONOTOXINS

Using molecular biology techniques, it has been possible to search in the venom duct of Conidae species for specific ADN coding for prepropeptides, which are precursors of mature toxins. Such prepropeptides have three structural segments : a signal sequence, a propeptide region and the mature toxin region. For a given family (for example the α-conotoxin family), the C-terminal end of the prepropeptide (signal sequence) is well

conserved, whereas the N-terminal part (coding for the mature toxin) is hypervariable (WOODWARD *et al.*, 1990). The conserved signal sequence may play an addressing role towards a determined region of the endoplasmic reticulum where the prepropeptide may undergo post-traductionnal modifications. Such modifications have been characterized in conotoxins, and include C-terminal amidation, glutamate carboxylation, prolyl hydroxylation (STONE *et al.*, 1982), tryptophan bromination (JIMENEZ *et al.*, 1996, 1997) and tyrosine sulfatation (LOUGHNAN *et al.*, 1998). The signal sequence could also help the specific formation of the disulfide bridges leading to the mature toxin. Indeed, a linear peptide containing 6 cysteines can fold into 15 different isomers. Notably, only one form possessing biological activity is found in the venom. Although the role of the propeptide is at present unknown, it may help the formation of disulfide bridges. The mature toxin region is hypervariable in its sequence within a conserved cysteine framework. The variability of the residues between the cysteines produces a great variety of toxin sequences. Thus, with a conserved structure (same cysteine framework), the toxin may exhibit a specificity for either sodium, calcium or potassium channels. This biochemical strategy can be summarized as follows: one common structure for diverse physiological activities.

In conclusion, the genus *Conus* represents an almost inexhaustible source of bioactive products because of its species richness, each species demonstrating an original set of conotoxins. Thus, the venoms of Conidae present a challenge both due to the number of toxins they contain and their pharmacological diversity. These conotoxins, by their selective pharmacological action, are of great interest for neuroscientists requiring selective tools to study nervous function. Moreover, conotoxins can have multiple applications, for example the probing of ionic channels with fluorescent toxins or mapping of binding sites between toxins and given ionic channels or receptors. In this way, conotoxins have already greatly improved our understanding of the function of some voltage-sensitive ionic channels. By way of peptide synthesis, most of the conotoxins are now available in large quantities for widespread use. Some conotoxins such as  $\omega$ -conotoxins have major therapeutic potential. Indeed, the  $\omega$ -conotoxin MVIIA is used in chronic neuropathic and malignant pain to relieve patients resistant to opioid treatments. To date, venoms from a dozen *Conus* species have been partially studied. Given the diversity of the genus *Conus* and the number of conotoxins found in the venom from each species, the search for new bioactive compounds in Conidae species offers great promise.

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